Design and evaluation of poly-nitrogenous adjuvants capable of potentiating antibiotics in Gram-negative bacteria

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ARTICLE

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Antibiotic resistance has been a growing public health crisis since the 1980s. Therefore, it is essential not only to continue to develop novel antibiotics but also to develop new methods for overcoming resistance mechanisms in pathogenic bacteria so antibiotics can be reactivated towards previously resistant strains of bacteria. One common cause of antibiotic resistance in Gram-negative bacteria is reduced permeability of the tightly packed, negatively charged lipopolysaccharide outer membrane (OM), which dramatically reduces or even prevents antibiotic accumulation within the cell. Adjuvants that promote passive diffusion through the OM, including phenylalanine-arginine-β-naphthylamide, tobramycin, and pentamidine, have proven useful in potentiating antibiotics against Gram-negative bacteria. Structural evaluation of these adjuvants, which all include multiple nitrogenous groups, indicates that the entry rules developed for improving antibiotic accumulation in *Escherichia coli* (EC), could also be used to guide adjuvant development. To this end, a series of structurally simple poly-nitrogenous diphenylsuccinamide compounds have been prepared and evaluated for their ability to potentiate a panel of classic antibiotics in wild-type EC and *Pseudomonas aeruginosa* (PA). Modest adjuvant activity was obsereved for all compounds surveyed when co-administered with known antibiotics to inhibit either wild-type EC or PA, and all were able to accumulate in both EC and PA.

Introduction

Multidrug resistant (MDR) bacterial infections are a growing health crisis that could lead to up to 10 million deaths worldwide by 2050 if the current trends in resistance and antibiotic development continue.[1,2] The leading cause of nosocomial MDR infections across the world are the ESKAPE pathogens (*Enterococcus facium*, *Staphylococcus aureus* (SA), *Klebsiella pneumoniae* (KP), *Acinetobacter baumannii* (AB), *Pseudomonas aeruginosa* (PA), and *Enterobacter* spp.) some of which have no clinical treatments available.[3–5] Gram-negative bacterial infections, such as those caused by *Escherichia coli* (EC), AB, and PA, are especially challenging to treat compared to Gram-positive bacterial infections due to the additional outer membrane (OM), reduced OM porin expression, and numerous efflux pumps of Gram-negative bacteria,[6,7] which lead to an overall reduction in accumulation of antibiotics within the cell. Improving antibiotic penetration of the OM, either through antibiotic modification[8–10] or antibiotic-adjuvant combination therapies,[11–14] is currently one of the main strategies for improving antibiotic activity against all Gram-negative pathogens.

The OM is composed of a bilayer of tightly packed, asymmetric polyanionic lipopolysaccharides (LPS) and glycerophospholipids, with embedded porins, substrate channels, and proteins, which together dramatically restrict diffusion of small molecules into the cell by charge and molecular weight.[8-10] Because of this, many groups have worked to develop "rules of entry" for the OM of EC and PA based on both computational and experimental evaluation of substrates that are able to cross either via passive diffusion or uptake. Broadly these entry rules for Gram-negative bacteria are: i) molecular weight < 500 g/mol; ii) $cLogD_{7.4}$ between -2 and 0; iii) ≤ 5 rotatable bonds; iv) high polar surface area (average 165 Å); v) low globularity; and vi) presence of a 1º amine or guanidinium.[8–10,15,16] These rules can be used in both early-stage library screening for potential new antibiotic compounds and late-stage modification of Gram-positive antibiotics to expand their activity to make them broad-spectrum antibiotics. Hergenrother and co-workers have recently employed the antibiotic modification strategy by synthetically modifying a wide range of structurally diverse Gram-positive antibiotics with either a primary amine,[10] pyridine, or guanidine[8] to improve accumulation in and activity against Gram-negative pathogens. However, this strategy requires the modification to be in an area that is not required for target binding.

