Identification and Evaluation of Brominated Carbazoles as a Novel Antibiotic Adjuvant Scaffold in MRSA

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Cite This: ACS Med. Chem. Lett. 2022, 13, 483−491

ABSTRACT: Antibiotic-resistant infections are a pressing global concern, causing millions of deaths each year. Methicillin-resistant Staphylococcus aureus (MRSA) is a leading cause of nosocomial infections in healthcare settings and is increasingly responsible for community-acquired infections that are often more difficult to treat. Antibiotic adjuvants are small molecules that potentiate antibiotics through nontoxic mechanisms and show excellent promise as novel therapeutics. Screening of low-molecular-weight compounds was employed to identify novel antibiotic adjuvant scaffolds for further elaboration. Brominated carbazoles emerged from this screening as lead compounds for further evaluation. Lead carbazoles were able to potentiate several β-lactam antibiotics in three medically relevant strains of MRSA. Gene expression studies determined that these carbazoles were dampening the transcription of key genes that modulate β-lactam resistance in MRSA. The lead brominated carbazoles represent novel scaffolds for elaboration as antibiotic adjuvants.

KEYWORDS: MRSA, antibiotic resistance, antibiotic adjuvants, carbazole, β-lactams

The World Health Organization has declared antibiotic resistance one of the top 10 global health threats facing humanity.1 In 2014, it was estimated that deaths from antibiotic-resistant infections would top 10 million per year by 2050 without decisive action.2 Unfortunately, the ongoing COVID-19 pandemic has likely accelerated the hypothesized timeline. Prolonged hospital stays, lengthy ventilator use, supportive care from indwelling medical devices such as catheters and IVs, and increased antibiotic treatments have all contributed to a notable increase in antibiotic-resistant infections worldwide.3−7 Novel approaches to combatting these infections are urgently needed.

Antibiotic adjuvants are an attractive tool for reinvigorating our current arsenal of antibiotics.8 Adjuvants are nontoxic to the bacteria on their own but potentiate the activity of antibiotics when coadministered. The therapeutic utility and efficacy of an adjuvant approach has been observed with Augmentin, a combination of amoxicillin (a β-lactam antibiotic) and clavulanic acid (a β-lactamase inhibitor). Augmentin has been in clinical use since the early 1980s and continues to be a highly prescribed and effective combination therapy for the treatment of bacterial infections.9 Novel adjuvants with varied mechanisms of action could be an effective way to revive existing antibiotics and provide clinicians with multiple combinations of medications to effectively treat drug-resistant bacterial infections.

Previously, we have disclosed the identification and study of two classes of U.S. Food and Drug Administration (FDA)-approved compounds that potentiated β-lactam antibiotics in methicillin-resistant Staphylococcus aureus (MRSA) by dampening the transcription of key resistance genes.10,11 The structural similarities between amoxapine and loratadine, the lead compounds identified in our previous studies, suggested that screening low-molecular-weight compounds with similar structural features could help identify novel scaffolds with similar activities for further synthetic elaboration.12−14 We assembled a small library of compounds that were enriched in fused aromatic scaffolds and evaluated their ability to inhibit MRSA growth in combination with a sublethal dose of oxacillin. Several candidate scaffolds emerged from this screen. On the basis of atom economy and the commercial availability of many derivatives, we chose to focus our attention on carbazoles as active fragments for further study.

Received: December 8, 2021
Accepted: February 2, 2022
Published: February 9, 2022
A small library of commercially available carbazoles (Figure 1) was screened for the ability to potentiate oxacillin in MRSA 43300, a common laboratory reporter strain (Table 1). Several compounds in the series demonstrated modest adjuvant activity, defined as four-fold reductions in the oxacillin minimum inhibitory concentration (MIC). This initial screening also revealed the importance of halogenation for activity. Compounds 1, 9, 13, and 14, which lack halogen substituents, did not lower the MIC of oxacillin. Interestingly, compounds 5 and 15 did not exhibit any adjuvant activity despite being halogenated. Compounds 6 and 7, which are halogenated at the same positions as 5 and 15, reduce the MIC of oxacillin two-fold, which is not considered significant in this assay. This suggests that halogenation at the three-position of the carbazole provides a slight improvement in activity and that this activity may depend on the identity of the halogen as well. Overall, the brominated carbazoles displayed the greatest adjuvant activity in the series. Compounds 2 and 8, both monobrominated carbazoles in the one- and four-positions of the carbazole, showed the greatest adjuvant activity, lowering the MIC of oxacillin by eight-fold and eight/four-fold, respectively. Compounds 10 and 12, both dibrominated carbazoles, were also able to lower the MIC of oxacillin four-fold.

