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The chemical synthesis and antibiotic activity of a diverse library of 2-aminobenzimidazole small molecules against MRSA and multidrug-resistant *A. baumannii*

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ABSTRACT

Multidrug-resistant bacterial infections continue to be a rising global health concern. Herein is described the development of a class of novel 2-aminobenzimidazoles with antibiotic activity. These active 2-aminobenzimidazoles retain their antibiotic activity against several strains of multidrug-resistant *Staphylococcus aureus* and *Acinetobacter baumannii* when compared to susceptible strains.

1. Introduction

There is a growing need for new and effective antibiotic agents due to the emergence of life threatening, multidrug-resistant (MDR) bacterial infections[1–6] such as methicillin-resistant *Staphylococcus aureus* (MRSA).[7–9] MRSA infections are the leading cause of nosocomial infections and cause more deaths than complications due to HIV infection in the US every year.[10,11] These infections are not restricted to hospital settings, as community-acquired MRSA infections are on the rise.[12] Vancomycin-resistant strains of *S. aureus* have also emerged, depriving the medical community of their last line of defense for treating MRSA infections.[10] Nosocomial infections from *Acinetobacter baumannii* have also received considerable attention as the incidence of multidrug-resistant infections from this Gram-negative pathogen has steadily been on the rise and has also resulted in several cases of severe infections to American soldiers that were deployed to Iraq and Kuwait.[13–17]

Over the past few years, our group has been interested in the design and synthesis of biologically active small molecules derived from 2-aminoimidazole marine alkaloids.[18–24] Bromoageliferin **1** and oroidin **2** are two such alkaloids that have been reported to possess both antibiotic and biofilm inhibition activities (Fig. 1).[25,26] Although this appears to be contradictory, our group has synthesized and evaluated several synthetic analogues of these natural products and determined that these analogues can be either toxic or non-toxic to planktonic bacteria depending on the compound concentration, bacterial species, and analogue architecture.[18–20] In continuing to develop small molecules inspired by bromoageliferin **1**, we have synthesized and screened a library focused on the 2-aminobenzimidazole (2ABI) ring system for antibacterial activity (Fig. 2).

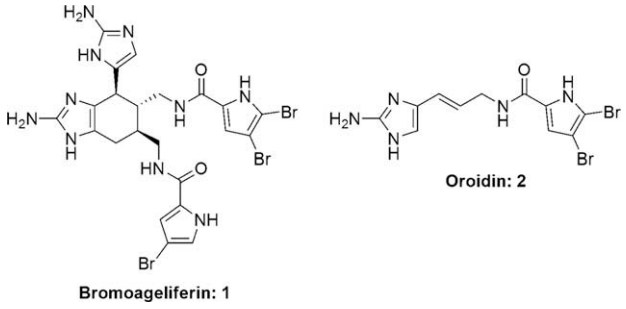

Figure 1. Bromoageliferin **1** and oroidin **2** have reported to have antibiotic and antibiofilm activities against various bacteria.

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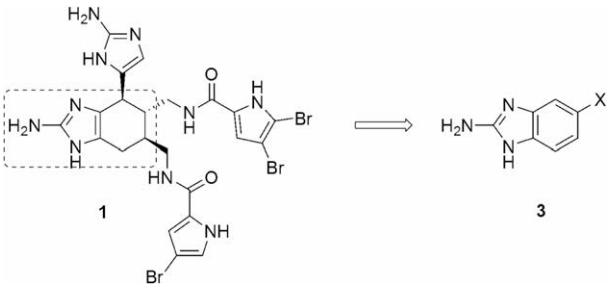


Figure 2. Structural simplification of the core ring system of bromoageliferin **1** to give the general structure of 2ABI **3**.

Benzimidazole derivatives have received attention in recent years due to their biological activities.[27–30] These heterocyclic compounds have demonstrated antimicrobial activity against many bacterial and fungal strains and prove to be promising starting points for studies toward simplified structures of bromoageliferin's tetrahydrobenzimidazole core.[31–38] Furthermore, we have shown that 2ABI small molecules can be appropriately designed to inhibit and disperse biofilms of Gram-positive bacteria.[39]

2. Results and discussion

2.1. Chemical synthesis of 2-aminobenzimidazole (2ABI) library

The chemical synthesis of simple 2ABIs was accomplished via the straightforward condensation between commercially available 1,2-phenyldiamines and cyanogen bromide (Scheme 1). The yields were generally good for this condensation, required no chromatography for purification, were scalable, and after Boc-protection allowed for analogue synthesis using **11** as the key building block of this synthesis. Amine **11** is a mixture of Boc-group constitutional isomers and was not separated, but used as a mixture to elaborate amide and sulfonamide libraries. Once these constitutional isomers were acylated, sulfonylated, or used to make various triazoles, deprotection generated one regiomeric product, thus we didn't feel it necessary to separate the constitutional isomers of **11** at any point.

The synthesis of the 2-aminobenzimidazole amide library was adapted from a previously reported route to **11** starting from **10** (Scheme 2).[40] It was envisioned to use amine **11** to systematically construct several small classes of 2ABI small molecules for screening. Amine **11** was acylated with various acid chlorides or anhydrides followed by Boc-group removal with TFA in methylene chloride, followed by anion exchange to give 2ABI amides as HCl salts in yields between 57% and 94%. Amine **11** was also treated with various sulfonyl chlorides to generate a diverse set of 10 2ABI sulfonamides (Scheme 3). These sulfonamides were synthesized in an analogous approach as the 2ABI amides; however, the overall yields were lower over the two steps (20–84% yield). As with the synthesis of the 2ABI amides, the sulfonylation reaction was quenched with dilute acid upon completion, extracted, passed through a small plug of silica gel and subsequently subjected to Boc-group deprotection by treatment with TFA in methylene chloride. The resulting TFA salt was then dissolved in HCl/methanol, concentrated then washed with ether to give pure 2ABI sulfonamide as an HCl salt (Scheme 3).

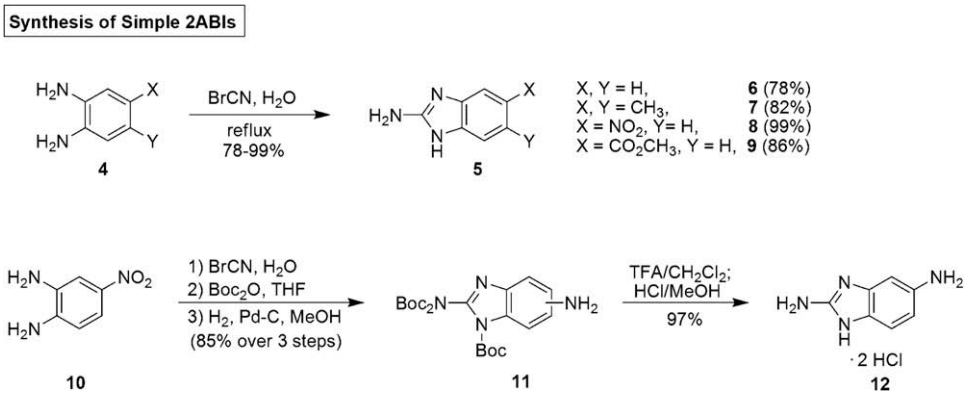
We were also interested in evaluating triazole molecules since our group has had success with triazole conjugates as biofilm modulators.[20,21] As an extension of the amide library, we synthesized alkyne **47** from amine **11** to be used in click reactions with various azides to make 2ABI triazoles (Scheme 4). Azide diversity was accessed through a two-step procedure to generate azide amides from chloroacetyl chloride **49**. This was accomplished by adding chloroacetyl chloride **49** dropwise to an amine at 0 °C to give the corresponding α-chloroamide **50**. This was then used, without purification, and treated with sodium azide in DMF overnight at elevated temperatures to give the α-azido amide **51** in 78–96% yield over two steps. These azide amides were triturated or recrystallized in hexanes and did not require chromatography for purification.

