Published in final edited form as:

Bioorg Med Chem. 2017 October 15; 25(20): 5749–5753. doi:10.1016/j.bmc.2017.08.055.

Small Molecule Adjuvants that Suppress Both Chromosomal and *mcr-1* Encoded Colistin-Resistance and Amplify Colistin Efficacy in Polymyxin-Susceptible Bacteria

William T. Barker^a, Sara E. Martin^a, Courtney E. Chandler^b, T. Vu Nguyen^a, Tyler L. Harris^a, Christopher Goodell^c, Roberta J. Melander^a, Yohei Doi^d, Robert K. Ernst^b, and Christian Melander^{a,*}

^aDepartment of Chemistry, North Carolina State University, Raleigh, NC, 27695-8024 USA

^bDepartment of Microbial Pathogenesis, University of Maryland-Baltimore, Baltimore, MD 21201 USA

^cBoston Children's Hospital/Harvard School of Dental Medicine, Boston, MA 02115 USA

^dDivision of Infectious Diseases, University of Pittsburgh Medical Center, Pennsylvania, USA

Abstract

Bacterial resistance to polymyxin antibiotics has taken on a new and more menacing form. Common are genomically-encoded resistance mechanisms to polymyxins, specifically colistin (polymyxin E), however, the plasmid-borne *mobile colistin resistance-1 (mcr-1)* gene has recently been identified and poses a new threat to global public health. Within six months of initial identification in Chinese swine in November 2015, the first human clinical isolation in the US was reported (Apr. 2016). Herein we report successful reversion of *mcr-1*-driven colistin resistance in *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Escherichia coli* with adjuvants we previously reported as modulators of chromosomally-encoded colistin resistance. Further screening of our in-house library of nitrogen-dense heterocycles has identified additional chemical scaffolds that actively attenuate colistin resistance. Ultimately, we present a diverse cohort of adjuvants that both sensitize colistin-resistant and colistin-susceptible bacteria to this antibiotic, thus providing a potential avenue to both reduce colistin dosage and toxicity, and overcome colistin resistance.

Graphical Abstract

*to whom correspondence should be addressed: ccmeland@ncsu.edu.

Conflicts of Interest

Christian Melander is a co-founder of Agile Sciences, a biotechnology company focused on commercial applications of nitrogen-rich small molecules, and currently sits on its board-of-directors.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Multidrug-resistant (MDR) bacterial strains pose one of the most pressing challenges to human health. Clinics and hospitals worldwide are plagued by an increasing number of bacterial isolates presenting resistance to one or multiple classes of antibiotics. The majority of recent successful antibiotic discovery efforts have targeted MDR Gram-positive bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA). The only new classes of antibiotics to be introduced to the clinic in the last two decades, the lipopeptides (i.e. daptomycin) and oxazolidinones (i.e. linezolid) are active solely against Gram-positive bacteria.¹ This lack of novel therapeutics for the treatment of MDR Gram-negative infections has forced clinicians to fall back on nearly obsolete antibiotics. A recent increase in the use of colistin, or polymyxin E, is a paradigmatic example of this problem. First put in service in the 1950s, colistin is now viewed as the “last resort” treatment for Gram-negative bacterial infections that are resistant to all other classes of antibiotics.² To date, increased clinical usage of colistin combined with its widespread use in commercial animal husbandry has resulted in an increasing number of colistin-resistant bacterial isolates.³

Recently, colistin-resistant strains of *Escherichia coli* discovered in Chinese swine spurred reassessment of the potential for rapid spread of colistin resistance. These isolates harbored a plasmid-mediated resistance gene encoding a lipid A phosphoethanolamine transferase dubbed *mobile colistin resistance-1* (*mcr-1*).⁴ This modification confers a net positive charge increase on the bacterial cell surface and significantly reduces the electrostatic binding interaction between colistin and the bacterium. The discovery of *mcr-1* marked a significant deviation from the previously characterized strains harboring chromosomally encoded colistin resistance and presented the first possibility of rapid dissemination of colistin resistance machinery via lateral gene transfer.⁵ This plasmid has garnered increasing attention as it was identified in a number of human patients across Asia and Europe, and was recently observed in the US when a Pennsylvania woman was found to have urinary tract infection due to *E. coli* containing *mcr-1* in April 2016.⁶ In June of 2016, the *mcr-2* gene with ~76% identity to *mcr-1* was identified in *E. coli* in Belgium,⁷ and *mcr-3*, having ~45% identity with *mcr-1*, was recently reported from porcine *E. coli* strains in China.⁸ The spread of colistin resistance coupled with the lack of new treatments for Gram-negative bacterial