We next sought to determine whether this class of compounds was capable of potentiating oxacillin in other medically relevant MRSA strains and whether the enhanced activity of the brominated carbazoles would be mirrored in these strains (Table 2). In addition to MRSA 43300, our expanded MRSA panel included USA100, the predominant hospital-acquired MRSA strain with high levels of resistance to oxacillin, USA300, the most common community-acquired MRSA strain in the United States, and COL, a hospital-acquired MRSA strain with high levels of oxacillin resistance. Gratifyingly, the brominated carbazoles also lowered the MIC of oxacillin in both USA100 and USA300. In USA100, 2 was most potent, lowering the MIC of oxacillin 512-fold. Compound 8 lowered the oxacillin MIC 16-fold, followed by compounds 10 and 12, which each lowered the oxacillin MIC four-fold. In USA300, 2, 8, and 10 each lowered the oxacillin MIC four-fold. Interestingly, the trends seen across 43300, USA100, and USA300 were absent in COL. In COL, most carbazoles were unable to lower the oxacillin MIC. Carbazoles 11 and 12 each lowered the MIC four-fold to 128 μg mL⁻¹, but there was no

![Figure 1. Library of commercially available carbazoles used to generate an initial structure–activity relationship (SAR).](https://doi.org/10.1021/acsmedchemlett.1c00680)

| compound | compound | concentration (μM) | MIC (μM) | fold reduction in oxacillin MIC |
|----------|----------|--------------------|----------|-----------------------------|
| 1        | >200     | 50                 | 32       | 1                           |
| 2        | >200     | 50                 | 4        | 8                           |
| 3        | >200     | 50                 | 16       | 2                           |
| 4        | >200     | 50                 | 16       | 2                           |
| 5        | >200     | 50                 | 32       | 1                           |
| 6        | >200     | 50                 | 16       | 2                           |
| 7        | >200     | 50                 | 16       | 2                           |
| 8        | 100      | 25                 | 4/8      | 8/4                         |
| 9        | >200     | 50                 | 32       | 1                           |
| 10       | >200     | 50                 | 8        | 4                           |
| 11       | 50       | 10                 | 16/32    | 2/1                         |
| 12       | >200     | 50                 | 8        | 4                           |
| 13       | >200     | 50                 | 64       | 0.5                         |
| 14       | >200     | 50                 | 32       | 1                           |
| 15       | 100      | 25                 | 32       | 1                           |
apparent correlation between adjuvant activity and halogen identity as seen in other strains.