Antibiotic adjuvants, which are compounds that do not have the ability to kill bacterial cells on their own but instead are able to increase the activity of a known antibiotic by improving entry

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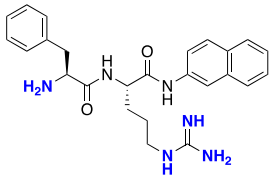
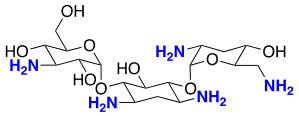
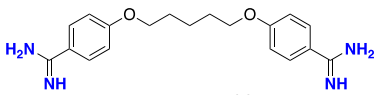
Electronic Supplementary Information (ESI) available: Supplementary materials include general synthetic procedures and characterization data including ^{1}H and ^{13}C NMR, IR, and MS for compounds **4**, **5**, **6**, **7**, **8**, **9**, and **10**; Adjuvant assay and accumulation assay methods and data for compounds **4**, **7**, **8**, and **10**; **SI Figure 1**–**3**, and **SI Table 1** and **2** associated with this article. See DOI: 10.1039/x0xx00000x



Phe-Arg-β-naphthylamide (1)



Tobramycin (2)



Pentamidine (3)

Adjuvant	Molecular Weight (g/mol)	Globularity[8]	Rotatable Bonds[8]	cLogP	tPSA (Å)
1	446.56	0.065	11	1.224	146.12
2	467.52	0.079	6	-4.716	268.17
3	340.43	0.057	10	2.021	118.2

Figure 1. Structure and physical properties of antibiotic adjuvants capable of penetrating the OM of Gram-negative bacteria. Globularity and RB determined by entry-way.org.[8] cLogP calculated in ChemDraw (vs. 19.0).

into the bacterial cell or inhibiting an active resistance mechanism, have also gained renewed attention in the past decade for targeting Gram-negative bacteria.[13,17] For example, phenylalanine-arginine-β-naphthylamide (**1**, **Figure 1**) has been shown to act as both an efflux pump inhibitor and an OM permeabilizer to potentiate fluoroquinolone and β-lactam antibiotics against PA.[18,19] Tobramycin (**2**, **Figure 1**), an aminoglycoside antibiotic capable of crossing the OM via self-promoted uptake, and tobramycin-efflux pump inhibitor conjugates have also been shown to be active adjuvants that potentiate antibiotic activity (including novobiocin, rifampicin, erythromycin, trimethoprim, and minocycline) against a wide range of Gram-negative pathogens.[11,12,14,20,21] In 2012 Katsu et al. demonstrated that pentamidine (**3**, **Figure 1**), an antiparasitic bisbenzamidine that works via DNA/RNA disruption[22,23], is not only capable of penetrating the OM of Gram-negative bacteria due to its polycationic and amphiphilic nature and physiological pH, but it is also able to potentiate activity of the aminocoumarin antibiotic novobiocin against EC.[24] Pentamidine's utility as an adjuvant has steadily been expanded over the last decade, and now it has been shown to be able to potentiate a wide range of Gram-positive antibiotics towards EC,[25] AB,[25] and *Enterobacteriaceae*[26] and non-antibiotics such as mitomycin C against clinical strains of EC, KP, PA, and AB.[27]

Evaluation of phenylalanine-arginine-β-naphthylamide, tobramycin, and pentamidine's molecular structures demonstrates that they do follow some of the eNTRy rules for OM permeability as described by Hergenrother. As shown in **Figure 1**, all three have molecular weights < 500 g/mol and low globularity. Tobramycin is the most rigid of the three, but all have more than 5 rotatable bonds. None of the three have ideal lipophilicity, but all three are poly-nitrogenous with phenylalanine-arginine-β-naphthylamide containing both an amine and guanidine functional group, tobramycin having 5 primary amines, and pentamidine having two amidines.

Based on the structures of previously identified OM penetrating antibiotic adjuvants and the eNTRy rules, we have hypothesized that even simple molecules will be able penetrate the OM of Gram-negative bacteria and possibly act as antibiotic adjuvants as long as they are relatively rigid, poly-nitrogenous structures with low globularity. To probe this hypothesis, we have developed a small series of readily accessible, poly-nitrogenous, para-substituted diphenylsuccinamide compounds that utilize features of pentamidine (para-substituted phenyl groups and amidine functionality) and phenylalanine-arginine-β-naphthylamide (amide backbone, and amino and guanidinium functionality) (**Figure 2**). We then evaluated their ability to penetrate the OM of wild-type (WT) EC and PA and act as adjuvants for classic antibiotics against both pathogens.