2.2. Screening the 2ABIs library for antibiotic activity against MRSA (ATCC BAA-44)

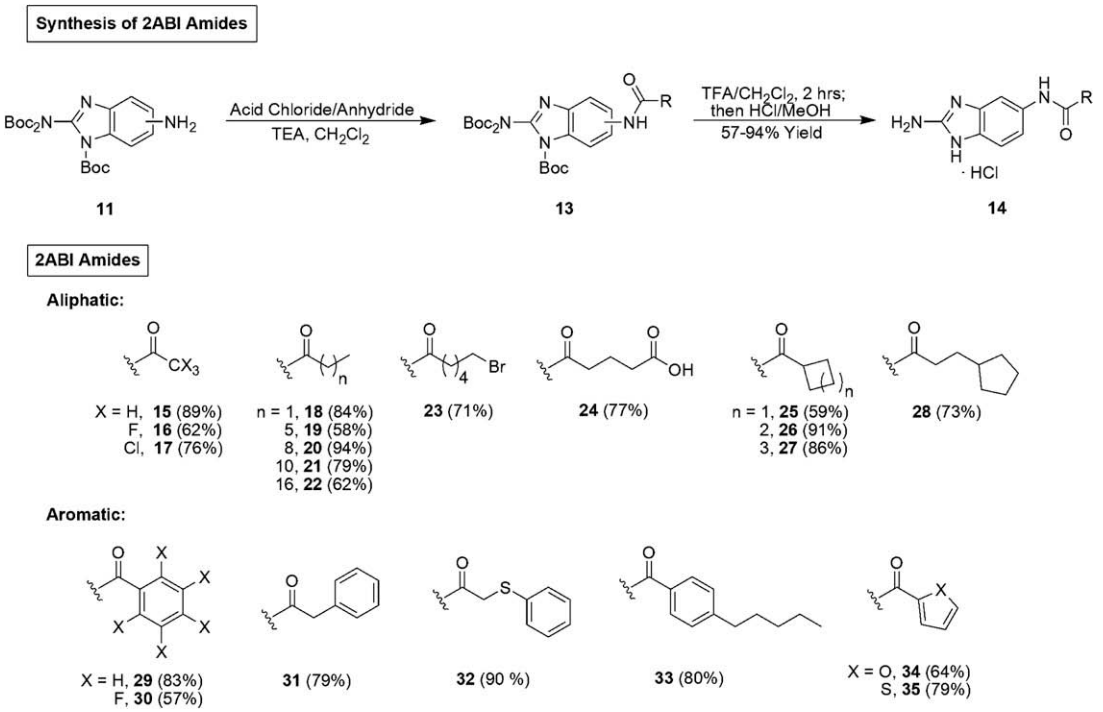
All 2ABIs were evaluated for antibiotic activity by determining MIC values using standard microdilution protocols.[41] With this in mind, we used a 96-well microtiter plate microdilution susceptibility testing starting at 400 μM of each compound and then making 12, twofold serial dilutions (entire range of this screen: 400 μM–391 nM) in PVC wells. MIC values were determined as the lowest concentration at which no growth was observed upon visual inspection after incubating for 16 h at 37 °C. Pellets formed on the bottom of wells were considered bacterial growth even if the wells were clear of turbidity.

Initially, 44 2ABIs were screened against MRSA (ATCC BAA-44). From this initial assay, 37 compounds were completely inactive against BAA-44 with MIC values of >400 μM. Two compounds showed marginal antibiotic activity (MIC = 400 μM) and two sulfonamide derivatives (**38** and **45**) demonstrated moderate antibiotic activity against BAA-44 (MIC = 100 μM). Three amide derivatives (**20**, **21**, and **33**), however, showed good antibiotic activity against BAA-44 with MIC values of 12.5–25 μM (Fig. 3).

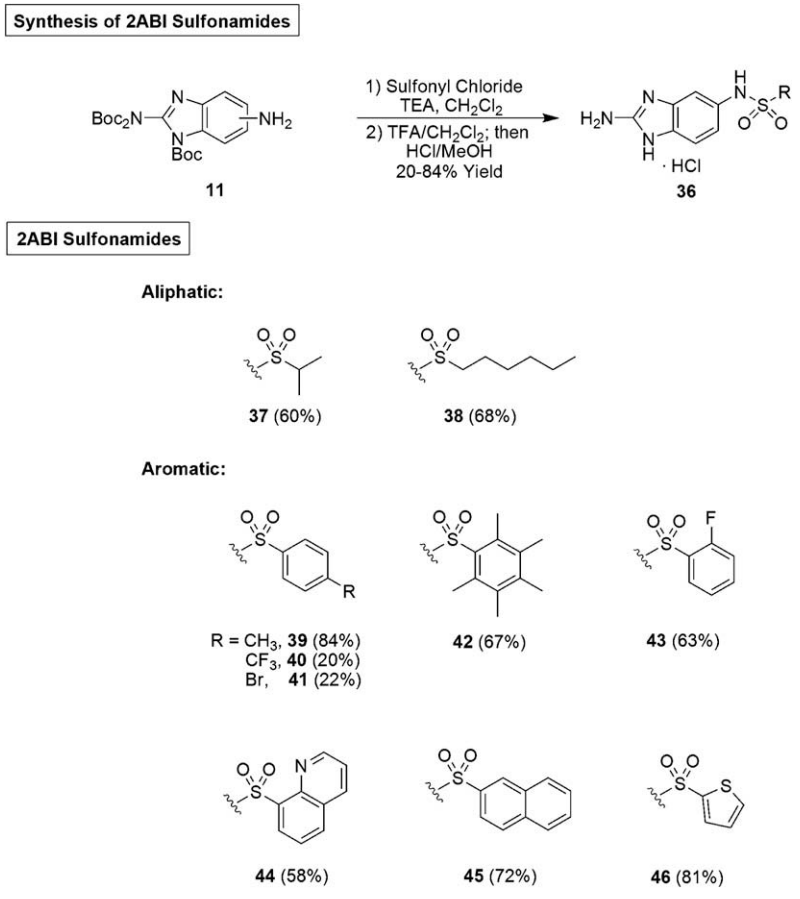
Two members from the 2ABI amide collection, **21** and **33**, demonstrated the most potent antibiotic activity against BAA-44 with MIC values of 12.5 μM while **20** had an MIC of 25 μM. Interesting,



Scheme 1. Synthesis of the simple 2ABIs.

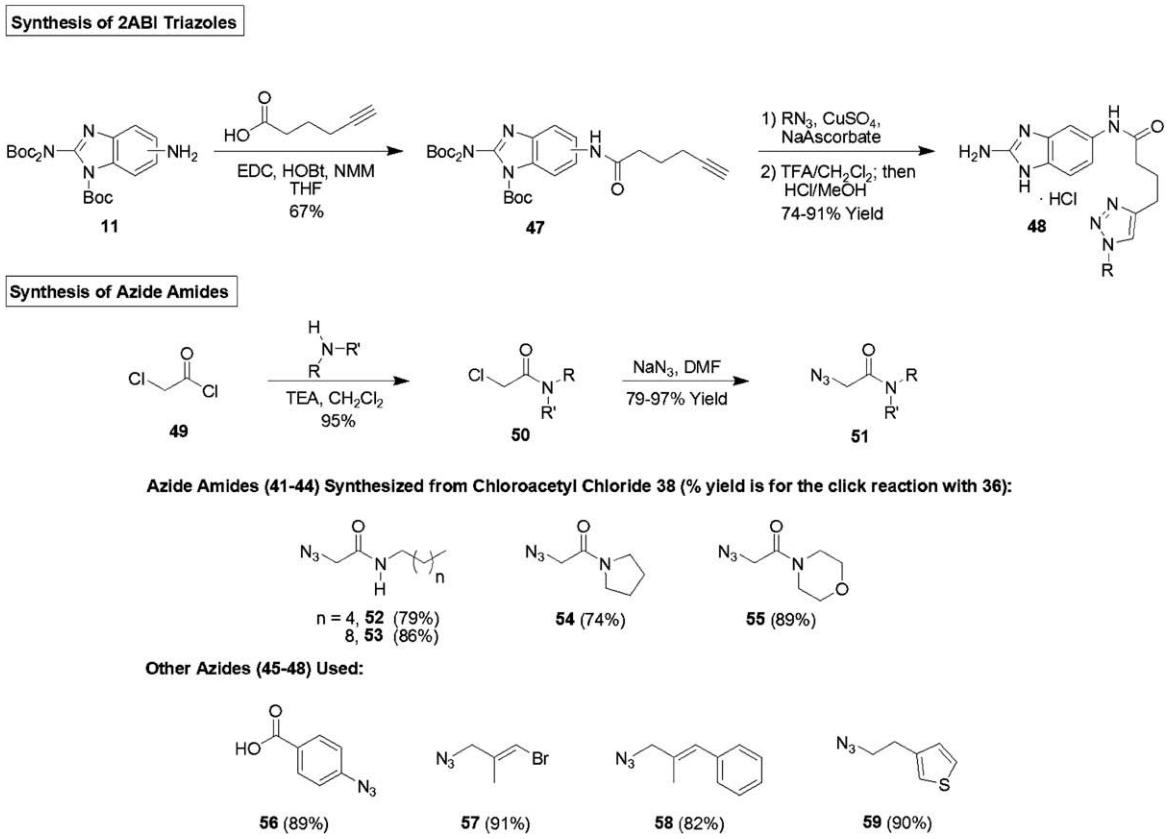


Scheme 2. 2ABI Amides provide structural diversity through amide formation.



Scheme 3. Synthesis of 2ABI sulfonamides.

these three compounds can occupy nearly the same chemical space when the alkyl chains of **20** and **21** are orientated to lay over four of the carbon atoms of the benzene ring of **33** (Fig. 3). There was no antibiotic activity against BAA-44 at the highest concentration



Scheme 4. Synthesis of 2ABI triazoles using click chemistry.

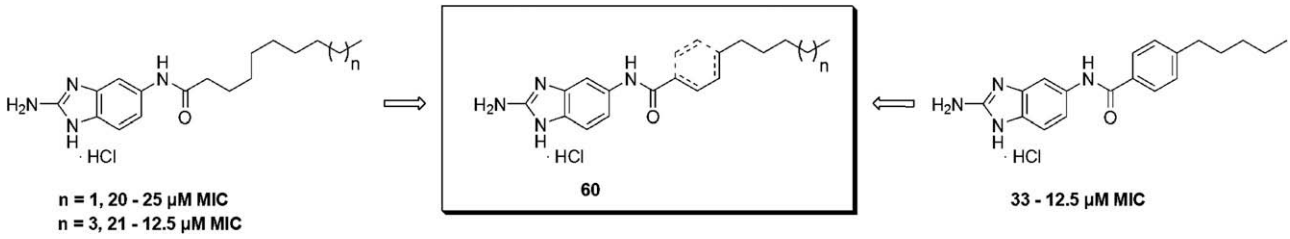


Figure 3. Lead 2ABIs amides against BAA-44.

tested (400 μM) from the shorter aliphatic chain of amide **19**. Lack of activity was also observed for the longer aliphatic chain in amide **22** at 400 μM.