On the basis of our results from the initial screens with oxacillin, we identified compounds 2, 8, and 12 as our lead carbazoles for further investigation. We utilized these compounds to expand our study of β-lactam potentiation across each of the previously studied MRSA strains (Table 3). The selected β-lactams target different transpeptidase enzymes in the peptidoglycan biosynthetic machinery, allowing us to determine whether our lead carbazoles display a narrow spectrum of activity or broadly potentiate β-lactam antibiotics. In 43300, the lead carbazoles primarily potentiated oxacillin, ampicillin, and cefoxitin. Little to no potentiation of methicillin and ceftazidime was observed. Carbazoles 2 and 8 lowered the MICs of oxacillin and ampicillin between four- and eight-fold followed by lowering the MIC of cefoxitin four-fold to 16 μg mL⁻¹. Dibrominated carbazole 12 lowered the MIC of oxacillin four-fold but elicited only one- to two-fold reductions in the MICs of ampicillin and cefoxitin. Little to no potentiation of methicillin and ceftazidime was observed. Carbazoles 2 and 8 were able to lower the MIC of ampicillin and cefoxitin between two- and four-fold but showed very little activity against the other tested β-lactams. Compound 8 was able to lower the MIC of ampicillin and cefoxitin between four- and eight-fold, and it lowered the MICs of oxacillin and methicillin both four-fold. Only methicillin was appreciably affected by 12, with a four-fold reduction in the MIC. All other β-lactam antibiotics tested were unaffected by treatment with 12. As previously seen, our lead carbazoles showed very little efficacy in COL. Compounds 2 and 8 were unable to potentiate any of the tested β-lactams beyond a two-fold reduction in the MIC. Dibrominated carbazole 12 demonstrated the greatest activity and was able to reduce the MICs of oxacillin and ampicillin four-fold and two- to four-fold, respectively.

Taken together, these results highlight some interesting patterns. Ceftazidime was not appreciably potentiated by our lead carbazoles, most often only leading to fold reductions in MIC in the one- to two-fold range (Table 3). Only compound 12 in USA100 was able to reduce the ceftazidime MIC above four-fold. These data suggest that our lead carbazoles are not likely interacting with any molecular targets involved in ceftazidime resistance. In USA100, oxacillin and methicillin MICs were strongly potentiated by treatment with the lead carbazoles as compared with the other tested β-lactam antibiotics; this may indicate that our compounds interfere with one or more pathways that involve resistance to oxacillin and methicillin. Compound 12 appears to have little effect on the MIC of cefoxitin, whereas 2 and 8 potentiate cefoxitin in all strains except COL. This is particularly notable in USA300, as cefoxitin targets PBP4, a low-molecular-weight transpeptidase. In hospital-acquired MRSA strains, PBP4 has negligible contributions to antibiotic resistance. However, in community-acquired MRSA strains, such as USA300, PBP4 is essential for the expression of β-lactam resistance. Interfering with resistance mechanisms that protect PBP4 from inactivation by β-lactam antibiotics such as cefoxitin could represent novel and important treatments for combating CA-MRSA infections. Intriguingly, COL is largely refractory to treatment with our lead carbazoles. This indicates that either COL does not contain the molecular target(s) of these compounds or that the strain evades their effect in some other way.

To gain more insight into how these carbazoles were potentiating β-lactam antibiotics, we analyzed key resistance genes’ mRNA levels via RT-qPCR (Figure 2). The bla and mec operons are the two main drivers of β-lactam resistance in MRSA. The bla operon contains the blaZ gene, which encodes a class C penicillinase that is expressed at high levels upon

| compound | S. aureus 43300 | S. aureus USA100 | S. aureus USA300 | S. aureus COL |
|----------|----------------|----------------|----------------|---------------|
| --       | 32             | 256            | 32             | 512           |
| 1        | 32 [1]         | 256 [1]        | 32 [1]         | 512 [1]       |
| 2        | 4 [8]          | 0.5 [512]      | 16 [4]         | 512 [1]       |
| 3        | 16 [2]         | 128 [2]        | 16/32 [2/1]    | 512 [2]       |
| 4        | 16 [2]         | 128 [2]        | 32 [1]         | 512 [1]       |
| 5        | 32 [1]         | 128 [2]        | 32 [1]         | 512 [1]       |
| 6        | 16 [2]         | 128 [2]        | 16 [2]         | 512 [1]       |
| 7        | 16 [2]         | 128 [2]        | 32 [1]         | 512 [1]       |
| 8        | 4/8 [8/4]      | 16 [16]        | 8 [4]          | 512 [1]       |
| 9        | 32 [1]         | 256 [1]        | 32 [1]         | 512 [1]       |
| 10       | 8 [4]          | 64 [4]         | 8 [4]          | 256 [2]       |
| 11       | 16/32 [2/1]    | 256 [1]        | 16 [2]         | 128 [4]       |
| 12       | 8 [4]          | 128 [4]        | 16 [2]         | 128 [4]       |
| 13       | 64 [1]         | 256 [1]        | 64 [1]         | 512 [1]       |
| 14       | 32 [1]         | 256 [1]        | 16/32 [2/1]    | 512 [1]       |
| 15       | 32 [1]         | 256 [1]        | 16/32 [2/1]    | 512 [1]       |