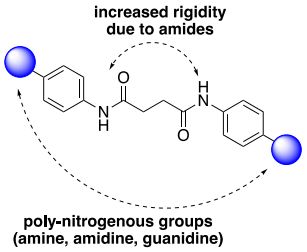


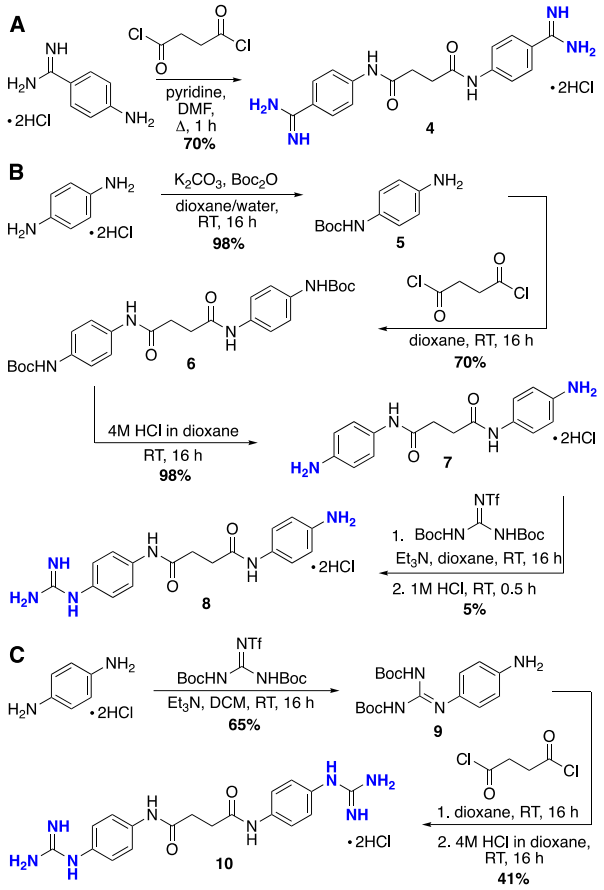
Figure 2. Design of poly-nitrogenous OM penetrating adjuvants.

Results and Discussion

Synthesis. As described in **Scheme 1**, all adjuvants were synthesized using a similar strategy that employed a condensation reaction between substituted anilines and succinyl chloride. Bisamidine (**4**) was synthesized in one step from a condensation reaction between 4-aminobenzamidine dihydrochloride and succinyl chloride in 70% yield (**Scheme 1**, **panel A**). Bisamine (**7**) and monoguanidine (**8**) were synthesized starting from *p*-phenylenediamine dihydrochloride. Mono-carbamate protection,[28] condensation with succinyl chloride, and subsequent deprotection yielded bisamine (**7**) in high yield. Monoguanidinylation of **7** was achieved using Hergenrother's conditions[8] and the guanidinium reagent to produce monoguanidine **8** in 5% yield over two steps (**Scheme 1**, **panel B**). Finally, bisguanidine **10** was synthesized starting also from *p*-phenylenediamine dihydrochloride, which was first monoguanidinylated using similar conditions as before to produce diBoc-protected **9** in 65% yield. Then **9** was subjected to the same condensation conditions with succinyl chloride to produce bisguanidine **10** in moderate yield after deprotection (**Scheme 1**, **panel C**).

Antibacterial activity. Although there are no reports to date of compounds **4**, **7**, **8**, and **10** specifically having antibacterial or

Scheme 1. Synthesis of poly-nitrogenous adjuvants **4**, **7**, **8**, and **10**.



adjuvant activity in the literature, McClusky et al. reported that other pentamidine-like poly-nitrogenous compounds are capable of eliciting antibiotic activity against both Gram-positive and Gram-negative bacteria through inhibition of the NusB–NusE protein interaction.[29] Therefore, the stand-alone antibacterial activity of **4**, **7**, **8**, and **10** were assessed using a standard broth microdilution assay against WT EC (ATCC 25922) and PA (ATCC 9027) (**SI Table 2**). Compounds **4**, **7**, and **8** were found to be inactive against both pathogens at 1000 μg/mL. Bisguanidine **10**, which is structurally the most similar to McClusky's compounds, did display weak activity against EC (~18% growth inhibition) at 100 μg/mL and against PA (~33% growth inhibition) at 1000 μg/mL. However, these concentrations are significantly higher than the concentration used for the adjuvant assays described below (20-100 μM of adjuvant); therefore we do not attribute any of the following bisguanidine **10** adjuvant activity to the bactericidal effect of the compound.