2.3. Second-generation library developed from 33 and simplified analogues to determine the necessary structural elements for antibiotic activity against BAA-44

Due to its structural rigidity, **33** was chosen as the lead structure for further SAR studies. A second-generation library of eight 2ABIs was synthesized in an attempt to enhance antibiotic activity, where the focus of this library was derivatizing the 4-position of the phenyl amide moiety in **33**. Both extension and retraction of hydrocarbon chain length, chain branching, substitution with another aromatic ring, and azide substitution was investigated (Fig. 4).

In addition to these modifications, we synthesized and assayed a number of control compounds to evaluate the necessity of the 2ABI subunit. We focused on the 2-aminoimidazole ring contained within the 2-aminobenzimidazole moiety of **33** by testing the tri-Boc protected synthetic precursor of **33** (**69**, Fig. 5). Although **69** is a mixture of two regioisomers, testing of this mixture in the microdilution susceptibility assays enables us to identify if an unprotected 2-aminoimidazole moiety is required for antibiotic activity against BAA-44. Two other control compounds were also synthesized that incorporated the active 4-pentylphenyl tail of **33** while eliminating the 2-aminobenzimidazole moiety. Complete removal of the 2-aminoimidazole moiety of **33** was accomplished by synthesizing **70**. The aniline amide moiety of **33** was completely eliminated in the design of control structure **71**, which is aimed at determining the role of the 2-aminoimidazole head fused to the 4-pentylphenyl tail in the antibiotic activity of BAA-44 (Fig. 5).

The synthesis of **70** was accomplished by simply adding 4-pentylbenzoyl chloride **72** to aniline (Scheme 5). Control analogue **71** was synthesized by adding **72** to diazomethane in ether and allowing the resulting reaction mixture to stir for one hour. This was followed by treatment with concentrated HBr (aq), and the resulting α-bromoketone was taken on without purification and treated with Boc-guanidine in DMF for three days at room temperature. Following flash chromatography, **71** was formed after Boc-removal

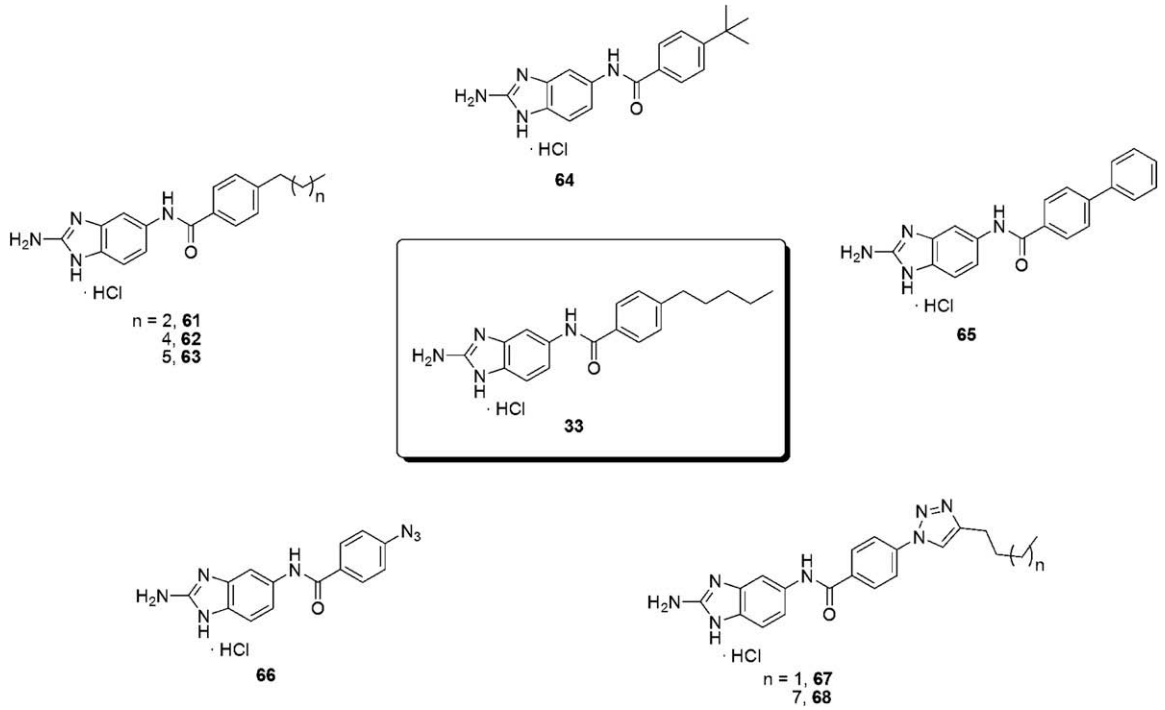


Figure 4. A focused library was synthesized based on the 4-position of the phenyl amide moiety in **33**.

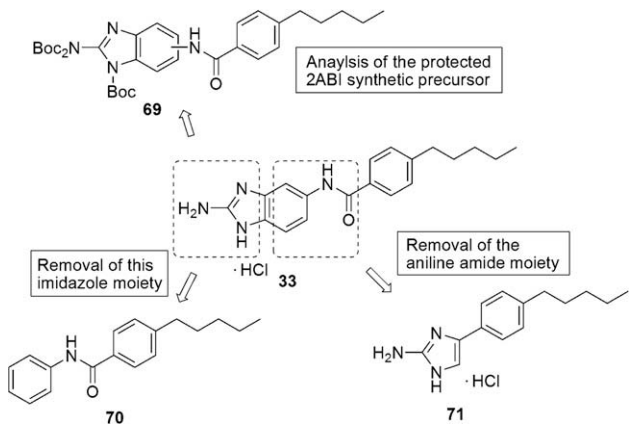
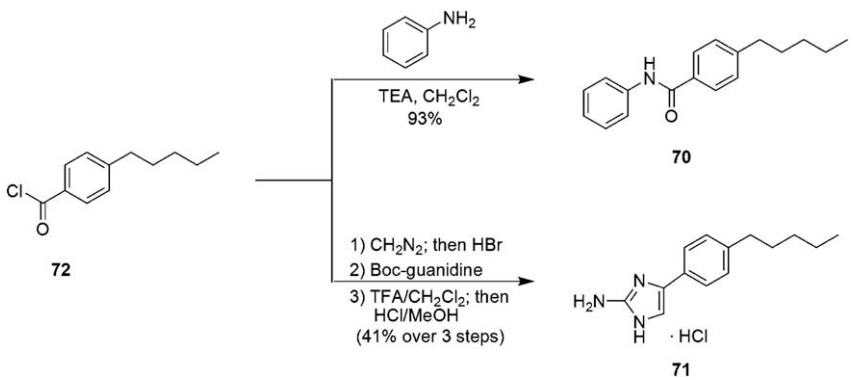


Figure 5. Determining the essential structural elements of **33** for antibiotic activity towards BAA-44 through the screening of various structural analogues related to **33**.

with TFA and final ion exchange to the HCl salt. 2-Aminoimidazole **71** was formed in 41% yield over these three steps.

This second-generation library was then screened in microdilution susceptibility assays to give a structure-activity relationship for these 2ABI amides against BAA-44. The two 4-substituted 2ABI triazoles **67** and **68** demonstrated no activity against BAA-44 as did the 4-phenyl derivative 2ABI amide **65** (MIC >400 μM). The substitution of a 4-azido group in 2ABI amide analogue **66** demonstrated weak antibiotic activity against BAA-44 (MIC = 400 μM).

There was an interesting structural trend in activity in the aliphatic substitution at the 4-position of the phenyl amide moiety of the lead **33**. The 4-*tert*-butyl group substitution of **64** showed a decrease in antibiotic activity from **33**, but still retained some antibiotic activity (MIC = 50 μM). The 4-butyl side chain of **61**, which is one methylene unit shorter than **33**, also demonstrated decreased potency against BAA-44 (MIC = 25 μM for **61** vs MIC = 12.5 μM for **33**). When 2ABI amide **62**, which has an additional methylene unit to its straight chain than **33**, was screened against BAA-44, it demonstrated the same potency as **33** (MIC = 12.5 μM). When this chain



Scheme 5. Synthesis of **70** and **71**.

length was extended out one methylene unit further the activity against BAA-44 began to decrease (MIC = 25 μM). It is apparent that chain length from the 4-position of the phenyl amide moiety is critical for optimal antibiotic activity against BAA-44 with *n*-pentyl and *n*-hexyl chains being optimal against this multidrug-resistant bacterium.

Compounds **69**, **70**, and **71** were also screened for anti-MRSA activity. As predicted, the tri-Boc-protected **69** had no activity against BAA-44 at 400 μM. As in the case with **69**, control **70** was also unable to demonstrate antibiotic activity against BAA-44 at 400 μM. However, 2-aminoimidazole control compound **71** demonstrated the same anti-MRSA activity as **33** with a MIC value of 12.5 μM. From these studies, we conclude that the 2-aminoimidazole heterocycle is required in its unprotected form, either as its own heterocycle or as part of a larger 2-aminobenzimidazole, along with the 4-pentylphenyl or 4-hexylphenyl tail to maximized anti-MRSA activity in microdilution susceptibility testing (Fig. 6).