“Oxacillin MIC values in μg mL⁻¹. βFold reduction in MIC provided in brackets. All compounds were assayed at 50 μM except compound 8 (25 μM) and compound 11 (10 μM).
exposure to β-lactam antibiotics. Sharing homologous architecture with the bla operon, the mec operon contains mecA, which encodes a modified penicillin binding protein, PBP2a, with reduced affinity for β-lactam antibiotics. We hypothesized that if the carbazoles were sensitizing MRSA to oxacillin, then these compounds could be downregulating the gene expression of the bla or mec operons. As expected, the blaZ expression was not affected by compound 8 or 2 alone but was significantly elevated when cultures were treated with oxacillin (Figure 2a,b). Upon cotreatment with compound 8 or 2 and oxacillin, blaZ levels were repressed. These changes in blaZ gene expression were observed for both 43300 and USA100 strains. (USA300 and COL do not contain the bla operon.) blaI mRNA levels were also affected by compounds 8 and 2, however, not as dramatically (Figure S4). This is consistent with results from our previous studies demonstrating that cotreatment with either amoxapine or loratadine and oxacillin leads to dampened blaZ transcription.10,11

We next measured mecA mRNA levels in four different MRSA strains. Like blaZ, mecA was not significantly affected by carbazole treatment alone but was elevated upon oxacillin treatment. Cotreatment with carbazole and oxacillin resulted in a dampening of mecA levels similar to untreated samples (Figure 2c,d). These gene expression changes were unique in the COL strain. Figure 2c shows that mecA levels were not elevated by oxacillin treatment. This is consistent with the lack of oxacillin potentiation these compounds displayed in COL (Tables 2 and 3) and suggests that COL is genetically distinct enough from other tested strains to be unaffected by these carbazoles.

Other transcripts found in the bla and mec operons, blaI and mecl, were also quantified using RT-qPCR. Whereas the same trends in gene expression changes were often observed, they were not as significant as in blaZ and mecA (Figure S4). Notably, compound 8 cotreatment resulted in more pronounced gene expression changes than compound 2. We also measured pbp2 mRNA levels, as this gene was not predicted to be regulated in the same manner as genes within the bla and mec operons. As expected, pbp2 mRNA levels remained unaffected by all treatments (Figure S4). The exception was in strain 43300, where the presence of oxacillin upregulated pbp2 levels, although less than two-fold on average.

We also tested compound 12 in these gene expression experiments, although it did not result in consistent gene expression changes (Figure S5), suggesting that it may be utilizing a different mechanism of action than compounds 8 and 2. This is consistent with our phenotypic assays in which 12 often exhibited different patterns of antibiotic potentiation as compared with 8 and 2 (Tables 3 and 4). We are continuing to study how this compound may be working at the molecular level.

Because relative mRNA levels should not be directly compared between different strains investigated in different experiments, we calculated the fold dampening upon cotreatment with these carbazoles and oxacillin. This calculation is made by taking the relative mRNA level measured in an oxacillin-treated sample compared with that in a cotreated sample. Table 4 shows that whereas both compounds 2 and 8 dampened the expression of multiple genes in the bla and mec operons, compound 8 elicited greater fold changes in gene expression. Additionally, compound 8 is used at half the concentration of compound 2, further highlighting its superior efficacy and potency. This also highlights the fact that blaZ levels are more drastically altered by carbazole and oxacillin cotreatment than other genes in these operons. Finally, these data