Adjuvant activity. Compounds **4**, **7**, **8**, and **10** were evaluated for their ability to potentiate the antibacterial activity of known antibiotics penicillin G, ampicillin, erythromycin, novobiocin, rifampicin and kanamycin against WT EC and PA using a standard minimum inhibitor concentration (MIC) adjuvant assay. We chose to evaluate a panel of mechanistically diverse and widely used antibiotics to determine whether the adjuvant activities of compounds **4**, **7**, **8**, and **10** are broad or specific to antibiotic class. Penicillin G and ampicillin are classic β-lactam antibiotics that inhibit cell wall synthesis by inactivating transpeptidase, and both suffer from widespread clinical resistance especially in Gram-negative pathogens due to reduced penetration of the OM and upregulation of β-lactamases.[30] Erythromycin, a macrolide antibiotic, and kanamycin, an aminoglycoside, inhibit bacterial protein synthesis by binding the 50S and 30S subunits of bacterial ribosomes respectively. Both erythromycin and kanamycin can either passively diffuse through the OM or enter via porin channels. Erythromycin is typically used to treat Gram-positive infections and is only bactericidal to non-resistant strains of WT PA at high concentrations. Kanamycin is active against Gram-negative bacteria including AB, KP, EC, and PA, but can lose activity due to a variety of resistance mechanisms including OM modification and reduction of porin channels.[31–33] Rifampicin and novobiocin, which both have been shown to be potentiated by pentamidine and tobramycin, are broad-spectrum antibiotics that inhibit RNA synthesis and DNA synthesis respectively.[11,24,25]

As shown in **Table 1**, our series of poly-nitrogenous compounds were able to modestly reduce the MIC of one or more antibiotics against WT EC or PA by 2-fold at a concentration of 100 μM (n > 3). Bisguanidine **10** was able to reduce the MIC of the largest number of antibiotics evaluated, potentiating erythromycin (EC and PA) and novobiocin (EC only). Monoguanidine **8** was able to potentiate erythromycin against both EC and PA, and bisamine **7** and bisamine **4** were only able to potentiate erythromycin against PA. Antidotally, it is worth noting that co-dosing of the adjuvants with many of the antibiotics surveyed did show increased pathogen inhibition even if MIC reduction was not observed. For example, there was an observable decrease in PA growth when rifampicin was combined with bisamine **7** and when novobiocin was combined with bisamine **7** and bisamidine **4**. Similarly, EC growth was decreased when rifampicin was combined with bisamine **7** and bisamidine **4**, when penicillin was combined with monoguanidine **8** and bisguanidine **10**, and when novobiocin was combined with bisamidine **4** and monoguanidine **8**. While the observed 2-fold MIC reduction for compounds **4**, **7**, **8**, and **10** is within broth microdilution standard error (as seen with ampicillin), this activity was seen consistently across multiple assays, and therefore we believe it indicates that these compounds could be further developed to increase adjuvant activity.

OM penetration. Based on the OM penetrating adjuvants described previously and the entry rules, we hypothesized that compounds **4**, **7**, **8**, and **10** could readily cross the OM of WT EC and PA. In addition to being poly-nitrogenous as seen in **Table 2**, all the adjuvants have a molecular weight that is less than 500 g/mol, have low globularity, and a cLogP between -2 and 0. Bisamine **7** has a lower than ideal polar surface area, and **4**, **8**, and **10** have more than 5 rotatable bonds. Although none of the adjuvants fit the rules for entry perfectly, all should readily penetrate the OM based on their physical properties. To experimentally confirm whether these molecules can penetrate the OM, each adjuvant was evaluated individually for its ability to accumulate in both WT EC and PA compared to ciprofloxacin,

a potent, high accumulating Gram-negative antibiotic, and ampicillin, a low accumulating antibiotic, using a modification of the method developed by Hergenrother (see the supporting information for method and calibration curves, **SI Figures 2/3**).[8,10] Using this method we found that compounds **4**, **7, 8**,