2.4. Activity of 2-aminobenzimidazoles screened against other *S. aureus* strains

The 2ABIs were further evaluated in the microdilution susceptibility assays against three other *S. aureus* strains (ATCC 29213, ATCC 25923, and ATCC 29740; Table 1) in comparison to five clinically used antibiotics. Results from the microdilution susceptibility testing demonstrated antibiotic-resistance of BAA-44 to every clinical antibiotic tested here (Table 1), except for vancomycin, when compared to the other *S. aureus* strains. As outlined by the ATCC, BAA-44 demonstrates resistant towards methicillin, ciprofloxacin, gentamycin and streptomycin while the other *S. aureus* strains (ATCC 29213, ATCC 25923, ATCC 29740) were found to be susceptible. Using the microdilution susceptibility testing, BAA-44 demonstrated >125-fold increase in resistance towards methicillin, 500-fold increase in resistance towards gentamycin, 400-fold increase in resistance to ciprofloxacin and >125-fold increase in resistance to streptomycin when compared to *S. aureus* strain ATCC 29213.

BAA-44 did not show drug-resistance to these 2ABIs as the relative potencies were consistent against all of the *S. aureus* strains. The lead 2ABIs were found to be more effective against BAA-44 in the microdilution susceptibility screens than methicillin, gentamycin, and streptomycin (Table 1). The active 2ABI leads were as active as ciprofloxacin in the microdilution susceptibility assays (all having MIC = 12.5 μM).

Table 1
MIC values of antibiotics and 2ABIs against *S. aureus* strains

Compound	MRSA BAA-44	ATCC 29213	ATCC 29740	ATCC 25923
Vancomycin	391 nM	391 nM	195 nM	391 nM
Methicillin	>400 μM	3.13 μM	n.a.	n.a.
Ciprofloxacin	12.5 μM	391 nM	391 nM	n.a.
Gentamycin	200 μM	391 nM	⩽195 nM	n.a.
Streptomycin	>200 μM	1.56 μM	391 nM	n.a.
2ABIs:				
20	25 μM	12.5 μM	12.5 μM	25 μM
21	12.5 μM	25 μM	12.5 μM	50 μM
33	12.5 μM	12.5 μM	6.25 μM	12.5 μM
38	100 μM	100 μM	100 μM	n.a.
45	100 μM	100 μM	n.a.	n.a.
61	25 μM	25 μM	12.5 μM	25 μM
62	12.5 μM	12.5 μM	6.25 μM	12.5 μM
63	25 μM	25 μM	12.5 μM	50 μM
64	50 μM	50 μM	50 μM	100 μM
65	>400 μM	>400 μM	>400 μM	>400 μM
66	400 μM	n.a.	n.a.	n.a.
67	>400 μM	n.a.	n.a.	n.a.
68	>400 μM	n.a.	n.a.	n.a.
69	>400 μM	n.a.	n.a.	n.a.
70	>400 μM	>400 μM	>400 μM	>400 μM
71	12.5 μM	n.a.	n.a.	n.a.

Note: n.a.—not applicable/not determined.

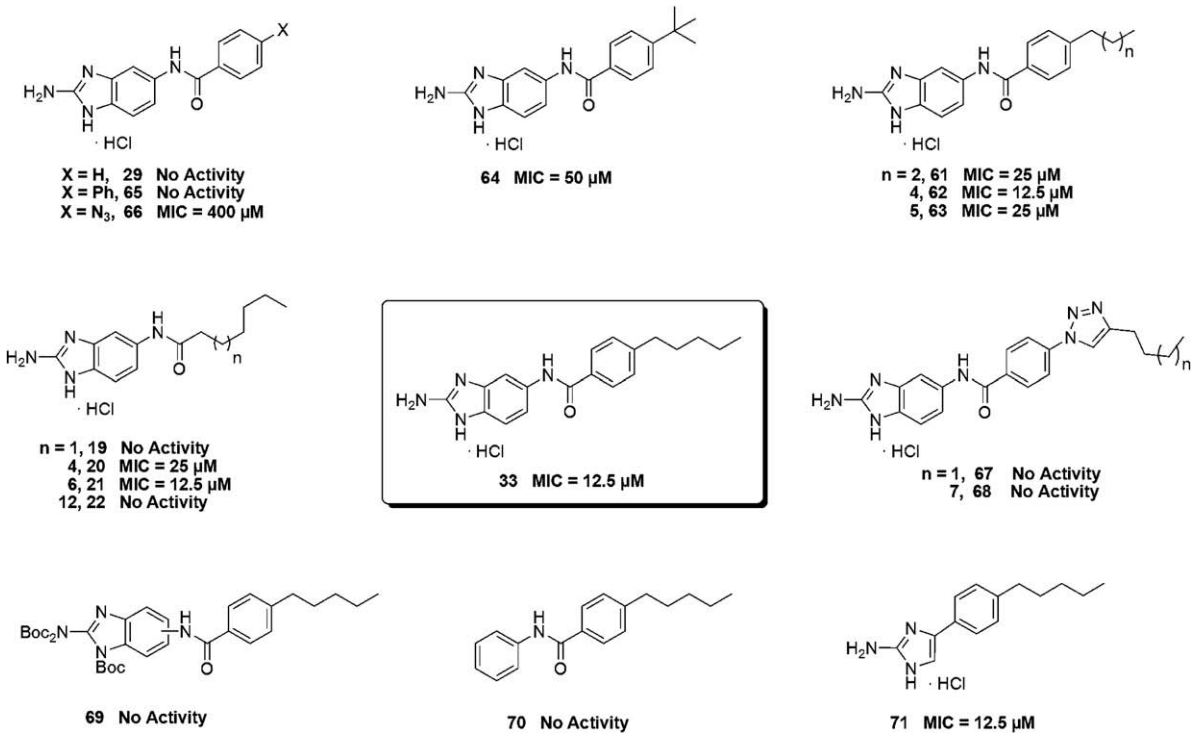


Figure 6. A detailed SAR for these novel 2ABIs against BAA-44.

2.5. Determining microbicidal activity and vancomycin enhancement with 33

Further evaluation of **33** against BAA-44 allowed us to determine its Minimum Bactericidal Concentration (MBC) to be 25 μM, thus demonstrating that **33** has bactericidal activity and not bacteriostatic activity against BAA-44. The MBC is the lowest concentration at which there is ⩾99.9% reduction in CFU mL^{-1} when compared to an untreated control.[42] Bacteriostatic activity is generally defined as an agent that inhibits bacterial growth, whereas bactericidal activity is defined as an agent which actually kills bacteria.[42] This activity is determined by MBC/MIC ratios for antibacterial agents. Antibacterial activity is considered to be bactericidal when a compound's MBC is ⩽4 times the concentration of the MIC, while a ratio is >4 the activity is said to be bacteriostatic. Since the MBC/MIC ratio for **33** was 2 (MBC = 25 μM/MIC = 12.5 μM against BAA-44) we conclude the activity of **33** is bactericidal.

The use of bactericidal agents, such as **33**, is preferred from a clinical standpoint compared to bacteriostatic agents since bacterial infections are better cured with greater reduction in viable bacteria or bacterial load.[42,43] At 12.5 μM (the MIC value against BAA-44), **33** demonstrated a 99.15 ± 0.75% reduction in BAA-44 CFU mL^{-1}. The MBC_{90} of **33** was determined to be 11.15 ± 0.12 μM and the MBC_{50} is 5.88 ± 0.50 μM.

There is also clinical value in combination therapy options when treating MRSA infections.[44] We were interested to see if **33** could synergistically enhance vancomycin activity against BAA-44 at concentrations well below the MIC value of **33**. BAA-44 was treated with 2, 4, and 6 μM of **33** and allowed to incubate with twofold dilutions of vancomycin against MRSA. After 16 h, all concentrations of **33** tested increased vancomycin activity by twofold. Without **33**, vancomycin's MIC is 391 nM, but with sub-MIC concentrations of two ABI **33**, vancomycin recorded an MIC value of 195 nM against BAA-44. It was determined that at 2 μM of **33** there was a 21% reduction in the CFU mL^{-1} of BAA-44, demonstrating a synergistic twofold enhancement in antibiotic activity of vancomycin when paired with **33**.

2.6. 2-Aminobenzimidazole microdilution susceptibility testing against *A. baumannii*

Next, we assessed the 2ABI's activity against a Gram-negative pathogen and used the microdilution susceptibility approach to screen selected 2ABIs against five *A. baumannii* strains. Four of these *A. baumannii* strains were MDR. Five clinically used antibiotics were tested in parallel to 12 2ABIs for direct comparison of antibiotic activity and to evaluate the drug-resistance of the clinical isolates.

First, we generated a preliminary structure–activity relationship of these 2ABIs against a non-MDR strain of *A. baumannii* (ATCC 19606). The 2ABI amides **20**, **21**, and **33** were found to demonstrate the most potent antibiotic activity against *S. aureus* strains and were also found to be the leads against *A. baumannii* with MIC values of 25 μM. Analogues **61**, **62**, and **63** were slightly less active with an MIC value of 50 μM, while **64** showed reduced antibiotic activity (MIC = 100 μM). Compounds **19** (with a shorter chain) and **22** (with a longer chain) demonstrated no antibiotic activity at 400 μM. Although the potency of these 2ABIs was slightly reduced, the structure–activity relationship was the same as it was against *S. aureus* strains.

