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Second generation modifiers of colistin resistance show enhanced activity and lower inherent toxicity

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ABSTRACT

We recently reported a 2-aminoimidazole-based antibiotic adjuvant that reverses colistin resistance in two species of Gram-negative bacteria. Mechanistic studies in *Acinetobacter baumannii* demonstrated that this compound downregulated the PmrAB two-component system and abolished a lipid A modification that is required for colistin resistance. We now report the synthesis and evaluation of two separate libraries of substituted 2-aminoimidazole analogues based on this parent compound. From these libraries, a new small molecule was identified that lowers the minimum inhibitory concentration of colistin by up to 32-fold greater than the parent compound while also displaying less inherent bacterial toxicity, thereby minimizing the likelihood of resistance evolution.

1. Introduction

Antibiotic resistant organisms represent a major threat to global health. The Center for Disease Control and Prevention (CDC) estimates that two million people acquire antibiotic-resistant infections each year, of which 23,000 are fatal.[1] The main culprits behind these infections are referred to as the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species).[2] The severity of the problem of multi-drug resistant (MDR) bacteria has been significantly exacerbated by the fact that there have only been two new classes of antibiotics introduced to the clinic in the past two decades, daptomycin and linezolid.[3] More concerning, these two classes of antibiotics are exclusively active towards Gram-positive bacteria, which leaves four of the ESKAPE pathogens untreated. As the well of clinically relevant antibiotics runs dry, the polymyxin antibiotic colistin has become a last line of defense against MDR Gram-negative infections.[4]

Unfortunately the frequency of colistin resistant strains of Gram-negative bacteria that have been observed in the clinic has increased as reliance upon colistin therapy has escalated.[5] The mechanistic basis of colistin resistance is thought to occur predominantly through modification of lipid A[6]; however, the two-component system (TCS) signaling that drives these modifications has recently been shown to activate additional mechanisms that are also required for resistance.[7] Our group has been focused on combating the inevitable development of antibiotic resistant bacteria by developing compounds capable of disrupting the mechanisms through which these organisms express resistance.[8–12] We recently established that the 2-aminoimidazole (2-AI) compound **1** is capable of reversing colistin resistance in multiple primary clinical isolates of two of the four Gram-negative ESKAPE pathogens: *K. pneumoniae*, and *A. baumannii* (Fig. 1).[11] Against several strains of both bacteria, the minimum inhibitory concentration (MIC) of colistin was lowered from 512 to ≤4 (in some cases as low as 0.25) μg/mL in the presence of 30 μM (8.4 μg/mL) **1**. Mechanistic studies revealed that treatment of colistin-resistant *A. baumannii* with **1** led to downregulation of the PmrAB two-component system while mass spectrometry demonstrated reversal of the phosphoethanolamine modification of lipid A responsible for colistin resistance in *A. baumannii*.

Despite this unprecedented activity, we noted that compound **1** itself harbored some inherent toxicity to the bacteria in the absence of colistin. Given that this toxicity may lead to an

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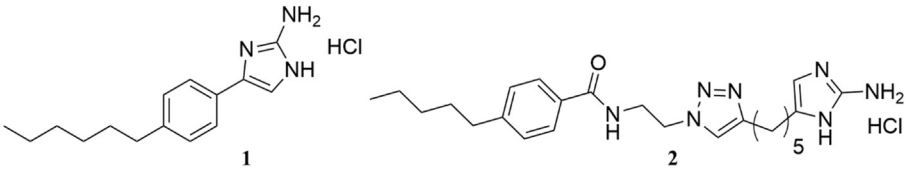


Fig. 1. Structures of compound **1** and **2**.

