ACS Medicinal Chemistry Letters
Cite This: ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX
Letter
pubs.acs.org/acsmedchemlett

Second-Generation Tryptamine Derivatives Potently Sensitize Colistin Resistant Bacteria to Colistin

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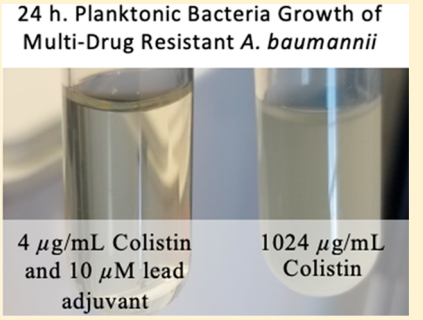
Supporting Information

ABSTRACT: Antibiotic resistance has significantly increased since the beginning of the 21st century. Currently, the polymyxin colistin is typically viewed as the antibiotic of last resort for the treatment of multidrug resistant Gram-negative bacterial infections. However, increased colistin usage has resulted in colistin-resistant bacterial isolates becoming more common. The recent dissemination of plasmid-borne colistin resistance genes (*mcr 1*−*8*) into the human pathogen pool is further threatening to render colistin therapy ineffective. New methods to combat antibiotic resistant pathogens are needed. Herein, the utilization of a colistin-adjuvant combination that is effective against colistin-resistant bacteria is described. At 5 μM, the lead adjuvant, which is nontoxic to the bacteria alone, increases colistin efficacy 32-fold against bacteria containing the *mcr-1* gene and effects a 1024-fold increase in colistin efficacy against bacteria harboring chromosomally encoded colistin resistance determinants; these combinations lower the colistin minimum inhibitory concentration (MIC) to or below clinical breakpoint levels (≤2 μg/mL).



KEYWORDS: *Antibiotic resistance, multidrug resistant bacteria, colistin, antibiotic adjuvant*

The impact of antibiotics cannot be understated; they have significantly contributed to the quality of life since the discovery of penicillin in the 1930s.[1] Despite the subsequent discovery of numerous antibiotic classes spanning multiple modes of action, bacteria have acquired resistance to every antibiotic in the clinician's arsenal. Antibiotic resistance will likely continue to become more common, as antibiotic usage rose 65% from 2000 to 2016, and history has shown us that resistance is more likely to be acquired when antibiotic consumption is increased.[2] Currently, the Centers for Disease Control and Prevention estimates approximately two million people present with an antibiotic resistant infection annually in the U.S., and the death toll from these infections is estimated to be at least 23,000.[3] A majority of these fatal infections are caused by the ESKAPE pathogens: the Gram-positive bacteria *Enterococcus faecium* and *Staphylococcus aureus*, and the Gram-negative bacteria *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.*

The arduous nature of developing effective therapies to treat infections caused by multidrug-resistant (MDR) Gram-negative bacteria has been well documented[4] and is hampered by the presence of both intrinsic and acquired resistance mechanisms that can render bacteria resistant to nearly, if not all, antibiotics on the market.[5] Indeed, in 2017, a woman in the United States succumbed to septic shock that stemmed from an infection caused by a strain of *K. pneumoniae* that was resistant to 26 antibiotics, including all aminoglycosides and polymyxins tested.[6]

The escalation of carbapenem resistance in Gram-negative bacteria has resulted in the increased usage of the "last resort" antibiotic colistin, a macrocylic cationic polypeptide with a bactericidal mechanism that involves direct physical disruption of the Gram-negative outer membrane.[7] Colistin is, however, nephrotoxic with ca. 25% of patients who are administered a full regimen experiencing some degree of nephrotoxicity (this does not include patients whose regimen was halted prematurely due to toxicity).[8] For this reason, physicians have traditionally avoided the use of colistin; however, the rise in resistance to other more well-tolerated antibiotic classes has forced colistin usage as of late. Prior to 2016, all known clinical resistance to colistin was mediated by chromosomally located genes. This situation changed dramatically with a report in 2016 of a patient presenting with a colistin-resistant *Escherichia coli* infection, in which the gene (*mcr-1*) encoding the colistin resistance machinery was located on a plasmid,[9] raising the possibility that colistin resistance could become rapidly spread through lateral gene transfer. Indeed, *mcr-1* and its variant genes (*mcr-2*−*8*) have now been reported globally.[10] The *mcr-1* gene encodes a phosphoethanolamine transferase that covalently modifies lipid A with phosphoethanolamine, which serves to decrease the overall net negative charge of the