Table 1. Adjuvant activity of compounds **4**, **7**, **8**, and **10** (100 μM) with known antibiotics against *E. coli* and *P. aeruginosa*. (n = 3)

Adjuvant MIC (μg/mL)[a,b]					
Antibiotic	*E. coli*				
	Antibiotic Only	Antibiotic + **7**	Antibiotic + **4**	Antibiotic + **8**	Antibiotic + **10**
Penicillin G	64	64[0]	64[0]	64[0]	64[0]
Ampicillin	16-32	16-32[0]	16-32[0]	16-32[0]	16-32[0]
Rifampicin	8	8[0]	4-8[0]	8[0]	8[0]
Erythromycin	128	128[0]	128[0]	64[2]	64[2]
Kanamycin	64	64[0]	64[0]	64[0]	64[0]
Novobiocin	128	128[0]	64-128[0]	64-128[0]	64[2]
	P. aeruginosa				
Penicillin G	>256	>256 [0]	>256 [0]	>256 [0]	>256 [0]
Ampicillin	128	128[0]	128[0]	128[0]	128[0]
Rifampicin	16	16[0]	16[0]	16[0]	16[0]
Erythromycin	128	64[2]	64[2]	64[2]	64[2]
Kanamycin	>64	>64[0]	>64[0]	>64[0]	>64[0]
Novobiocin	256	256 [0]	256 [0]	256 [0]	256 [0]

[a]MIC = minimum inhibitory concentration of >90% pathogen growth inhibition of at OD = 590nm compared to (-)-control (DMSO + pathogen); [b]Fold reduction of MIC in brackets

Table 2. Physical properties and accumulation (n ≥ 3) of compounds **4**, **7**, **8**, and **10** in *E. coli* and *P. aeruginosa* compared to ciprofloxacin (high accumulator) and ampicillin (low accumulator).

Compound	**MW (g/mol)**	**Glob[a]**	**RB[a]**	**tPSA[b] (Å)**	**cLogP[b]**	**EC Accumulation (nmol /10^{12} CFU)**	**PA Accumulation (nmol /10^{12} CFU)**
7	298.34	0.05	5	110.24	-0.04	3.3 ± 1.9	2.0 ± 1.1
4	352.39	0.007	7	157.94	-0.39	1.0 ± 0.2	0.9 ± 0.2
8	340.39	0.054	7	146.12	-0.44	1.3 ± 0.6	0.3 ± 0.06
10	382.42	0.043	9	182	-0.84	1.1 ± 0.1	1.1 ± 0.1
Ciprofloxacin	331.34	0.04	3	72.88	-1.12	2.4 ± 0.8	1.8 ± 0.2
Ampicillin	349.41	0.111	4	112.73	-1.20	< 0.06[c]	< 0.06[c]

MW = molecular weight; Glob = globularity; RB = number of rotatable bonds; tPSA = total polar surface area; cLogP = calculated log of the partition coefficient. [a]Globularity and RB determined by entry-way.org;[8] [b]tPSA and cLogP calculated in ChemDraw (vs. 19.0); [c]No accumulation detected at lowest evaluated standard concentration.

and **10** accumulated within an order of magnitude of ciprofloxacin for both pathogens and 5–50 times higher than ampicillin, which accumulated at concentrations lower than our limit of detection of 0.06 nmol per 10^{12} CFU. Specifically, in WT EC all the adjuvants and ciprofloxacin accumulated between 1–2 nmol per 10^{12} CFU. However, in PA monoguanidine **8** showed slightly reduced accumulation compared to the other adjuvants and ciprofloxacin. The ability of compounds **4**, **7**, **8**, and **10** to accumulate in both WT EC and PA could be leveraged in future studies to develop covalent antibiotic/adjuvant hybrids as has been done with tobramycin and other adjuvants previously.[13]

Conclusions

While there is a dire need for the continued discovery of new antibiotics, development of antibiotic adjuvants that overcome resistance mechanisms and broaden the use of current antibiotics on the market is an equally essential weapon in our battle against antibiotic resistant bacteria. Herein, we detailed the synthesis and biological evaluation of poly-nitrogenous diphenylsuccinamide adjuvants that were specifically designed to promote diffusion of co-dosed antibiotics into Gram-negative bacterial cells by using the rules of OM entry and the structural motifs found in other OM penetrating molecules. While all the adjuvants examined were found to be capable of only modest potentiation of one or more antibiotics in WT EC or PA and accumulating in both WT EC and WT PA, it was observed that slight modifications in the nitrogenous groups employed (amine vs. amidine vs. guanidine) and symmetry (monoguanidine vs. bisguanidine) did have an impact on the scope of adjuvant activity regarding which antibiotics were potentiated against WT EC. Additionally, increasing nitrogen content increased the number of antibiotics potentiated with bisguanidine **10** showing the broadest activity.