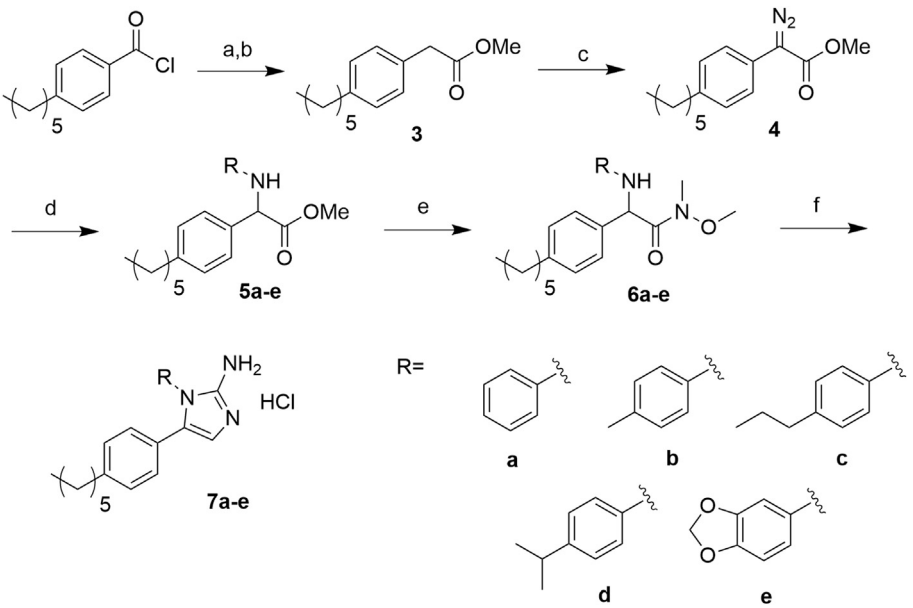
accelerated rate of resistance evolution, we wondered whether we could augment the activity of **1** while decreasing inherent toxicity through analogue synthesis. In this regard, our group has also recently developed several 2-AIs based upon compound **2** (Fig. 1) that are capable of reversing β-lactam resistance in methicillin-resistant *S. aureus* (MRSA).[10,13,14] In these studies, we were able to modify adjuvant activity through imprinting either a 1,4- or 1,5-substitution pattern on the 2-AI ring. Specifically, compound **2** was able to lower the MIC of oxacillin against MRSA four-fold at 25 μM, while from the library of 1,5 substituted derivatives of compound **2**, a compound emerged that is capable of lowering the MIC of oxacillin against MRSA up to 512-fold at 5 μM.[10] Inspired by these results, we set out to determine whether imparting either a 1,4- or 1,5-substitution pattern upon the 2-AI of **1** would deliver compounds with augmented activity and reduced inherent toxicity. Herein we report the synthesis of both 1,5- and 1,4-substituted analogues of **1**, as well as the evaluation of their biological activity in terms of colistin resistance suppression. Moreover, we report a compound capable of lowering the MIC of colistin against resistant strains of both *A. baumannii* and *P. aeruginosa* to a greater degree than compound **1**.

2. Results and discussion

2.1. Synthesis and biological evaluation of 1,5 2-AIs

As we had the most success in our previous MRSA studies with the 1,5-substitution pattern where the introduced appendage was an aromatic substituent, we chose to initially evaluate a pilot library of five aryl-1,5-substituted 2-AIs that were synthesized according to Scheme 1. Briefly, commercially available 4-hexylbenzoyl chloride was reacted with diazomethane, and the resulting diazoketone was subjected to standard Arndt–Eistert conditions (silver benzoate in methanol).[15] The homologated ester, **3** underwent diazotransfer reaction, accomplished by reaction with *para*-acetamidobenzenesulfonyl azide (*p*-ABSA) in the presence of DBU, to yield diazoketone **4**.[16] Analog diversity was then introduced via a Ru-catalyzed N–H insertion reaction.[17] Conversion of the ester to the *N*-methoxy-*N*-methylamide (Weinreb amide) proceeded without the need for protection of the newly installed amine. Finally, reduction of the Weinreb amide to the corresponding aldehyde using diisobutylaluminum hydride (DIBAL-H), followed by cyclization with cyanamide afforded the 1,5-2AI derivatives **7a**–**e**.[10]

Our pilot library of 1,5 2-AIs was evaluated for the ability to break resistance to colistin against the colistin-resistant strains of *A. baumannii* that we employed in our previous study.[11] These strains, obtained from the Walter Reed Army Institute of Research (WRAIR), have colistin MICs significantly higher (512–1024 μg/mL) than the Clinical and Laboratory Standards Institute (CLSI) defined threshold for resistance for *A. baumannii* (≥4 μg/mL).[18] As is common practice for evaluating adjuvant activity of our 2-AIs, we first established the intrinsic antibiotic activity of our library alone. Whereas the parent compound **1** has an MIC of 100 μM against all strains, all members of our library had MICs of ≥200 μM. We then determined the MIC of colistin against two strains of *A. baumannii* in the presence of 30 and 60 μM of each compound (Table 1). Surprisingly, the 1,5 substitution pattern essentially eradicated activity against *A. baumannii* in the context of colistin resensitization. At 30 μM compounds **7a**–**e** only reduced the colistin MIC four-fold, from 512 to 128 μg/mL, whereas the parent compound at the same concentration was able to lower the MIC to 4 μg/mL.



Scheme 1. Synthesis of 1,5-substituted 2-aminoimidazoles. a) i. CH_2N_2, rt, 1 h. ii. AcOH, rt, 1 h. b) AgOBz, Et_3N, MeOH, rt, 16 h. c) *p*-ABSA, DBU, MeCN, rt, 16 h. d) $[Ru(p\text{-cymene})Cl_2]_2$, R–$NH_2$, CH_2Cl_2, rt, 1 h. e) $HN(OCH_3)CH_3$, i-PrMgCl, THF, −40 °C, 8 h. f) i. DIBAL-H, THF −78 °C, 1 h. ii. EtOH/H_2O, pH 4.3, H_2NCN, 95 °C, 2 h. iii. MeOH/HCl.

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