Received: March 26, 2019
Accepted: April 12, 2019

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DOI: 10.1021/acsmedchemlett.9b00135
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

membrane and greatly impacts the ability of colistin to disrupt the bacterial membrane due to decreased electrostatic interactions.[11]

As the antibiotic pipeline for the treatment of MDR Gram-negative infections is sparse, the development of novel approaches to combat these bacteria are warranted. One such method is the antibiotic-adjuvant approach, in which a conventional antibiotic is codosed with a nontoxic small molecule that increases the antibiotic's efficacy.[12,13] In a previous study from our lab, tryptamine derivative **1a** (Figure 1) exhibited a 32-fold and a 2-fold reduction in the colistin

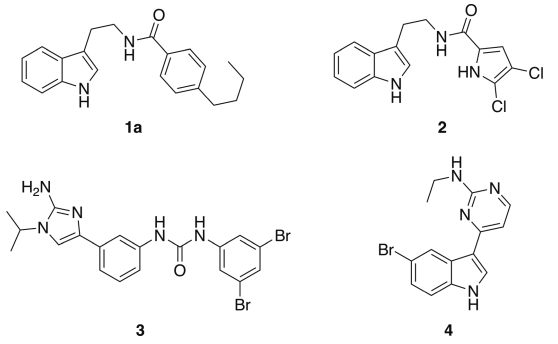


Figure 1. Active adjuvants that reduce the MIC of colistin against various MDR Gram-negative bacteria.

minimum inhibitory concentration (MIC) against *Francisella philomiragia* and *F. novicida*, respectively, when tested at 50 μM.[14] Additional indole and 2-aminoimidazole (2-AI) derivatives (**2**, **3**, and **4**, Figure 1) have also demonstrated the ability to potentiate colistin activity against several MDR Gram-negative bacterial strains.[15−17] Building upon these initial studies, we report the synthesis and screening of 32 novel tryptamine derivatives for their ability to enhance colistin efficacy against a range of MDR Gram-negative bacteria. During these studies, we identified seven compounds that, at 30 μM or less, increased colistin efficacy, reducing the MIC below the susceptible breakpoints defined by the Clinical and Laboratory Standards Institute (2 μg/mL for the bacteria tested).[18] The lead compound from this series, at 5 μM, elicited a 1024-fold reduction of the colistin MIC against a chromosomally resistant *K. pneumoniae* strain, reducing the MIC from 512 to 0.5 μg/mL. Herein, we describe the structure−activity relationship (SAR) study that led to the discovery of these novel small molecule adjuvants that increase colistin efficacy *in vitro*.

We first evaluated compound **1a** for its ability to potentiate colistin against three representative Gram-negative strains that contain the *mcr-1* plasmid as well as their parent strains (Table 1).[19] This compound showed modest activity and, at 50 μM, reduced the colistin MICs between two- and 16-fold, with the greatest activity being a 16-fold MIC reduction observed against the parent *E. coli* strain. In a parallel SAR study on compound **2**, we noted that replacement of the halogenated pyrrole with diverse halogenated heterocycles abrogated colistin potentiation activity against several of the same bacterial strains.[15] Therefore, it was interesting that compound **1a** exhibited activity against these six Gram-negative strains.

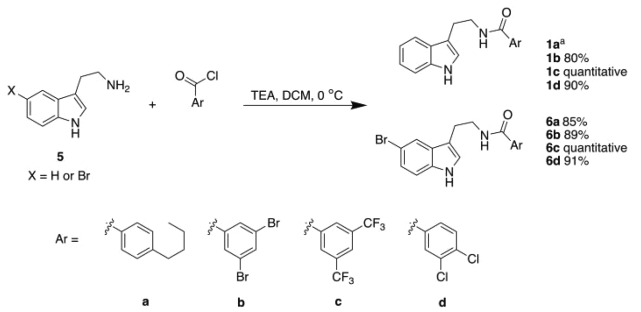
We next elected to investigate whether substitution of the alkyl tail was tolerated as well as bromination of the indole ring. Previous studies in our group have shown that bromination of the indole ring as well as substituting alkyl

Table 1. Potentiation of Colistin (MICs μg/mL) with Pilot and Brominated Library Adjuvants Tested at 50 μM