infections has created a situation in which contraction of an incurable, pan-resistant bacterial infection is a possibility. One such case was recently reported in Nevada, when a 70-year-old woman was infected with a *Klebsiella pneumoniae* strain resistant to all antibiotics clinically available in the US. Having no viable options for antibiotic treatment, this patient ultimately succumbed to the infection.⁹ Methods to tackle antibiotic-resistant Gram-negative bacteria are clearly needed.

In this study, we establish that small molecule adjuvants previously described by our group have the ability to reverse colistin resistance in *E. coli*, *Acinetobacter baumannii*, and *K. pneumoniae* strains harboring *mcr-1* plasmid vectors. Furthermore, a more comprehensive screening of our small molecule library has identified multiple additional scaffolds that also have the ability to break colistin resistance. Finally, we demonstrate that these molecules have the ability to further sensitize colistin-susceptible strains to the effects of colistin, providing a potential avenue to reduce therapeutic doses of colistin *in vivo* and thus mitigate toxicity.

Results and Discussion

Five Gram-negative bacterial strains were transfected with an engineered plasmid vector containing both the *mcr-1* gene and a gentamicin resistance cassette to afford selection for cells containing the plasmid.¹⁰ The MICs of colistin against both the parent and transfected strains are summarized in Table 1. Briefly, bacteria were cultured in cation-adjusted Mueller-Hinton broth (CAMHB) for approx. 5 hours in duplicate. Gentamicin sulfate was added to these cultures to a final concentration of 30 µg/mL to select for the *mcr-1* vector. After five hours of incubation with shaking, each culture was diluted in fresh CAMHB to 5×10^5 CFUs and split into two separate, identical aliquots to afford a duplicate assay. These aliquots were then treated with colistin in serial dilutions to determine the MIC. This assay follows guidelines set out by the Clinical and Laboratory Standards Institute, which defines colistin susceptibility as 2 µg/mL and resistance as 4 µg/mL. As can be seen, the *mcr-1* plasmid indeed confers increased colistin resistance in all strains at or above the CLSI breakpoint, and patients infected with these strains could not be successfully treated with colistin in its highest tolerated dosage.

Next, we determined the ability of compounds **1** and **2** (Figure 1) to resensitize each of the *mcr-1*-carrying strains to colistin challenge. Both compounds were previously disclosed by our group as potent modulators of colistin resistance in isolates with endogenous colistin resistance.¹¹ The colistin MIC of each strain was determined in the presence of either 30 or 15 µM adjuvant. At 30 µM, we observed reductions of 16 to >2,048-fold with compound **1** and 8 to 512-fold with compound **2** (Table 2). These fold reductions mirror each compound's respective activity against clinically-derived colistin-resistant isolates. Each compound showed reduced activity at 15 µM. Next, we determined the MIC of adjuvant alone. Compound **2** was non-toxic to all strains, returning MIC values of >200 µM, whereas compound **1** had minimal toxicity against *E. coli* ATCC 25922 (50 µM) and *E. coli* YD626 (100 µM). Against the other three strains, the MIC of **1** was >200 µM.

Previously, we demonstrated that compound **2** abrogated phosphoethanolamine modification of lipid A and reversed colistin resistance in that process. As *mcr-1* encodes a phosphoethanolamine transferase (PEtN) that modifies lipid A¹⁰ we studied the extent of lipid A modification post treatment with compound **2**. As a test strain, *A. baumannii* ATCC 17978^{+mcr-1} was grown in the presence or absence of 30 μ M compound **2** for 24 hours, at which point lipid A was isolated and analyzed. Mass spectra of each sample (Figure 2) revealed again that compound **2** reduced the extent of lipid A modification.