In addition to evaluating potential new antibiotic adjuvants, the goal of this study was also to examine whether structurally simple molecules could be rationally designed to penetrate the OM of Gram-negative bacteria and promote antibiotic uptake using the established antibiotic rules of entry. The appeal of this approach is that since OM penetrating adjuvants only need to promote antibiotic uptake, the most basic molecular structure

can be employed, which will be easier to access and modify through synthesis compared to the antibiotics themselves that tend to be structurally complex due to their mechanisms of action. Based on the accumulation and adjuvant activity of the four poly-nitrogenous diphenylsuccinamide compounds presented in herein, we believe that this approach could be a new avenue for developing adjuvants that reactivate antibiotics towards Gram-negative pathogens, and that this work specifically could be furthered through covalent antibiotic/adjuvant hybrid development.

Author Contributions

BAP and KEM synthesized compounds **4**, **7**, **8**, and **10**. BAP, ABR, and ALW assayed compounds **4**, **7**, **8**, and **10** in cell death and adjuvant assays. BAP, MAS, and MRG performed the accumulation assay on compounds **4**, **7**, **8**, and **10**. JWB provided guidance and design for the MS work. KEM analyzed data and wrote the synthetic SI. ALW designed experiments, analyzed data, and wrote the manuscript. All authors contributed to manuscript revision.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