	E. coli YD626		*K. pneumoniae* 2210291		*A. baumannii* 17978	
compound	parent	+mcr-1	parent	+mcr-1	parent	+mcr-1
	1	8	1	16	2	16
1a	0.0625	4	0.125	8	0.25	4
1b	0.25	4	0.5	4	1	4
1c	0.0625	4	0.25	16	0.25	8
1d	0.5	2	0.5	2	1	2
6a	0.125	4	0.25	4	0.5	4
6b	0.5	4	0.5	4	1	4
6c	0.0625	4	0.25	8	0.5	8
6d	0.25	2	0.5	1	0.5	1

groups attached to a phenyl ring with either trifluoromethyl groups or various halogens can augment activity in the context of antibiofilm activity and colistin potentiation (2-AI **3** and meridianin D analog **4**, Figure 1).[16] To explore the effects of these structural modifications to tryptamine in the context of colistin potentiation, seven additional compounds were readily synthesized by acylating the appropriate tryptamine derivative (Scheme 1) and were then assayed for activity.

Scheme 1. General Synthetic Route for Pilot Library



[a]Compound was previously synthesized.[14]

Initially, we measured the MIC of each adjuvant against three engineered bacterial strains harboring the *mcr-1* gene, along with the corresponding parent strains.[19] All adjuvants were essentially nontoxic themselves, exhibiting MICs > 200 μM. We then screened each nonbrominated tryptamine derivative (**1b−d**) at 50 μM (≤25% of their MIC so that potential toxic effects of the adjuvants toward the bacteria would be minimal) and observed that every adjuvant showed at least modest activity against all strains tested (Table 1). Compound **1d** displayed the greatest activity across all *mcr-1* containing strains, lowering the colistin MIC to 2 μg/mL in all cases (between 4- and 8-fold reductions), which is the susceptibility breakpoint (2 μg/mL), and demonstrated that replacement of the alkyl group is tolerated. The brominated indolic derivatives, compounds **6a−d**, exhibited similar activity to their nonbrominated analogues (potentiation was within 2-fold against all six strains) (Table 1), with most exhibiting 2-fold reductions in activity. However, compound **6d** did exhibit a 2-fold increase in activity against two of the three *mcr-1* containing strains when compared to its nonbrominated counterpart **1d**.

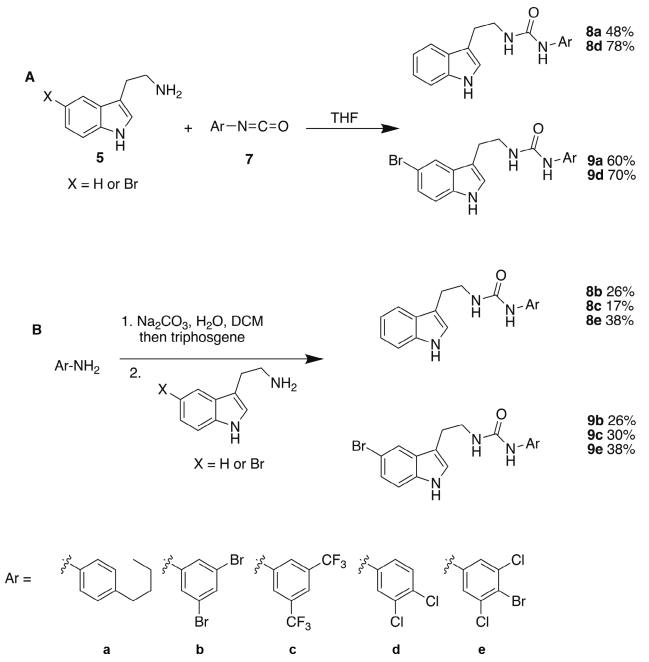
With negligible traction gained from brominating the indole ring or replacing the phenalkyl tail, we next turned our

DOI: 10.1021/acsmedchemlett.9b00135
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

attention to the amide linker. In the context of colistin adjuvants based upon the 2-AI scaffold, it has been noted that replacing the amide linker with a urea (i.e., compound **3**, Figure 1) increased colistin potentiation activity substantially.[17] Therefore, we elected to test whether this structural modification translated between scaffolds, and we set out to make both the brominated and nonbrominated tryptamine derivatives containing a urea linker.

Compounds were synthesized via one of two methods depending on the commercial availability of the starting materials. If the appropriate isocyanate was available, it was coupled directly with tryptamine or 5-bromotryptamine (Scheme 2A); otherwise, in a one-pot procedure, the

Scheme 2. Synthesis of Urea-containing Indole Adjuvants



isocyanate was formed *in situ* from the corresponding aniline and triphosgene, and then immediately coupled with tryptamine or 5-bromotryptamine (Scheme 2B). These methods afforded products **8a–e** and **9a–e**.