An MDR strain of *A. baumannii* was purchased from ATCC (BAA-1605) and screened to determine the 2ABI analogue's activity. As with BAA-44, all but one clinically used antibiotic showed increased resistance when subjected to microdilution susceptibility testing. Tobramycin showed increased activity against this strain, but this MDR *A. baumannii* strain demonstrated >125-fold increase in resistance towards imipenem, >4-fold increase in resistance towards gentamycin and >125-fold increase in resistance towards tetracycline (Table 2).

As previously discovered when the 2ABIs were screened against BAA-44, the active 2ABIs against *A. baumannii* strain ATCC 19606 were at least as active against BAA-1605. Analogues **21**, **33**, **61**, and **62** all demonstrated a twofold increase in antibiotic activity. The most active 2ABI analogues against this MDR *A. baumannii* strain were found to be **21**, **33**, and **62** which had MIC values of 12.5 μM and demonstrated more potent antibacterial activity than imipenem (MIC = 50 μM) and the other antibiotics tested except for tobramycin (MIC = 6.25 μM).

Once we had established increased antibiotic activity against the MDR strain of *A. baumannii* we evaluated these 2ABIs against three additional clinical isolates (AB0043, UH8407, and 3340). The most well characterized clinical specimen in this study was AB0043, an isolate from a patient suffering an *A. baumannii* infection at Walter Reed Army Medical Center. This bacterial strain was isolated in 2004 from the blood of a soldier that was deployed to Iraq/Kuwait, and was found to be resistant to 7 of the 9

Table 2
MIC value of antibiotics and 2ABIs against *A. baumannii*

Compound	*A. baumannii* ATCC 19606	MDRAB BAA-1605	UH8407	3340	AB0043
Imipenem	391 nM	50 μM	3.125 μM	200 μM	50 μM
Ampicillin	>400 μM	>400 μM	>400 μM	>400 μM	>400 μM
Tobramycin	25 μM	6.25 μM	200 μM	>400 μM	200 μM
Gentamycin	100 μM	>400 μM	>400 μM	>400 μM	200 μM
Tetracycline	3.13 μM	>400 μM	>400 μM	25 μM	6.25 μM
2ABIs:					
19	>400 μM	>400 μM	>400 μM	>400 μM	400 μM
20	25 μM	25 μM	25 μM	25 μM	25 μM
21	25 μM	12.5 μM	25 μM	12.5 μM	12.5 μM
22	>400 μM	>400 μM	>400 μM	>400 μM	>400 μM
33	25 μM	12.5 μM	25 μM	25 μM	12.5 μM
61	50 μM	25 μM	50 μM	100 μM	25 μM
62	50 μM	12.5 μM	25 μM	50 μM	12.5 μM
63	50 μM	n.a.	n.a.	50 μM	12.5 μM
64	100 μM	100 μM	100 μM	100 μM	50 μM
65	>400 μM	n.a.	n.a.	>400 μM	>400 μM
51	>400 μM	>400 μM	>400 μM	n.a.	>400 μM
59	>400 μM	>400 μM	>400 μM	n.a.	>400 μM
70	>400 μM	n.a.	n.a.	n.a.	n.a.

Note: n.a.—not applicable/not determined.

antibiotics used in the study to determine its MDR phenotype. Genetic analysis showed that this isolate demonstrated enhanced efflux pump activity via *adeR* expression, which typically makes isolates resistant to aminoglycosides, quinolones, tetracycline and trimethoprim. Several aminoglycosides-modifying enzymes (AMEs) were expressed in this clinical isolate as well. Isolate AB0043 was found to express the AME gene *aadB* which was often associated with tobramycin resistance of *A. baumannii* clinical isolates.[45]

The isolates UH8407, 3340, and AB0043 were found to be resistant against tobramycin and gentamycin by use of the microdilution susceptibility assay. There was increased resistance towards imipenem with isolate 3340 showing >500-fold more resistance when compared to the wild-type ATCC 19606 strain. Tetracycline demonstrated antibiotic activity against two of the four MDRAB strains. None of the clinical antibiotics maintained their antibiotic activity against all clinical isolates and MDR *A. baumannii* strains compared to the wild-type *A. baumannii* ATCC 19606 strain.

When the 2ABI analogues were screened against the clinical specimens we observed the same level of antibiotic activity against the wild-type (ATCC 19606) and MDRAB strains. In terms of compound potency, 2ABI analogues **20**, **21**, **33**, and **62** consistently demonstrate more potent antibiotic activity towards the *A. baumannii* clinical isolates than any of the clinically used antibiotics during this study. These analogues do not appear to be affected by the developed mechanisms of drug-resistance, including increased drug efflux, and maintain their relative antibiotic activity across the five *A. baumannii* strains (Table 2).

2.7. Potential of lead compound 33 to lyse red blood cells and synthetic vesicles

Given that active 2ABI analogues contain a lipophilic tail coupled to a polar head group, we investigated whether these compounds were potentially eliciting their activity through a pore-forming mechanism. Two experiments were run to probe this mode of action, a red blood hemolysis assay[46] and a dye dispersion assay[47] from synthetic vesicles. Compound **33** was subjected to red blood cells (RBCs) in hemolysis assays along with inactive compound **29** which served as our control. Each compound was assayed at 25 μM, the highest MIC observed for **33**. At this concentration, both compounds showed <1% hemolysis.

Next, we evaluated the potential of **33** to induce dye leakage from synthetic vesicles. Two types of vesicles were prepared, one containing 1-Palmitoyl-2-Oleoyl-sn-Glycero-3-Phosphocholine (POPC) and the other containing 1-Palmitoyl-2-Oleoyl-sn-Glycero-3-Phosphoglycerol (POPG). POPC mimics typical cell membranes encountered for mammalian red blood cells, while POPG mimics the negatively charged lipids encountered in bacterial membranes. At 25 μM **33** induced only 16% dye leakage in the POPG lipids while it induced 9% dye leakage in POPC lipids. Furthermore, >50% dye leakage in the POPG vesicles was only observed at >500 μM **33**, concentrations that are significantly above its MIC. Taken together, this data suggests that **33** is not eliciting its antibacterial activity through a pore-forming mechanism.

3. Conclusion

In conclusion, a collection of 55 small molecules has been synthesized and screened for antibiotic activity against several multidrug-resistant bacterial strains. Active 2ABIs from this library demonstrate broad-spectrum antibiotic activity. In all, nine strains of *S. aureus* and *A. baumannii* were used to determine the effectiveness of this library using microdilution susceptibility testing.

From these studies, we have identified 2ABI **33** which demonstrates bactericidal activity against the MRSA strain BAA-44. In addition, 2ABI **33** is active against several MDR *A. baumannii* strains, including clinical isolates. This same compound was also found to synergistically increase vancomycin activity two-fold at 2 μM, a concentration well under its MIC value (MIC = 12.5 μM).

4. Experimental

4.1. Chemistry

All chemicals and solvents used were purchased from commercially available sources and used without further purification. Silica gel was used for column chromatography and was performed with 60 Å mesh standard grade silica gel from Sorbtech. ^{1}H and ^{13}C NMR spectra were performed using Varian 300 MHz and 400 MHz spectrometers. Chemical shifts are reported in parts per million relative to $CDCl_3$ (δ 7.26), CD_3OD (δ 3.31) and DMSO-d_6 (δ 2.50) for ^{1}H NMR and relative to $CDCl_3$ (δ 77.23), CD_3OD (δ 49.51) and DMSO-d_6 (δ 39.51) for ^{13}C NMR with TMS as an internal standard. Abbreviations used for ^{1}H NMR splitting are as follows: s = singlet, br s = broad singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet, br m = broad multiplet. High-resolution mass spectra were obtained at the North Carolina State Mass Spectrometry Laboratory for Biotechnology. Fluorescence measurements were recorded using a Hitachi Fluorescence Spectrometer (F-2500). All compounds that were tested for antibacterial activity were synthesized using standard techniques and are described in complete detail in the Supplementary data. Selected 2ABIs are characterized here.

4.1.1. General procedure for the formation of simple 2ABIs from 1,2-phenylenediamine starting materials. 5-Nitro-1*H*-benzo[*d*]imidazol-2-amine (8)

Cyanogen bromide (3.55 g, 33.5 mmol) was added to a solution of 4-nitro-1,2-phenylenediamine (5.06 g, 33.1 mmol) in water/acetonitrile (80:10 mL). This reaction was refluxed for 4 h while turning a deep red color. After concentrating nearly half of the water/acetonitrile reaction solvent, concentrated ammonia hydroxide (aq) was added to the solution until a yellow precipitate began to form. The mixture was then filtered, and the precipitate was dried under reduced pressure to give 5.97 g of **8**.