With successful reversion of colistin resistance in strains harboring *mcr-1*, we then conducted a more comprehensive screen of our in-house compound library to identify additional small molecule scaffolds with ability to reverse colistin resistance using *A. baumannii* ATCC 17978^{+mcr-1} as our test strain. Of the 220 compounds screened, numerous scaffolds possessed the ability to reverse colistin resistance. Compounds **3** and **4** (Figure 3) were selected for further evaluation. Interestingly, compound **3** was previously reported by our group to highly suppress oxacillin resistance in MRSA.¹² Compound **4** was synthesized to probe structure activity relationships in the context of indole signaling.¹³ At 60 μ M, compound **3** reduced the MIC of *A. baumannii* ATCC 17978^{+mcr-1} 64-fold (to 0.25 μ g/mL), and compound **4** reduced the MIC 32-fold.

Observing significant suppression of colistin resistance by compound **3** in *A. baumannii* ATCC 17978^{+mcr-1}, we evaluated it against our panel of *mcr-1*-carrying strains (Table 3). Although this compound was less potent at 30 μ M in comparison to either compound **1** or **2**, across all strains it retained activity at 15 μ M. Furthermore, the MIC of compound **3** alone against all six strains was >200 μ M.

Noting its potent activity in *mcr-1*-carrying strains, we elected to evaluate **3** against a panel of colistin-resistant primary clinical isolates of *A. baumannii* and *K. pneumoniae* with an established heritage of chromosomally-encoded colistin resistance. These strains boast colistin MICs much higher than the *mcr-1*-carrying strains. As shown in Table 4, we again noted significant decrease in colistin MICs with concomitant treatment of compound **3** in a dose-dependent manner. Interestingly, compound **3** was less potent against these strains than compounds **1** or **2**.

Comparing the absolute values of colistin MICs of *mcr-1*-carrying strains in the presence of adjuvant (**1**, **2**, or **3**, Tables 2/3) to that of their corresponding parent (Table 1) reveals that these adjuvants, despite being non-toxic, are generating colistin MICs in resistant strains far below those of colistin-susceptible strains. Of note, colistin is viewed as the antibiotic of last resort for treatment of MDR Gram-negative bacterial infections as it exhibits dose-dependent toxicity.¹⁴ Here we observe that these adjuvants hypersensitize Gram-negative bacteria to colistin *in vitro*, raising the possibility that combined adjuvant treatment could potentially lower the effective dosage of colistin for *in vivo* MDR Gram-negative infections thus mitigating its toxicity.

To quantify this hypersensitivity, we determined the colistin MIC of all six parent strains (Table 1) in the presence of 30 μ M compounds **1**, **2**, or **3** (Table 5). We observed suppression of 64 to >2,048-fold against all strains, the greatest being a final colistin MIC of <0.49

ng/mL. We subsequently evaluated the MIC of each compound against all parent strains. Compound **1** returned MICs of 100 μ M against the *A. baumannii* strains, 200 μ M against the *E. coli* strains, and >200 μ M against the *K. pneumoniae* strains. Compound **2** returned MICs of 200 μ M against all strains, while compound **3** registered MICs of >200 μ M.

Finally, we quantified the ability of compounds **1**, **2**, and **3** to reduce colistin MICs in three MDR isolates: *A. baumannii* 5075,¹⁵ *A. baumannii* ATCC BAA1605, and *K. pneumoniae* ATCC BAA2146. Both *A. baumannii* strains are primary MDR clinical isolates, while the *K. pneumoniae* strain contains the New Delhi metallo-beta-lactamase gene *bla*_{NDM-1}.¹⁶ All three strains are colistin-susceptible returning MICs of 1 μ g/mL. Dose response studies were conducted on these strains to determine the minimum concentration of each adjuvant needed to lower the colistin MIC to 0.125 μ g/mL. These data are shown in Table 6. We first note successful resensitization in all strains with adjuvant concentrations as little as 5 μ M (2.9 μ g/mL **3**). Holistically, compound **3** is more potent than **1** or **2** while being non-toxic against all three strains at >200 μ M (116 μ g/mL). Compound **1** outperforms **2** with a lower effective dose across all strains, but is toxic against both *A. baumannii* isolates at 200 μ M (54 μ g/mL) where as **2** returned an MIC of >200 μ M (57 μ g/mL). Compounds **1**, **2**, and **3** all registered MICs of >200 μ M in *K. pneumoniae* 2146.