### Table 3. MIC of selected β-lactam antibiotics alone and in combination with carbazoles in selected MRSA strains a,b

| Antibiotic (MIC) | S. aureus 43300 | S. aureus USA100 | S. aureus COL |
|------------------|----------------|----------------|--------------|
| oxacillin (PBP2) | 0.5 128/256 | 4 128/256 | 4 128/256 |
| ampicillin (PBP2) | 16 64/32 | 4 32/16 | 4 32/16 |
| methicillin (PBP1/3) | 256 64/32 | 4 32/16 | 4 32/16 |
| cefotaxime (PBP1/3) | 1024 512 | 256 128/64 | 256 128/64 |
| cefoxitin (PBP4) | 512 64 | 4 4 | 4 4 |

a. Antibiotic MIC values in μg mL⁻¹.
b. Fold reduction in the MIC provided in brackets.

c. Molecular target of the given antibiotic provided in parentheses.
support the phenotypic results that the lead compound 8 most dramatically potentiates oxacillin (Table 2).

Figure 2. Cotreatment with carbazole compounds and oxacillin results in downregulation of blaZ and mecA. (a,b) Average levels of blaZ relative to 16S rRNA are displayed on a log2 scale. (c,d) Average levels of mecA relative to 16S rRNA are displayed on a log2 scale. In all panels, error bars represent the standard error of the mean. *p ≤ 0.05; **p ≤ 0.01. Compound 8 was assayed at 25 μM, and compound 2 was assayed at 50 μM.

Figure 3. Carbazoles do not inhibit Stk1 kinase domain activity in vitro. (a) ADP-Glo assay of truncated Stk1 kinase domain and nonspecific phosphosubstrate myelin-basic protein (MBP) untreated (DMSO) or in the presence of 50 μM of the indicated compounds. RLU, relative luminescence units. Error bars represent the standard deviation of triplicate measurements. (b) Stk1Kin was incubated with DMSO or 50 μM of the indicated compounds before the addition of ATP and MBP. No Stk1Kin was used as a control.

Table 4. Cotreatment with Compound 8 and Oxacillin Dampens Resistance Gene Expression to a Greater Extent than Cotreatment with Compound 2

| gene of interest | fold dampening of gene expression |
|------------------|----------------------------------|
|                  | oxacillin + 2 (50 μM) | oxacillin + 8 (25 μM) |
| blaZ             | 3.86                      | 8.06                    |
| blal             | 1.36                      | 1.36                    |
| mecA             | 1.39                      | 4.00                    |
| mecI             | 0.67                      | 1.28                    |

"Fold dampening represents the relative mRNA level of the gene listed in oxacillin-treated cells divided by that in cotreated cells.

Table 5. Oxacillin Potentiation by Lead Brominated Carbazoles in USA300 Mutant Strains

| USA300 strain       | oxacillin MIC (μg mL⁻¹) |
|---------------------|-------------------------|
|                     | − | +2 | +8 |
| parent, USA300      | 32 | 16  [2] | 8  [4] |
| USA300 Δstk1        | 0.25/0.5 | 0.25/0.5  [1] | 0.25/0.5  [1] |
| USA300 Δ/+stk1      | 4  | 1  [4] | 0.5  [8] |

*Compound 2 was assayed at 50 μM, and compound 8 was assayed at 25 μM. **Fold change in MIC provided in brackets.