4.1.2. General procedure for the formation of 2ABI amides (14)

To a vial of **11** (100 mg, 0.22 mmol) and triethylamine (70 μL, 0.50 mmol) in methylene chloride (5 mL) was added the respective acid chloride or anhydride (0.21 mmol) either dropwise or as a solid. The reaction was then allowed to stir overnight (16–20 h) at ambient temperatures. Upon the completion of the reaction, it was quenched with 5% HCl (aq) and transferred to a separatory funnel. The crude amide was formed as two regioisomers (no attempts at separating these isomers was attempted) and extracted two times with methylene chloride and the combined organic layers were dried with sodium sulfate and concentrated. This mixture was quickly flashed through a plug of silica gel and concentrated in a vial. The mixture of regioisomer amide products were taken on without characterization, however, a select few were characterized. The resulting tri-Boc–protected 2ABI amide was then dissolved in 2 mL of methylene chloride and 2 mL of triflouroacetic acid was added slowly to give a 1:1 mixture (CH_2Cl_2/TFA). This was allowed to stir for 2 h at room temperature after which the solvent was removed under reduced pressure to give the 2ABI amide as a TFA salt. To this was added 2 mL of saturated HCl/methanol to give the HCl salt of the 2ABI amide. Then methanol and

ether was added to the vial to triturate the resulting product **14**. This two step procedure gave yields between 57% and 94%.

4.1.3. *N*-(2-Amino-1*H*-benzo[*d*]imidazol-5-yl)acetamide·HCl (15)

The general procedure for the formation for 2ABI amides was used for the synthesis of **15** which was formed as a white solid in 89% yield. White solid: mp 313–318 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.39 (br s, 2H), 10.13 (s, 1H), 8.43 (s, 2H), 7.91 (s, 1H), 7.25 (s, 2H), 2.05 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.3, 150.6, 135.3, 129.7, 125.1, 114.5, 111.2, 102.4, 24.0; HRMS (ESI) calcd for $C_9H_{10}N_4O$ (MH^+) 190.0855, found 190.0857.

4.1.4. *N*-(2-Amino-1*H*-benzo[*d*]imidazol-5-yl)-2,2,2-trifluoroacetamide·HCl (16)

The general procedure for the formation for 2ABI amides was used for the synthesis of **16** which was formed as a white solid in 62% yield. White solid: mp 234–238 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.93 (s, 1H), 7.44 (d, J = 8.4 Hz, 1H), 7.36 (d, J = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 157.1, 156.7, 152.9, 134.1, 131.3, 128.7, 118.3, 112.7, 105.9; HRMS (ESI) calcd for $C_9H_7F_3N_4O$ (MH^+) 244.0572, found 244.0575.

4.1.5. *N*-(2-Amino-1*H*-benzo[*d*]imdazol-5-yl)propionamide·HCl (18)

The general procedure for the formation for 2ABI amides was used for the synthesis of **18** which was formed as a white solid in 84% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.93 (m, 1H), 7.26 (m, 2H), 2.40 (q, J = 14.8 Hz, J = 7.6 Hz, 2H), 1.21 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 175.6, 152.5, 136.6, 131.2, 127.2, 117.3, 117.3, 112.4, 104.8, 31.1, 10.4; HRMS (ESI) calcd for $C_{10}H_{12}N_4O$ (MH^+) 204.1011, found 204.1009.

4.1.6. *N*-(2-Amino-1*H*-benzo[*d*]imidazole-5-yl)heptanamide·HCl (19)

The general procedure for the formation for 2ABI amides was used for the synthesis of **19** which was formed as a white solid in 58% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.51 (br s, 2H), 10.14 (s, 1H), 8.47 (s, 2H), 7.91 (s, 1H), 7.31 (dd, J = 8.8 Hz, 1.8 Hz, 1H), 7.25 (d, J = 8.4 Hz, 1H), 2.31 (t, J = 7.6 Hz, 2H), 1.56 (m, 2H), 1.27 (m, 6H), 0.84 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.2, 150.5, 135.3, 129.7, 125.1, 114.4, 111.2, 102.4, 36.4, 31.0, 28.3, 25.1, 22.0, 13.9; HRMS (ESI) calcd for $C_{14}H_{20}N_4O$ (MH^+) 260.1637, found 260.1642.

4.1.7. *N*-(2-Amino-1*H*-benzo[*d*]imidazol-5-yl)decanamide·HCl (20)

The general procedure for the formation for 2ABI amides was used for the synthesis of **20** which was formed as a white solid in 94% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.93 (m, 1H), 7.28 (dd, J = 8.8 Hz, J = 0.8 Hz, 1H), 7.26 (dd, J = 8.8 Hz, J = 2.0 Hz, 1H), 2.39 (t, J = 3.6 Hz, 2H), 1.70 (m, 2H), 1.36–1.28 (m, 12 H), 0.89 (t, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 174.9, 152.5, 136.6, 131.1, 127.2, 117.3, 112.4, 104.8, 38.1, 33.1, 30.7, 30.6, 30.5, 30.5, 27.1, 23.8, 14.6; HRMS (ESI) calcd for $C_{17}H_{26}N_4O$ (MH^+) 302.2107, found 302.2109.

4.1.8. *N*-(2-Amino-1*H*-benzo[*d*]imidazol-5-yl)dodecanamide·HCl (21)

The general procedure for the formation for 2ABI amides was used for the synthesis of **21** which was formed as a light orange solid in 79% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.93 (s, 1H), 7.29 (d, J = 8.4 Hz, 1H), 7.26 (dd, J = 8.4 Hz, J = 2.0 Hz, 1H), 2.39 (t, J = 7.6 Hz, 2H), 1.70 (quintet, J = 7.2 Hz, 2H), 1.36–1.26 (m, 16H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 165.3, 143.0, 127.0, 121.6, 117.7, 107.7, 102.9, 95.3, 28.5, 23.5, 21.2 (2), 21.1, 20.9 (2), 20.8, 17.4, 14.2, 4.9; HRMS (ESI) calcd for $C_{19}H_{30}N_4O$ (MH^+) 330.2420, found 330.2422.

4.1.9. *N*-(2-Amino-1*H*-benzo[*d*]imidazol-5-yl)-6-bromohexanamide·HCl (23)

The general procedure for the formation for 2ABI amides was used for the synthesis of **23** which was formed as a light yellow solid in 71% yield. Light yellow solid: mp 222–225 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.93 (m, 1H), 7.28 (d, J = 8.4 Hz, 1H), 7.27 (dd, J = 8.8 Hz, J = 2.0 Hz, 1H), 3.46 (t, J = 6.4 Hz, 2H), 2.41 (t, J = 7.2 Hz, 2H), 1.90 (quintet, J = 7.2 Hz, 2H), 1.74 (quintet, J = 7.6 Hz, 2H), 1.54 (quintet, J = 7.2, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 174.6, 152.6, 136.6, 131.3, 127.3, 117.3, 112.5, 104.9, 37.8, 34.3, 33.8, 28.9, 26.1; HRMS (ESI) calcd for $C_{13}H_{17}BrN_4O$ (MH^+) 324.0586, found 324.0590.

4.1.10. *N*-(2-Amino-1*H*-benzo[*d*]imidazol-5-yl)cyclobutanecarboxamide·HCl (25)

The general procedure for the formation for 2ABI amides was used for the synthesis of **25** which was formed as a white solid in 59% yield. White solid: mp 199–203 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.93 (s, 1H), 7.28 (d, J = 8.8 Hz, 1H), 7.24 (dd, J = 1.6 Hz, J = 1.2 Hz, 1H), 2.34 (quintet, J = 1.6 Hz, 2H), 2.23 (m, 2H), 2.18–2.01 (sextet, J = 8.8 Hz, 2H), 1.92 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 176.3, 152.5, 136.6, 131.1, 127.1, 117.4, 112.4, 104.9, 41.7, 26.2, 19.1; HRMS (ESI) calcd for $C_{12}H_{14}N_4O$ (MH^+) 230.1168, found 230.1166.

4.1.11. *N*-(2-Amino-1*H*-benzo[*d*]imidazol-5-yl)cyclopentanecarboxamide·HCl (26)

The general procedure for the formation for 2ABI amides was used for the synthesis of **26** which was formed as a white solid in 91% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.86 (d, J = 1.6 Hz, 1H), 7.30 (dd, J = 8.6 Hz, J = 1.8 Hz, 1H), 7.24 (d, J = 8.4 Hz, 1H), 2.86 (quintet, J = 8.0 Hz, 1H), 1.97–1.90 (br m, 2H), 1.84–1.70 (br m, 4H), 1.66–1.57 (br m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 177.9, 152.4, 136.7, 131.1, 127.1, 117.4, 112.4, 104.9, 47.3, 31.7, 27.2; HRMS (ESI) calcd for $C_{13}H_{16}N_4O$ (MH^+) 244.1324, found 244.1321.

4.1.12. *N*-(2-Amino-1*H*-benzo[*d*]imidazol-5-yl)cyclohexanecarboxamide·HCl (27)

The general procedure for the formation for 2ABI amides was used for the synthesis of **27** which was formed as a white solid in 86% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.88 (s, 1H), 7.29 (d, J = 8.4 Hz, 1H), 7.28 (d, J = 8.4 Hz, 1H), 2.41 (t, J = 11.6 Hz, 1H), 1.87 (d, J = 12.8 Hz, 2H), 1.80 (d, J = 12.8 Hz, 2H), 1.70 (d, J = 12.0 Hz, 1H), 1.51 (q, J = 25.0 Hz, J = 12.4 Hz, 2H), 1.40–1.22 (m, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 177.9, 152.5, 136.7, 131.1, 127.2, 117.5, 112.4, 104.9, 47.2, 30.8, 27.0, 26.9; HRMS (ESI) calcd for $C_{14}H_{18}N_4O$ (MH^+) 258.1481, found 258.1480.