In conclusion, we report successful *in vitro* colistin resensitization in five engineered strains of *mcr-1*-carrying Gram-negative bacteria using compounds **1**, **2** and **3**. Compound **1** reverses lipid A modification in *A. baumannii* ATCC 17978^{+mcr-1}, while compound **3**, as previously reported for compounds **1** and **2**, sensitizes chromosomally encoded colistin-resistant strains to colistin's effects. We further demonstrate successful hypersensitization of colistin-susceptible strains *in vitro*, opening an avenue to explore adjuvant efficacy *in vivo* en route to a solution for colistin toxicity in treating MDR Gram-negative infections.

Comparing these three adjuvants, the increased activity of compound **1** may be due in part to its inherent toxicity, which is reflected in its lower MIC values against all strains studied. Compounds **2** and **3** appear to be essentially non-toxic to all strains interrogated as they return MICs of 200 μ M. Interestingly, we have found that neither compound **2** nor **3** abrogates lipid A modification in *E. coli* ATCC 25922^{+mcr-1}, indicating that there is an alternative mode of action for colistin repotentialization that excludes lipid A modification. Dual efforts are currently underway to elucidate alternative resensitization pathways and validate *in vivo* adjuvant/colistin combination therapy in MDR Gram-negative infection models.

Experimental

All antibiotics, culture media, and other reagents used in this study were obtained from the commercial sources denoted. Adjuvants were synthesized by previous methods as cited.

Minimum Inhibitory Concentration (MIC)

This experiment follows Clinical and Laboratory Standards Institute (CLSI) guidelines for determining MIC. Bacterial strains were cultured in duplicate aliquots of CAMHB (supplemented with 30 μ g/mL gentamicin sulfate (Sigma-Aldrich, St. Louis, MO) where appropriate) for 5 hours and then subcultured to 5×10^5 CFU in fresh CAMHB (Becton-

Dickson, Franklin Lakes, NJ). Separate aliquots of the subculture were collected and inoculated with one compound or colistin (Sigma-Aldrich, St. Louis, MO). Serial dilutions of each compound in 100 μ L volumes of subculture were performed on a 96-well microtiter plate and incubated stationary overnight. Upon visual inspection, the lowest dilution point with no colony growth was recorded as the MIC for each condition.

Resensitization MIC

Simultaneous bacterial challenge to adjuvant and antibiotic followed the previously described MIC procedure with one exception: Before colistin was added, the subculture was split into separate aliquots and each was treated with adjuvant at a given concentration. This afforded serial dilution of colistin in uniform 100 μ L volumes of subculture with a fixed concentration of adjuvant.

Lipid A Mass Spectrometry

Strains were cultured for 16 hours at 37°C with shaking in 3 mL volumes of CAMHB (supplemented with 30 μ g/mL gentamicin sulfate where appropriate) and then subcultured to 1×10^6 CFU in 160 mL aliquots of fresh CAMHB. Subcultures were split, inoculated with adjuvant, and incubated for 24 hours with shaking. After 8 hours, 80 mLs of each subculture was collected and centrifuged (4000 rpm, 5°C). Supernatants were discarded and cell pellets were frozen on dry ice. The remaining subcultures were collected at 24 hours and cell pellets were retrieved in the same manner. Cell pellets were thawed, resuspended in 400 μ L 70% isobutyric acid/1M ammonium hydroxide (5:3 [vol/vol]), and incubated at 100°C for 45 minutes. Samples were centrifuged at $2,000 \times g$ for 15 min and supernatants were added to endotoxin-free water (1:1 [vol/vol]), snap-frozen on dry ice, and lyophilized overnight. The resultant material was washed twice with 1 mL methanol. Lipid A was extracted with 100 μ L of chloroform/methanol/water mixture (3:1.5:0.25 [vol/vol/vol]). One μ L of extracted lipid A was spotted on a steel matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) plate followed by 1 μ L of 10 mg/mL norharmane matrix in chloroform-methanol (2:1, [vol/vol]) (Sigma-Aldrich, St. Louis, MO). Samples were air dried and then analyzed on a Bruker Microflex mass spectrometer (Bruker Daltonics, Billerica, MA) in negative ion mode. Mass calibration was achieved using an electrospray tuning mix (Agilent, Palo Alto, CA). All spectral data were analyzed with Bruker Daltonics FlexAnalysis software. Estimations of lipid A structures in each condition were made comparing Spectra with predicted structures and molecular weights of known species of lipid A. Structural heterogeneity of lipid A within a single bacterial membrane has been well-described.^{17, 18} Two unique sample sets of each bacterial strain and treatment condition were generated and analyzed independently.