As observed with other tryptamine derivatives, compounds **8a–e** and **9a–e** were nontoxic to bacteria and displayed standalone MICs > 200 μM against the six bacterial strains. When tested at 50 μM in combination with colistin, most compounds showed increased colistin potentiation when compared to their amide counterparts (Table 2). Compounds **8b**, **8c**, **8d**, **8e**, **9d**, and **9e** resensitized all three *mcr-1* strains toward colistin, dropping the MIC to ≤2 μg/mL, demonstrating that the urea linkage generally is more active for colistin potentiation than the amide.

Since the replacement of the amide with a urea moiety provided analogs with increased colistin potentiation activity, we next explored the effect of linker length between the indole and urea and the position at which the urea was attached to the indole ring. Three additional sets of compounds were synthesized following the routes outlined in Scheme 2, but with the urea placed either one methylene group closer to the indole ring (**11a–e**, Figure 2) or directly attached to the indole ring, at either the 5 or 6 position (**12**/**13a–e**, Figure 2). As with all previously synthesized analogs, adjuvant standalone

Table 2. Potentiation of Colistin (MIC μg/mL) with Urea-containing Adjuvants Tested at 50 μM

	E. coli YD626		*K. pneumoniae* 2210291		*A. baumannii* 17978	
compound	parent	+mcr-1	parent	+mcr-1	parent	+mcr-1
	1	8	1	16	2	16
8a	1	8	1	16	2	16
8b	0.25	2	0.5	1	0.5	1
8c	0.0313	1	0.25	1	0.125	0.5
8d	0.125	1	0.25	0.5	0.5	0.5
8e	0.125	1	0.5	1	0.5	1
9a	1	8	0.5	16	2	16
9b	0.25	2	0.5	2	0.5	2
9c	0.0313	4	0.5	2	0.25	2
9d	0.25	0.5	0.5	0.5	0.5	1
9e	0.25	1	0.5	0.5	0.5	0.5

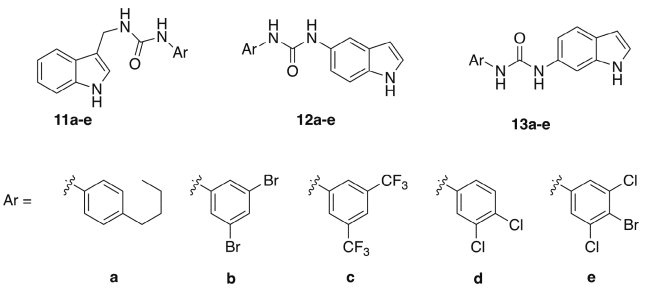


Figure 2. Second-generation urea-containing adjuvants synthesized for SAR analysis.

MICs were >200 μM, and all were therefore tested at 50 μM in combination with colistin. With the exception of compound **11c**, all compound **11** derivatives exhibited a decrease in activity. Compound **11c** rivaled its tryptamine derivative **8c** in terms of activity. As for derivatives **12a–e** and **13a–e**, direct attachment of the urea to the indole ring at the 5 or 6 position abrogated activity, showing ≤2-fold reduction in the MIC of colistin (Table 3).

We next performed a dose–response study on the seven lead compounds in combination with colistin against the three strains that contain the *mcr-1* plasmid (Table S1). The concentration of the adjuvant was sequentially lowered to determine the point at which the colistin MIC exceeded the breakpoint for clinical colistin susceptibility (2 μg/mL). All adjuvants maintained similar activity to the original 50 μM dose when tested at 30 μM, and adjuvants **8b**, **8c**, **8e**, **9d**, and **9e** successfully lowered the colistin MIC to the susceptibility breakpoint at 15 μM against all three strains. Compound **9d** was the second most potent adjuvant and lowered the colistin MIC to 1 μg/mL against all *mcr-1* strains at 15 μM, while adjuvant **9e** displayed significant potentiation activity down to 5 μM, where it reduced the MIC of colistin from 8, 16, and 16 to 1, 2, and 1 μg/mL against *mcr-1* plasmid containing *E. coli*, *K. pneumoniae*, and *A. baumannii*, respectively.