1 CDC. Antibiotic Resistant Threats in the United States. **2019**.
2 M. E. A. de Kraker, A. J. Stewardson, and S. Harbarth, *PLoS Medicine,* 2016, **13**(11), 1–6.
3 D. J. Payne, M. N. Gwynn, D. J.Holmes, and D. L. Pompliano, D. L, *Nature Reviews Drug Discovery,* 2007, **6**(1), 29–40.
4 K. Lewis, *Nature Reviews Drug Discovery,* 2013, **12**(5), 371–387.
5 S. Santajit and N. Indrawattana, *BioMed Research International,* 2016, **2016**, 1–8.
6 L. L. Silver, *Expert Opinion on Drug Discovery,* 2008, **3**(5), 487–500.
7 A. V. Vargiu, V. K. Ramaswamy, G. Malloci, I. Malvacio, A. Atzori, and P. Ruggerone, *Research in Microbiology*, 2018, **169**(7–8), 384–392.
8 Key reference: S. J. Perlmutter, E. J. Geddes, B. S. Drown, S. E. Motika, M. R. Lee, and P. J. Hergenrother, *ACS Infectious Diseases*, 2021, **7**(1), 162–173; E. J. Geddes, Z. Li, and P. J Hergenrother, *Nature Protocols*, 2021, **16**, 4833–4854.
9 Key reference: S. J. Cooper, G. Krishnamoorthy, D. Wolloscheck, J. K. Walker, V. v. Rybenkov, J. M. Parks, and H. I. Zgurskaya, *ACS Infectious Diseases,* 2018, **4**(8), 1223–1234.
10 Key reference: M. F. Richter, B. S. Drown, A. P. Riley, A. Garcia, T. Shirai, R. L. Svec, and P. J. Hergenrother, *Nature,* 2017, **545**(7654), 299–304.
11 Y. Lyu, X. Yang, S. Goswami, B. K. Gorityala, T. Idowu, R. Domalaon, G. G. Zhanel, G. A. Shan, and F. Schweizer, *Journal of Medicinal Chemistry,* 2017, **60**(9), 3684–3702.
12 T. Idowu, D. Ammeter, H. Rossong, G. G. Zhanel, and F. Schweizer, *Journal of Medicinal Chemistry,* 2019, **62**(20), 9103–9115.
13 R. Domalaon, T. Idowu, G. G. Zhanel, and F. Schweizer, *Clinical Microbiology Reviews,* 2018, **31**(2), 1–45.
14 B. K. Gorityala, G. Guchhait, D. M. Fernando, S. Deo, S. A. McKenna, G. G. Zhanel, A. Kumar, and F. Schweizer, *Angewandte Chemie - International Edition,* 2016, **55**(2), 555–559.
15 R. O'Shea, and H. E. Moser, *Journal of Medicinal Chemistry,* 2008, **51**(10), 2871–2878.
16 S. Acosta-Gutiérrez, I. v. Bodrenko, and M. Ceccarelli, *Antibiotics,* 2021, **10**(635), 1–10.
17 R. J. Melander, and C. Melander, *ACS Infectious Diseases,* 2017, **3**(8), 559–563.
18 R. P. Lamers, J. F. Cavallari, and L. L. Burrows, *PLoS ONE,* 2013, **8**(3), 1–7.
19 S. Schuster, J. A. Bohnert, M. Vavra, J. W. Rossen, and W. v. Kern, *Molecules,* 2019, 1–12.
20 X. Yang, S. Goswami, B. K. Gorityala, R. Domalaon, Y. Lyu, A. Kumar, G. G. Zhanel, and F. Schweizer, *Journal of Medicinal Chemistry,* 2017, **60**(9), 3913–3932.
21 T. Idowu, D. Ammeter, G. Arthur, G. G. Zhanel, and F. Schweizer, *Journal of Antimicrobial Chemotherapy,* 2019, **74** (9), 2640–2648.
22 T. L. Huang, J. J Vanden Eynde, A. Mayence, M. S. Collins, M. T. Cushion, D. Rattendi, I. Londono, L. Mazumder, C. J. Bacchi, and N. Yarlett, *Bioorganic and Medicinal Chemistry Letters,* 2009, **19**(20), 5884–5886.
23 J. J. Vanden Eynde, A. Mayence, M. Mottamal, C. J. Bacchi, N. Yarlett, M. Kaiser, R. Brun, and T. L. Huang, *Pharmaceuticals,* 2016, **9**(2), 1–11.
24 M. Ando, R. Kamei, K. Komagoe, T. Inoue, K. Yamada, and T. Katsu, *Journal of Microbiological Methods,* 2012, **91**(3), 497–500.
25 J. M. Stokes, Macnair, B. Ilyas, S. French, J. P Côté, C. Bouwman, M. A. Farha, A. O Sieron, C. Whitfield, B. K. Coombes, and E. D. Brown, *Nature Microbiology,* 2017, **2**, 1–21.
26 T. Cebrero-Cangueiro, R. Álvarez-Marín, G. Labrador-Herrera, Y. Smani, E. Cordero-Matía, J. Pachón, and M. E. Pachón-Ibáñez, *Frontiers in Cellular and Infection Microbiology,* 2018, **8** (October), 363.
27 C. Wu, L. Xia, W. Huang, Y. Xu, Y. Gu, C. Liu, L. Ji, W. Li, Y. Wu, K. Zhou, and X. Feng, *European Journal of Clinical Microbiology and Infectious Diseases,* 2020, 1771–1779.
28 K. E. Linder, E. Metcalfe, P. Nanjappan, T. Arunachalam, K. Ramos, T. M. Skedzielewski, E. R. Marinelli, M. F. Tweedle, A. D. Nunn, and R. E. Swenson, *Bioconjugate Chemistry,* 2011, **22** (7), 1287–1297.
29 P. J. Cossar, M. K. Abdel-Hamid, C. Ma, J. A. Sakoff, T. N. Trinh, C. P. Gordon, P. J. Lewis, A. McCluskey, *ACS Omega,* 2017, **2** (7), 3839–3857.
30 D. W. Green, *Expert Opinion on Therapeutic Targets,* 2002, **6**(1), 1–20.
31 S. Garneau-Tsodikova, and K. J. Labby, *MedChemComm,* 2016, **7** (1), 11–27.
32 D. Jelić, and R. Antolović, *Antibiotics,* 2016, **5**(3).
33 Y. Morita, J. Tomida, and Y. Kawamura, *Frontiers in Microbiology,* 2013, **4** (JAN), 1–8.