Acknowledgments

The authors would like to thank the National Institutes of Health for support (R01 GM055769 to CM, R01 GM111066 to BE, and R01 AI104895 to YD).

Notes and References

1. Coates ARM, Halls G, Hu Y. Novel classes of antibiotics or more of the same? *Br J Pharmacol*. 2011; 163:184–194. [PubMed: 21323894]
2. Garonzik SM, Li J, Thamlikitkul V, Paterson DL, Shoham S, Jacob J, Silveira FP, Forrest A, Nation RL. Population pharmacokinetics of colistin methanesulfonate and formed colistin in critically ill patients from a multicenter study provide dosing suggestions for various categories of patients. *Antimicrob Agents Chemother*. 2011; 55:3284–3294. [PubMed: 21555763]
3. Poirel L, Jayol A, Nordmann P. Polymyxins: Antibacterial Activity, Susceptibility Testing, and Resistance Mechanisms Encoded by Plasmids or Chromosomes. *Clin Microbiol Rev*. 2017; 30:557–596. [PubMed: 28275006]
4. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH, Shen J. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis*. 2016; 16:161–168. [PubMed: 26603172]
5. Hu Y, Liu F, Lin IY, Gao GF, Zhu B. Dissemination of the *mcr-1* colistin resistance gene. *Lancet Infect Dis*. 2016; 16:146–147. [PubMed: 26711359]
6. Kline KE, Shover J, Kallen AJ, Lonsway DR, Watkins S, Miller JR. Investigation of First Identified *mcr-1* Gene in an Isolate from a U.S. Patient - Pennsylvania, 2016. *MMWR Morb Mortal Wkly Rep*. Jun.2016;977–978.
7. Xavier BB, Lammens C, Ruhel R, Kumar-Singh S, Butaye P, Goossens H, Malhotra-Kumar S. Identification of a novel plasmid-mediated colistin-resistance gene, *mcr-2*, in *Escherichia coli*, Belgium, June 2016. *Euro Surveill*. 2016; 21:30281.
8. Yin W, Li H, Shen Y, Liu Z, Wang S, Shen Z, Zhang R, Walsh TR, Shen J, Wang Y. Novel Plasmid-Mediated Colistin Resistance Gene *mcr-3* in *Escherichia coli*. *mBio*. 2017; 8:e00543–17. [PubMed: 28655818]
9. Chen L, Todd R, Kiehlbauch J, Walters M, Kallen A. Notes from the Field: Pan-Resistant New Delhi Metallo-Beta-Lactamase-Producing *Klebsiella pneumoniae* - Washoe County, Nevada, 2016. *MMWR Morb Mortal Wkly Rep*. 2017; 66:33. [PubMed: 28081065]
10. Liu YY, Chandler CE, Leung LM, McElheny CL, Mettus RT, Shanks RM, Liu JH, Goodlett DR, Ernst RK, Doi Y. Structural Modification of Lipopolysaccharide Conferred by *mcr-1* in Gram-Negative ESKAPE Pathogens. *Antimicrob Agents Chemother*. 2017; 61:e00580–17. [PubMed: 28373195]
11. Brackett CM, Furlani RE, Anderson RG, Krishnamurthy A, Melander RJ, Moskowitz SM, Ernst RK, Melander C. Second generation modifiers of colistin resistance show enhanced activity and lower inherent toxicity. *Tetrahedron*. 2016; 72:3549–3553. [PubMed: 27429479]
12. Harris TL, Worthington RJ, Melander C. Potent Small-Molecule Suppression of Oxacillin Resistance in Methicillin-Resistant *Staphylococcus aureus*. *Angew Chem, Int Ed*. 2012; 51:11254–11257.
13. Richards JJ, Ballard TE, Huigens RW III, Melander C. Synthesis and screening of an oroidin library against *Pseudomonas aeruginosa* biofilms. *ChemBioChem*. 2008; 9:1267–1279. [PubMed: 18431726]
14. Lim LM, Ly N, Anderson D, Yang JC, Macander L, Jarkowski A III, Forrest A, Bulitta JB, Tsuji BT. Resurgence of colistin: a review of resistance, toxicity, pharmacodynamics, and dosing. *Pharmacotherapy*. 2010; 30:1279–1291. [PubMed: 21114395]
15. Jacobs AC, Thompson MG, Black CC, Kessler JL, Clark LP, McQueary CN, Gancz HY, Corey BW, Moon JK, Si Y, Owen MT, Hallock JD, Kwak YI, Summers A, Li CZ, Rasko DA, Penwell WF, Honnold CL, Wise MC, Waterman PE, Lesho EP, Stewart RL, Actis LA, Palys TJ, Craft DW, Zurawski DV. AB5075, a Highly Virulent Isolate of *Acinetobacter baumannii*, as a Model Strain for the Evaluation of Pathogenesis and Antimicrobial Treatments. *MBio*. 2014; 5:e01076–14. [PubMed: 24865555]
16. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, Chaudhary U, Doumith M, Giske CG, Irfan S, Krishnan P, Kumar AV, Maharjan S, Mushtaq S, Noorie T,