To further investigate the lead compounds, we selected compounds **8b**, **9d**, and **9e** to test against chromosomally colistin resistant *K. pneumoniae* and *A. baumannii* strains, which are typically significantly more resistant to colistin than isolates with resistance encoded by the *mcr-1* gene. All three compounds were highly active at 20 μM, eliciting between 64 and ≥2048-fold reductions in the MIC of colistin (Table 4). Compound **9e** was again the most active compound and

DOI: 10.1021/acsmedchemlett.9b00135
ACS Med. Chem. Lett. XXXX, XXX, XXX–XXX

Table 3. Potentiation of Colistin (MIC μg/mL) with Second-Generation Urea-containing Adjuvants Tested at 50 μM

	E. coli YD626		*K. pneumoniae* 2210291		*A. baumannii* 17978	
compound	parent	+mcr-1	parent	+mcr-1	parent	+mcr-1
-	1	8	1	16	2	16
11a	1	8	0.5	16	2	16
11b	0.25	4	0.5	16	1	8
11c	0.0313	1	0.125	1	0.125	1
11d	0.5	4	0.5	16	2	16
11e	0.25	8	0.5	8	1	8
12a	0.5	8	0.5	16	1	16
12b	1	8	1	16	1	16
12c	0.5	4	0.5	8	1	8
12d	0.5	8	1	16	2	16
12e	0.5	8	1	16	1	8
13a	0.5	8	1	16	2	8
13b	1	8	1	16	2	16
13c	0.5	4	1	16	1	8
13d	1	8	1	16	2	16
13e	1	8	1	16	2	16

resensitized all four chromosomally colistin resistant strains to or below the CLSI breakpoint ($\leq$2 μg/mL) when tested at a concentration of 20 μM, and resensitized three out of the four strains to breakpoint colistin levels at a concentration of 7.5 μM. Against *K. pneumoniae* B9 and *A. baumannii* 4106, compound **9e** returned over a 2000-fold reduction of the colistin MIC at 10 μM. Overall, these compounds, especially adjuvant **9e**, displayed potent *in vitro* colistin potentiation activity against chromosomally resistant bacterial strains.

Additional assays were performed with lead compound **9e** to confirm activity and examine toxicity. To rule out activity due to nonspecific compound aggregation effects, the resensitization of *A. baumannii* 17978$^{+mcr\text{-}1}$ to colistin was performed in the presence of 0.01% Tween 80.[20] Compound **9e** displayed equipotent colistin sensitization activity in the absence and presence of Tween 80 when tested at 30 μM. Next, lead adjuvant **9e** and inactive compound **9a** were tested for toxicity against red blood cells at a concentration of 200 μM, and both compounds affected <2.5% cell lysis. Lastly, the potential for eukaryotic cell toxicity was explored using a mouse mammary gland tumor cell line, 4T1 (ATCC). Five analogs (**8b**, **8c**, **8d**, **9d**, and **9e**), in addition to an active compound from one of our previous reports (**14**,[17] Figure S1), were each tested in monotherapy and in combination with colistin (1 μg/mL). After 18 h of incubation with 4T1 cells, the culture medium was aspirated, and cells were treated with a 0.5 mg/mL solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in culture media to afford a colorimetric assay for cell viability (Figure S2). The concentration at which each compound resulted in 50% cell viability was recorded as that compound's CT_{50} value (Table 5). From these data, we

Table 5. CT_{50} (μM) of Analogs in 4T1 Cells Dosed with and without 1 μg/mL Colistin

	compound alone	compound + colistin
8b	86.2	64.2
8c	60.5	61.8
8d	117.3	104.8
9d	125.5	84.6
9e	94.1	64.9
14	23.2	15.6

first note that the respective CT_{50} value of each compound was not significantly altered by the presence of colistin in culture media. Second, each compound was considerably less toxic than **14**, a compound previously shown to potentiate colistin.[17]

Finally, we sought to determine if our antibiotic–adjuvant combination would translate into an *in vivo* model. We chose a *Galleria mellonella* model of *A. baumannii* infection to test our antibiotic–adjuvant combination as previous studies at the Walter Reed Army Institute of Research (WRAIR) have shown results from this model to be predictive of outcomes in murine models of infection.[21,22] Since the use of *A. baumannii* 4106 in a *G. mellonella* model has not, to the best of our knowledge, been previously reported, we first performed a dose escalation study to determine the minimum lethal dose of bacteria in the worms. Much to our surprise, this strain was notably virulent, and 6×10^5 colony forming units (CFUs) brought about 100%

Table 4. Dose Response of Lead Compounds 8b, 8d, and 9e with Colistin (MIC μg/mL) against Chromosomally Colistin Resistant Strains