4.1.13. *N*-(2-Amino-1*H*-benzo[*d*]imidazole-5-yl)-3-cyclopentylpropanamide·HCl (28)

The general procedure for the formation for 2ABI amides was used for the synthesis of **28** which was formed as a light yellow solid in 73% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 9.35 (s, 2H), 8.75 (s, 1H), 8.19 (d, J = 1.2 Hz, 1H), 8.17 (d, J = 1.6 Hz, 1H), 8.07 (d, J = 8.4 Hz, 1H), 3.13 (t, J = 7.6 Hz, 2H), 2.48 (br m, 3H), 2.37 (m, 2H), 2.30 (m, 2H), 2.20 (m, 2H), 1.83 (br m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 181.1, 160.2, 145.1, 139.3, 134.7, 124.2, 120.8, 112.1, 49.0; HRMS (ESI) calcd for $C_{15}H_{20}N_4O$ (MH^+) 272.1637, found 272.1630.

4.1.14. *N*-(2-Amino-1*H*-benzo[*d*]imidazol-5-yl)benzamide·HCl (29)

The general procedure for the formation for 2ABI amides was used for the synthesis of **29** which was formed as a white solid in 83% yield. White solid: mp 245–250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.53 (br s, 2H), 10.41 (s, 1H), 8.49 (s, 2H), 8.03 (d,

J = 2.0 Hz, 1H), 7.98 (s, 1H), 7.96 (s, 1H), 7.59 (m, 1H), 7.53 (m, 3H), 7.33 (d, J = 8.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.5, 150.7, 134.9, 131.6, 129.7, 128.4, 127.7, 125.8, 115.9, 111.1, 103.7; HRMS (ESI) calcd for $C_{14}H_{12}N_4O$ (MH^+) 252.1011, found 252.1017.

4.1.15. *N*-(2-Amino-1*H*-benzo[*d*]imidazol-5-yl)-4-pentylbenzamide·HCl (33)

The general procedure for the formation for 2ABI amides was used for the synthesis of **33** which was formed as a white solid in 80% yield. White solid: mp 263–267 °C; IR (KBr) 3303, 3142, 2952, 1686, 1639, 1562, 1356 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 8.01 (s, 1H), 7.88 (d, J = 2.8 Hz, 1H), 7.85 (d, J = 2.8 Hz, 1H), 7.45 (dd, J = 8.4 Hz, J = 1.6 Hz, 1H), 7.34 (m, 2H), 7.32 (m, 1H), 2.69 (t, J = 6.8 Hz, 2H), 1.66 (quintet, J = 7.2 Hz, 2H), 1.35 (m, 4H), 0.91 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 169.0, 152.6, 148.8, 136.6, 133.5, 131.2, 129.8, 128.9, 127.6, 118.5, 112.4, 105.9, 36.9, 32.7, 32.2, 23.7, 14.5; HRMS (ESI) calcd for $C_{19}H_{22}N_4O$ (MH^+) 322.1794, found 322.1788.

4.1.16. *N*-(2-Amino-1*H*-benzo[*d*]imidazol-5-yl)furan-2-carboxamide·HCl (34)

The general procedure for the formation for 2ABI amides was used for the synthesis of **34** which was formed as a white solid in 64% yield. White solid: mp 225–229 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.36 (s, 1H), 8.43 (s, 2H), 7.97 (d, J = 2.0 Hz, 1H), 7.94 (m, 1H), 7.52 (dd, J = 8.8 Hz, J = 1.6 Hz, 1H), 7.40 (d, J = 3.2 Hz, 1H), 7.31 (d, J = 8.4 Hz, 1H), 6.70 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.3, 150.9, 147.4, 145.8, 134.2, 129.8, 126.0, 115.9, 114.9, 112.1, 111.2, 103.9; HRMS (ESI) calcd for $C_{12}H_{10}N_4O_2$ (MH^+) 242.0804, found 242.0810.

4.1.17. *N*-(2-Amino-1*H*-benzo[*d*]imidazol-5-yl)thiophene-2-carboxamide·HCl (35)

The general procedure for the formation for 2ABI amides was used for the synthesis of **35** which was formed as a white solid in 79% yield. White solid: mp 99–104 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.42 (s, 1H), 8.45 (s, 2H), 8.09 (d, J = 3.6 Hz, 1H), 7.95 (s, 1H), 7.86 (d, J = 5.2 Hz, 1H), 7.51 (dd, J = 8.8 Hz, J = 2.0 Hz, 1H), 7.32 (d, J = 8.8 Hz, 1H), 7.23 (t, J = 4.4 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.0, 150.8, 140.1, 134.5, 131.8, 129.8, 129.5, 128.2, 125.9, 116.0, 111.2, 103.9; HRMS (ESI) calcd for $C_{12}H_{10}N_4OS$ (MH^+) 258.0575, found 258.0578.

4.1.18. *N*-(2-Amino-1*H*-benzo[*d*]imidazol-5-yl)-4-butylbenzamide·HCl (61)

The general procedure for the formation for 2ABI amides was used for the synthesis of **61** which was formed as a white solid in 83% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.95 (s, 1H), 7.85 (d, J = 8.4 Hz, 2H), 7.46 (dd, J = 8.6 Hz, J = 1.8 Hz, 1H), 7.29 (m, 3H), 2.65 (t, J = 7.8 Hz, 2H), 1.59 (quintet, J = 7.6 Hz, 2H), 1.35 (sextet, J = 7.6 Hz, 2H), 0.95 (t, J = 12.8 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 167.0, 148.7, 136.5, 133.4, 131.1, 130.9, 129.8, 128.9, 127.6, 118.5, 112.4, 106.0, 37.0, 34.7, 23.4, 14.4; HRMS (ESI) calcd for $C_{18}H_{20}N_4O$ (MH^+) 308.1637, found 308.1636.

4.1.19. *N*-(2-Amino-1*H*-benzo[*d*]imidazol-5-yl)-4-hexylbenzamide·HCl (62)

The general procedure for the formation for 2ABI amides was used for the synthesis of **62** which was formed as a white solid in 91% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.95 (s, 1H), 7.85 (d, J = 8.4 Hz, 2H), 7.46 (dd, J = 8.8 Hz, J = 2.0 Hz, 1H), 7.28 (m, 3H), 2.63 (t, J = 7.6 Hz, 2H), 1.60 (br m, 2H), 1.29 (m, 6H), 0.87 (t, J = 3.4 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 168.9, 148.7, 136.5, 133.4, 131.1, 130.9, 129.7, 128.9, 127.5, 118.5, 112.4, 105.9, 36.9, 32.9, 32.4, 30.1, 23.8, 14.5; HRMS (ESI) calcd for $C_{20}H_{24}N_4O$ (MH^+) 336.1950, found 336.1945.

4.1.20. *N*-(2-Amino-1*H*-benzo[*d*]imidazol-5-yl)-4-heptylbenzamide·HCl (63)

The general procedure for the formation for 2ABI amides was used for the synthesis of **63** which was formed as a white solid in 82% yield. White solid: mp 279–283 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.96 (d, J = 2 Hz, 1H), 7.86 (d, J = 8.0 Hz, 2H), 7.46 (dd, J = 8.8 Hz, J = 2.0 Hz, 1H), 7.29 (dd, J = 8.4 Hz, J = 5.2 Hz, 3H), 2.65 (t, J = 8.0 Hz, 2H), 1.62 (quintet, J = 7.2 Hz, 2H), 1.29 (br m, 8H), 0.87 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 169.0, 152.6, 148.8, 136.6, 133.4, 131.2, 129.8, 128.9, 127.6, 118.5, 112.4, 105.9, 36.9, 33.1, 32.5, 30.4 (2), 23.8, 14.6; HRMS (ESI) calcd for $C_{21}H_{26}N_4O$ (MH^+) 350.2107, found 350.2101.

4.1.21. *N*-(2-Amino-1*H*-benzo[*d*]imidazol-5-yl)-4-*tert*-butylbenzamide·HCl (64)

The general procedure for the formation for 2ABI amides was used for the synthesis of **64** which was formed as a white solid in 88% yield. White solid: mp 337–341 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.99 (s, 1H), 7.89 (d, J = 8.4 Hz, 2H), 7.55 (d, J = 6.8 Hz, 2H), 7.47 (dd, J = 8.4 Hz, J = 1.2 Hz, 1H), 7.33 (d, J = 8.4 Hz, 1H), 1.35 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD) δ 167.0, 156.9, 136.6, 133.2, 131.2, 128.7, 127.7, 126.7, 126.5, 118.5, 112.5, 106.0, 36.0, 31.7; HRMS (ESI) calcd for $C_{18}H_{20}N_4O$ (MH^+) 308.1637, found 308.1633.