Paterson DL, Pearson A, Perry C, Pike R, Rao B, Ray U, Sarma JB, Sharma M, Sheridan E, Thirunarayan MA, Turton J, Upadhyay S, Warner M, Welfare W, Livermore DM, Woodford N. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect Dis.* 2010; 10:597–602. [PubMed: 20705517]

17. Dixon DR, Darveau RP. Lipopolysaccharide heterogeneity: Innate host responses to bacterial modification of lipid A structure. *J Dent Res.* 2005; 84:584–595. [PubMed: 15972584]
18. Shaffer SA, Harvey MD, Goodlett DR, Ernst RK. Structural Heterogeneity and Environmentally Regulated Remodeling of *Francisella tularensis* subspecies *novicida* Lipid A Characterized by Tandem Mass Spectrometry. *J Am Soc Mass Spectrom.* 2007; 18:1080–1092. [PubMed: 17446084]

Figure 1.
Previously disclosed colistin adjuvants.

Figure 2. MS analysis of *A. baumannii* ATCC 17978^{+mcr-1} treated with compound **2**. PEtN-modified species shown in red. All spectra were acquired in negative ion mode.

Figure 3.
Compounds **3** and **4**.

Table 1 Colistin MICs ($\mu\text{g/mL}$) in *mcr-1*-carrying strains and their corresponding parent.

	Parent	Parent ^{+mcr-1}
<i>A. baumannii</i> ATCC 17978	2	16
<i>A. baumannii</i> SM1536	2	64
<i>E. coli</i> ATCC 25922	1	8
<i>E. coli</i> YD626	1	4
<i>K. pneumoniae</i> 2210291	1	16

Table 2 Colistin MICs, given as $\mu\text{g/mL}$ (fold-reduction), of *mcr-I* strains treated with **1** and **2**.

	30 μM 1 (8.1 $\mu\text{g/mL}$)	15 μM 1 (4.05 $\mu\text{g/mL}$)	30 μM 2 (8.6 $\mu\text{g/mL}$)	15 μM 2 (4.3 $\mu\text{g/mL}$)
<i>A. baumannii</i> ATCC 17978 ^{+mcr-I}	0.5 (32)	4 (4)	0.5 (32)	4 (4)
<i>A. baumannii</i> SM1536 ^{+mcr-I}	0.25 (256)	8 (8)	1 (64)	8 (4)
<i>E. coli</i> ATCC 25922 ^{+mcr-I}	$<3.9 \times 10^{-3}$ (>2,048)	4 (2)	0.016 (512)	8 (0)
<i>E. coli</i> YD626 ^{+mcr-I}	$<1.9 \times 10^{-3}$ (>2,048)	0.5 (8)	0.016 (256)	2 (2)
<i>K. pneumoniae</i> 2210291 ^{+mcr-I}	0.125 (128)	4 (4)	0.5 (32)	8 (2)

Table 3 Colistin MICs, given as $\mu\text{g/mL}$ (fold-reduction), of *mcr-I* strains treated with compound **3**.