On the basis of the observed transcriptional changes, we hypothesized that the adjuvant activity of the carbazoles may be attributed to the inhibition of one or more regulatory kinases in S. aureus. Carbazoles have been previously explored as kinase inhibitors in mammalian systems. Additionally, brominated
Figure 4. Brominated carbazoles disrupt β-lactam resistance gene transcription through an unknown mechanism. (a) MRSA utilizes combinations of PBP2a and β-lactamase to evade and inactivate β-lactam antibiotics. In step 1, β-lactam antibiotics (pink circle) bind to extracellular domains of MecR1, BlaR1, and Stk1. In step 2, the intracellular kinase domain of Stk1 phosphorylates the catalytic domain of BlaR1. BlaR1 cleaves BlaI and MecI, thereby permitting transcription of their respective operons. It is unknown if Stk1 directly phosphorylates MecR1. In step 3, transcription of blaZ and mecA leads to the formation of β-lactamase and PBP2a, respectively. (b) The four MRSA strains utilized in this work and their respective β-lactam resistance architectures are represented. 43300 and USA100 have full-length Stk1, a functional bla operon, and Type II SCCmec, which confers a fully functional mec operon. USA300 does not have the bla operon and thus does not have BlaR1. USA300 possesses the Type IV SCCmec, causing a truncated MecR1 without the extracellular domain and no MecI. COL contains SCCmec I, which has the same MecR1 truncation as that in USA300. It does not contain the bla operon, and its stk1 has a premature stop codon that results in truncation of the extracellular domains. In each of these strains, treatment with β-lactam antibiotics (pink circle) leads to transcription of the mec operon and, if present, the bla operon. (c) Cotreatment with lead-brominated carbazoles (cyan circle) and β-lactam antibiotics (pink circle) dampens the transcription of bla and mec operons as compared with treatment with β-lactam alone in all MRSA strains with a full-length Stk1. COL, which has a truncated Stk1, shows no significant differential transcription of the mec operon upon cotreatment with β-lactams and lead brominated carbazoles. (d) On the basis of chemical genetics assays, we hypothesize that our lead carbazoles impact cellular signaling mechanisms that control the transcription of β-lactam resistance genes. Lead carbazoles may block antibiotic binding to the extracellular portion of Stk1, inhibit autophosphorylation or phosphotransfer from Stk1 to downstream targets, or inhibit signal transduction from a necessary second messenger protein that has yet to be elucidated.
derivatives of the kinase inhibitor meridianin D, which bears structural similarity to carbazoles, have been shown to inhibit biofilm formation in MRSA. An evaluation of regulatory kinases in S. aureus suggested that the PASTA kinase Stk1 could be the molecular target of the lead carbazoles. Stk1 is a global regulator of cell-wall homeostasis and biofilm formation and has been implicated in the regulation of resistance to β-lactams and vancomycin. Furthermore, expression of the biaA operon is known to be directly regulated by Stk1. To test this hypothesis, we utilized a forward chemical genetics study to evaluate the effect of our lead carbazoles on two mutant strains of USA300 (Table 5). USA300 Δstk1 is a deletion mutant, and USA300 Δ/stk1 is the same deletion mutant that has been transformed with a plasmid bearing stk1 under the control of an inducible promoter. In the stk1 deletion mutant, the MIC of oxacillin drops precipitously to 0.25 μg mL⁻¹, which is consistent with previous studies demonstrating that Stk1 is essential for maintenance of the β-lactam resistance phenotype. None of our lead carbazoles showed any further reduction in the MIC of oxacillin in the USA300 Δstk1 strain. The induction of stk1 expression restored the MIC of oxacillin to 4 μg mL⁻¹ in the USA300 Δ/stk1 strain. Additionally, lead carbazoles 2 and 8 were able to efficiently lower the MIC of oxacillin in this strain four- and eight-fold, respectively. Taken together, these results suggested that carbazoles 2 and 8 may inhibit Stk1 or downstream resistance genes, thereby potentiating β-lactam antibiotics.

To rule out the possibility that stk1 transcription was being suppressed rather than the protein kinase, we quantified stk1 mRNA levels. In 43300 (unpublished data), USA100, and USA300, the stk1 mRNA levels remained the same with all treatments tested. The COL strain, however, showed subtle yet statistically significant changes in the stk1 gene expression. Compound 8 treatment alone led to a 1.5-fold increase in stk1 mRNA compared with untreated cells, whereas oxacillin treatment alone led to a two-fold decrease in stk1 levels. Cotreatment returned stk1 to the untreated control level (Figure S4). Because COL produces a truncated version of Stk1, it remains to be investigated how these carbazoles act on it differently compared with a full-length protein. Signals and transcription factors that regulate the expression of Stk1 in S. aureus remain elusive.