		K. pneumoniae B9	*K. pneumoniae* C3	*A. baumannii* 3941	*A. baumannii* 4106
-compound	concentration (μM)	512	512	512	1024
8v	20	$\leq$0.25	2	8	4
	15	0.5	4	8	4
	10	1	8	16	8
	7.5	4			
	5	32			
9d	20	$\leq$0.25	1	2	2
	15	0.5	2	4	2
	10	1	4	8	4
	7.5	2			
	5	8			
9e	20	$\leq$0.25	0.5	2	$\leq$0.5
	15	$\leq$0.25	0.5	4	$\leq$0.5
	10	$\leq$0.25	1	4	1
	7.5	$\leq$0.25	2	16	1
	5	0.5	4		4

DOI: 10.1021/acsmedchemlett.9b00135
ACS Med. Chem. Lett. XXXX, XXX, XXX–XXX

death of moth larva by day 4, which is similar to the highly virulent *A. baumannii* 5075 strain.[21,22] Next, we sought to find a positive control for this model; however, this strain was essentially resistant to all antibiotic classes, with the antibiotic tigecycline demonstrating the most potent *in vitro* MIC of 8 μg/mL. With this antibiotic as our only positive control option, we determined that a dose of 100 mg/kg provided minimal survival after 4 days (20% survival at day 4, Figure 3).

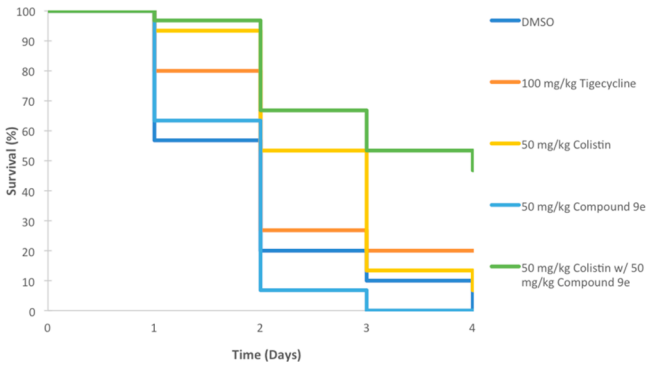


Figure 3. Kaplan–Meier curve of worms inoculated with 6×10^5 colony forming units of *A. baumannii* 4106.

Treatment of infected worms with either 50 mg/kg colistin or compound **9e** provided only 7% and 0% survival, respectively, after 4 days. On the contrary, when 50 mg/kg colistin was paired with 50 mg/kg compound **9e**, the combination increased worm survival to 47% after 4 days, considerably exceeding that of the tigecycline control.

In conclusion, we report novel tryptamine derivatives that are highly active in combination with colistin against colistin-resistant bacteria. We found that incorporation of a urea linker significantly augmented activity when compared to analogs containing an amide linker. Three of the adjuvants disclosed in this study display high colistin potentiation activity at 20 μM against all colistin resistant strains tested. Lead compound **9e** resensitized all three *mcr-1* containing bacterial strains to colistin at a concentration of 5 μM and resensitized three out of four chromosomally resistant strains at a concentration of 7.5 μM, which makes **9e** the most active adjuvant reported for reversal of colistin resistance. Further studies showed that compound **9e** was generally nontoxic to red blood cells and the 4T1 cell line. Finally, an *in vivo G. mellonella* model showed 47% survival of inoculated wax worms after 4 days when given a combination treatment of colistin and compound **9e**. This is a significant increase in survival when compared to day 4 of monotherapy of colistin (7% survival), compound **9e** alone (0% survival), and 100 mg/kg of tigecycline (20% survival). Efforts to delineate the mechanism of action of these tryptamine derivatives as well as evaluation in murine models of infection are currently ongoing.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.9b00135.

Chemistry experimental; biology experimental; *Galleria mellonella* day-by-day results; compound NMRs (PDF)

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Author Contributions

All authors have given approval to the final version of the manuscript.

Funding

The authors would like to thank the National Institutes of Health (GM055769 and AI136904) for funding.

Notes

The authors declare the following competing financial interest(s): Dr. Melander is co-founder and board of directors member of Agile Sciences, a biotechnology company seeking to commercialize antibiotic adjuvants.

■ ACKNOWLEDGMENTS

The authors would like to thank Robert Ernst (University of Maryland, Baltimore) and Yohei Doi (University of Pittsburgh) for *mcr-1* containing bacterial strains.

■ ABBREVIATIONS

MDR, multidrug-resistant; MIC, minimum inhibitory concentration; SAR, structure–activity relationship

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DOI: 10.1021/acsmedchemlett.9b00135
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