	60 μM 3 (34.4 $\mu\text{g/mL}$)	30 μM 3 (17.2 $\mu\text{g/mL}$)	15 μM 3 (8.6 $\mu\text{g/mL}$)
<i>A. baumannii</i> ATCC 17978 ^{+mcr-I}	0.25 (64)	0.5 (32)	2 (8)
<i>A. baumannii</i> SM1536 ^{+mcr-I}	1 (64)	2 (32)	8 (8)
<i>E. coli</i> ATCC 25922 ^{+mcr-I}	0.031 (256)	0.25 (32)	0.5 (16)
<i>E. coli</i> YD626 ^{+mcr-I}	$<2.0 \times 10^{-3}$ (>2,048)	$<2.0 \times 10^{-3}$ (>2,048)	0.063 (64)
<i>K. pneumoniae</i> 2210291 ^{+mcr-I}	0.125 (128)	0.25 (64)	1 (16)

Table 4 Colistin MICs, given as $\mu\text{g/mL}$ (fold-reduction), of colistin resistant clinical isolates treated with **3**.

	Untreated	60 μM 3 (34.4 $\mu\text{g/mL}$)	30 μM 3 (17.2 $\mu\text{g/mL}$)	15 μM 3 (8.6 $\mu\text{g/mL}$)
<i>A. baumannii</i> PM3941	512	8 (64)	16 (32)	32 (16)
<i>A. baumannii</i> PM3942	512	4 (128)	16 (32)	32 (16)
<i>A. baumannii</i> PM4112	1,024	8 (128)	16 (64)	32 (32)
<i>A. baumannii</i> PM4119	1,024	2 (512)	4 (256)	16 (64)
<i>K. pneumoniae</i> B9	512	4 (128)	8 (64)	32 (16)
<i>K. pneumoniae</i> C3	512	8 (64)	16 (32)	64 (8)
<i>K. pneumoniae</i> C5	128	2 (64)	4 (32)	32 (4)

Table 5 Colistin MICs, given as $\mu\text{g/mL}$ (fold-reduction), of MDR isolates treated with 30 μM **1**, **2** and **3**.

	Compound 1	Compound 2	Compound 3
<i>A. baumannii</i> ATCC 17978	0.0039 (512)	0.016 (128)	$<9.8 \times 10^{-4}$ ($>2,048$)
<i>A. baumannii</i> SM1536	0.0078 (256)	0.016 (128)	$<9.8 \times 10^{-4}$ ($>2,048$)
<i>E. coli</i> ATCC 25922	9.8×10^{-4} (2,048)	0.0039 (256)	$<4.9 \times 10^{-4}$ ($>2,048$)
<i>E. coli</i> YD626	4.9×10^{-4} (2,048)	4.9×10^{-4} (2,048)	$<4.9 \times 10^{-4}$ ($>2,048$)
<i>K. pneumoniae</i> 2210291	0.031 (32)	0.031 (64)	0.016 (64)

Table 6

Adjuvant concentration necessary to achieve colistin MICs of 0.125 µg/mL in MDR strains.

	Compound 1	Compound 2	Compound 3
<i>A. baumannii</i> 5075	15 µM (4.05 µg/mL)	20 µM (5.7 µg/mL)	10 µM (5.8 µg/mL)
<i>A. baumannii</i> BAA1605	15 µM (4.05 µg/mL)	25 µM (7.2 µg/mL)	5 µM (2.9 µg/mL)
<i>K. pneumoniae</i> BAA2146	15 µM (4.05 µg/mL)	20 µM (5.7 µg/mL)	10 µM (5.8 µg/mL)