4.1.22. General procedure for the formation of 2ABI sulfonamides (36)

To a vial containing **11** (100 mg, 0.22 mmol) and triethylamine (70 μL, 0.50 mmol) stirring in methylene chloride (5 mL) was added sulfonyl chloride (0.23 mmol) either dropwise or as a solid to the reaction mixture which was allowed to stir overnight (~16 h) at ambient temperatures. The reaction was then quenched with 5% HCl (aq) and transferred to a separatory funnel. The resulting sulfonamide was extracted two times with dichloromethane and the combined organic layers were dried with sodium sulfate and concentrated. This mixture was then flashed through a plug of silica gel and concentrated in a vial. This gave an inseparable mixture of regioisomer sulfonamide products. The resulting sulfonamide 2ABI was then dissolved in 2 mL of methylene chloride and 2 mL of triflouroacetic acid was added slowly to give a 1:1 mixture (CH_2Cl_2/TFA). This was allowed to stir for 2 h at room temperature after which the solvent was removed under reduced pressure to give the TFA salt of the desired 2ABI sulfonamide. To this was added 2 mL of saturated HCl/methanol and the TFA salt was exchanged to give the HCl salt of the 2ABI sulfonamide. Then ether was added to the vial to triturate the resulting product and the ether was then decanted giving pure 2ABI sulfonamide (**36**) as an HCl salt between 20% and 84% yield.

4.1.23. *N*-(2-Amino-1*H*-benzo[*d*]imidazol-5-yl)hexane-1-sulfonamide·HCl (38)

The general procedure for the formation for 2ABI sulfonamides was used for the synthesis of **38** which was formed as a light yellow solid in 68% yield. Light yellow solid: mp 214–219 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.44–7.34 (m, 3H), 3.62 (t, J = 7.2 Hz, 2H), 1.85 (m, 2H), 1.47 (m, 2H), 1.35 (m, 4H), 0.90 (m, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 153.4, 132.3, 131.4, 131.0, 128.5, 116.0, 112.8, 56.5, 32.4, 28.9, 24.4, 23.5, 14.4; HRMS (ESI) calcd for $C_{13}H_{20}N_4O_2S$ (MH^+) 296.1307, found 296.1316.

4.1.24. *N*-(2-Amino-1*H*-benzo[*d*]imidazol-5-yl)-4-methylbenzenesulfonamide·HCl (39)

The general procedure for the formation for 2ABI sulfonamides was used for the synthesis of **39** which was formed as a red residue

in 84% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.61 (s, 1H), 7.59 (m, 1H), 7.27 (s, 1H), 7.25 (s, 1H), 7.20 (d, J = 1.2 Hz, 1H), 7.18 (d, J = 8.4 Hz, 1H), 6.90 (dd, J = 8.4 Hz, J = 2.0 Hz, 1H), 2.35 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 171.7, 143.9, 136.6, 134.1, 130.2, 129.4, 128.5, 127.1, 118.1, 111.4, 105.4, 20.2; HRMS (ESI) calcd for $C_{14}H_{14}N_4O_2S$ (MH^+) 302.0838, found 302.0829.

4.1.25. *N*-(2-Amino-1*H*-benzo[*d*]imidazole-5-yl)naphthalene-2-sulfonamide·HCl (45)

The general procedure for the formation for 2ABI sulfonamides was used for the synthesis of **45** which was formed as a white solid in 72% yield. White solid: mp 265–269 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.36 (s, 1H), 8.08 (dd, J = 8.8 Hz, J = 2.4 Hz, 1H), 8.03 (d, J = 8.4 Hz, 1H), 7.92 (d, J = 8.0 Hz, 1H), 7.87 (m, 1H), 7.76 (m, 1H), 7.66 (m, 1H), 7.29 (dd, J = 8.4 Hz, J = 2.4 Hz, 1H), 6.99 (m, 1H), 6.93 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 153.4, 137.3, 137.1, 133.4, 132.6, 131.9, 131.4, 131.2, 131.1, 130.8, 130.8, 129.3, 129.3, 128.9, 124.2, 116.2, 112.8; HRMS (ESI) calcd for $C_{17}H_{14}N_4O_2S$ (MH^+) 338.0838, found 338.0834.

4.2. Biology

4.2.1. Bacterial strains

The *S. aureus* strains were purchased from ATCC (ATCC 29213, ATCC 25923, and ATCC 29740), including MRSA strain BAA-44. Two *A. baumannii* strains were also purchased from ATCC (ATCC 19606 and BAA-1605). Three *A. baumannii* clinical isolates (AB0043, UH8407, and 3340) were obtained from Dr. Robert Bonomo.

4.2.2. Reagents

All compounds that were tested were stored as 100 mM stock solutions in dimethyl sulfoxide (DMSO) and frozen at −80 °C until used for screening.

4.2.3. Microdilution susceptibility testing

All antibiotic susceptibility testing was completed with a starting inoculum of 5×10^5 CFU mL^{-1} according to NCCLS[41] standards and incubated for 16–20 h at 37 °C. After this time bacterial growth was visually inspected and the lowest concentration at which no observable bacterial growth or turbidity was observed was considered to be the MIC value. Pellets that formed at the bottom of 96 well microtiter plates were considered growth and although there was essentially no turbidity, the pellet was considered bacterial growth in these studies. Microdilutions were made in the 96 well microtiter plates from a starting concentration of 400 μM with the addition of 200 μL to a predetermined well. From this, twofold serial dilutions were made by transferring 100 μL of the initially treated well (of 400 μM) into the next well and mixing once using a multichannel pipet, this next well then contained 200 μL of bacterial, media and test 2ABI at a concentration of 200 μM. This transfer of 100 μL was done in succession for a total of 12 twofold serial dilutions giving a range of 400 μM–391 nM for tested antibiotic in 96 well microtiter plates. The final dilution then had 200 μL (of the 391 nM wells) and then 100 μL was removed and thrown away. A plastic lid was then used to cover the wells and the plates with their lids were wrapped in suran wrap and placed in a humidified chamber and incubated for 16–20 h at 37 °C. In one plate, 8 antibiotics could be run in parallel with 12 twofold dilutions. After the incubation time, the wells were observed for bacterial growth and MICs were determined accordingly (described above).

4.2.4. Determining MBC values for 33

The MBC was determined by taking 50 μL from individual microtiter wells from untreated BAA-44 and treated BAA-44 wells with **33** at several concentrations after a 16 h incubation from microdilution susceptibility testing. Serial dilutions were made with the samples that were taken from the wells and they were then plated out on TSA plates and incubated overnight to grow BAA-44 colonies. From these BAA-44 colonies, CFU mL^{-1} were determined. The MBC is the lowest concentration that gave $\geqslant$99.9% reduction in CFU mL^{-1}. The dose–response was plotted with concentrations of **33** that gave less than the $\geqslant$99.9% reduction in CFU mL^{-1}. This was then used to give MBC_{50} and MBC_{90} values for **33**. The MBC value for **33** was found to be 25 μM. The MBC_{50} was found to be 5.88 ± 0.50 μM and the MBC_{90} was found to be 11.15 ± 0.12 μM.

4.2.5. Vancomycin enhancement assay with 33

Serial dilutions were made with Vancomycin according to the methods previously described to determine MBC values. Inoculated media was prepared and added to the appropriate row of wells in the microtiter plates with 2, 4, and 6 μM **33** before vancomycin was added to a well at 400 μM. Serial dilutions were then made and incubated for 16–20 h. The plates were then removed after incubation and inspected for bacterial growth. Vancomycin was enhanced with each treatment of **33** (2, 4, and 6 μM) twofold. Vancomycin's MBC without **33** is 391 nM against BAA-44, but after adding **33** to the vancomycin treated wells the MBC is 195 nM.

4.3. Red blood hemolysis

Hemolysis assays were performed on mechanically difibrinated sheep blood (Hemostat Labs: DSB100). Difibrinated blood (1.5 mL) was placed into a microcentrifuge tube and centrifuged for 10 min at 10,000 rpm. The supernatant was then removed and then the cells were resuspended with 1 mL of phosphate-buffered saline (PBS). The suspension was centrifuged, the supernatant was removed and cells were resuspended two additional times. The final cell suspension was then diluted 10-fold. Test compound solutions were made in PBS in small culture tubes and then added to aliquots of the 10-fold suspension dilution of blood. PBS was used as a negative control and a zero hemolysis marker. Triton X (a 1% sample) was used as a positive control serving as the 100% lysis marker. Samples were then placed in an incubator at 37 °C while being shaken at 200 rpm for one hour. After one hour, the samples were transferred to microcentrifuge tubes and centrifuged for 10 min at 10,000 rpm. The resulting supernatant was diluted by a factor of 40 in distilled water. The absorbance of the supernatant was then measured with a UV spectrometer at a 540 nm wavelength.

4.4. Dye leakage from synthetic vesicles

The leakage of vesicle contents was monitored by the release of calcein encapsulated in large unilamellar vesicles. Vesicles were prepared by reverse-phase evaporation. After evaporating the organic solvent, residue was hydrated with the calcein solution (100 mM) in buffer (10 mM Tris–HCl pH 7.4). The free calcein was removed by elution through a Sephadex G-50 size-exclusion column in the same buffer. The leakage process was monitored by following the increase of calcein fluorescence intensity at 515 nm (excitation at 490 nm) after 20 μL of compound at different concentrations were added to 10 μL of vesicle solution mixed in 2 mL of TBS buffer. Complete leakage was achieved by addition of 100 μL of 20% Triton X-100 to the 2 mL solution, and the corresponding fluorescence intensity was used as 100% leakage for the calculation of leakage fraction.

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Supplementary data

^{1}H and ^{13}C NMR data and HRMS for all compounds tested within this article along with the dose–response curve for MBC values of **33**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.12.003.

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