We next sought to directly investigate whether our lead carbazoles inhibit Stk1-mediated phosphorylation of the nonspecific substrate myelin basic protein (MBP). Using an ADP-Glo assay, we monitored the in vitro kinase activity of the purified Stk1 kinase domain (Stk1Kin) in the presence of several carbazoles. The phosphotransfer activity of the kinase domain was not significantly changed in the presence of any carbazoles (Figure 3a). Further studies using differential scanning fluorimetry were conducted to determine if the carbazoles were binding to the kinase domain even if they were insufficient to inhibit activity. As seen in Table S2, the carbazoles did not significantly alter the melting temperature of Stk1Kin, indicating that there is no significant binding between the carbazoles and the kinase domain. We next considered whether truncation of Stk1 may impact the inhibition. Several studies have been published evaluating inhibitors against both full-length Stk1 and the isolated kinase domain. Compound MAB-12 was previously reported by Mobashery and colleagues to inhibit full-length Stk1 autophosphorylation and phosphotransfer to MBP with IC₅₀ values of ~16 μM (6 ± 1 μg mL⁻¹). Interestingly, MAB-12 also failed to inhibit the phosphotransfer activity of Stk1Kin (Figure S7) in the ADP-Glo assay. To validate our findings, we monitored the phosphorylation of MBP directly using protein electrophoresis and probing with a phosphopeptide gel stain. Consistent with our previous results, no significant inhibition of MBP phosphorylation was observed when treated with the carbazoles or MAB-12 (Figure 3b). It remains to be determined if the lead carbazoles are able to bind and inhibit full-length Stk1 or if they are interacting with targets downstream of Stk1.

The continued threat of antibiotic-resistant infections necessitates novel treatments to combat a constantly evolving enemy. In an effort to identify new scaffolds for extrapolation as antibiotic adjuvants, we screened an in-house library for low-molecular-weight compounds with β-lactam adjuvant activity in MRSA. We identified three brominated carbazoles as promising candidates for further study and demonstrated that these compounds potentiate β-lactam antibiotics in several medically relevant MRSA strains. Importantly, cotreatment with lead brominated carbazoles and oxacillin dampened the β-lactam resistance response in MRSA, leading to the observed susceptibility to β-lactams. We hypothesized that our lead carbazoles may be inhibiting Stk1, a master regulator of cell-wall homeostasis and antibiotic resistance in MRSA (Figure 4). Interestingly, experiments involving the kinase domain of Stk1 revealed no meaningful interactions with the tested carbazoles. This suggests that either the carbazoles interact with different target(s) in MRSA or that the Stk1 kinase domain alone is insufficient to observe an interaction with Stk1 and the carbazoles. Further studies are under way to elaborate the structure of our lead carbazoles into novel adjuvant molecules for further studies. Additionally, we are continuing work to elucidate the molecular target of the brominated carbazoles and will report these findings in due course.

**ASSOCIATED CONTENT**

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.1c00680.

Experimental details, biological data, binding and inhibition studies, and NMR data for all carbazoles (PDF)

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https://doi.org/10.1021/acsmedchemlett.1c00680
ACS Med. Chem. Lett. 2022, 13, 483–491
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Funding

This work was funded by the National Institutes of Health (R15GM134503 to M.S.B. and H.B.M.), the National Science Foundation (1919685 to M.S.B.), the Beckman Foundation (R15GM134503 to M.S.B. and H.B.M.), the National Science Foundation (1919685 to M.S.B.), the Beckman Foundation (R15GM134503 to M.S.B. and H.B.M.), the National Science

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Dr. Ambrose Cheung for providing the stk1-mutant strains and Dr. Shahriar Mobashery for providing MAB-12.

ABBREVIATIONS

MRSA, methicillin-resistant Staphylococcus aureus; MIC, minimum inhibitory concentration; CA-MRSA, community-acquired methicillin-resistant Staphylococcus aureus; mRNA, messenger ribonucleic acid; RT-qPCR, real-time quantitative polymerase chain reaction; PBP2a, penicillin binding protein 2a; PBP2, penicillin binding protein 2; Stk1, serine-threonine kinase 1; ADP, adenosine diphosphate; DMSO, dimethyl sulfoxide; RLU, relative luminescence units

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