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Discovery and Initial Structure-Activity Relationships of *N*-Benzyl Tricyclic Indolines as Antibacterials for Methicillin-Resistant *Staphylococcus aureus*

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most prevalent drug resistant bacteria. In 2012, over 11,000 fatalities in the United States were directly attributable to MRSA. In an effort to develop novel structural and mechanistic classes of antibacterial agents to fight against MRSA, we have optimized a hit compound, Of4, previously discovered in a screening campaign of a bio-inspired polycyclic indoline library previously developed in our lab. We took advantage of our concise and versatile synthetic strategy to conduct initial structure-activity relationship studies of Of4, and we now report the discovery of compound **4k** as a more potent antibacterial agent against *S. aureus*. **4k** also displayed equivalent activity in four MRSA and a methicillin-susceptible strains while demonstrating an improved mammalian cytotoxicity profile compared to Of4. Interestingly, **4k** shares the same tricyclic indoline core as Of1, a β -lactam-selective resistance-modifying agent, but harbors a distinct modification pattern conferring unique bioactivity. This phenomenon is reminiscent of many bioactive natural products.

Graphical abstract

Development of antibiotic resistance in pathogenic microorganisms is an ongoing public health threat.¹⁻⁴ In 2012, *Staphylococcus aureus* was a leading cause of hospital-acquired infections in the United States; and methicillin-resistant *S. aureus* (MRSA) alone caused 80,461 infections leading to 11,285 fatalities.⁵ Recently, *S. aureus* strains resistant to last-line-of-defense antibacterial agents have emerged.⁶⁻⁸ To exacerbate the issue, research in the

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field of antibiotic-resistant microorganisms has seen a marked decline.⁹ To keep pace with emerging drug resistance, unified and multidisciplinary strategies must be implemented. Various approaches can be undertaken in order to better control the emergence and spread of drug-resistant pathogenic microorganisms. Firstly, prudent use of current antibacterial agents in healthcare and agriculture will better maintain the current treatment options.¹⁰ Secondly, increased research on novel approaches such as anti-virulence and resistance modification not only will provide better understanding of how microbes become resistant to our known antibacterials, but also may yield novel solutions such as adjuvant therapy.¹¹⁻¹³ And finally, the discovery of novel antibacterials must go on to further expand treatment options.^{14, 15}

Since the introduction of penicillin in the 1940's, the majority of antibacterials in clinical use have been natural products or derivatives of natural products.^{16, 17} More recently, high throughput screening efforts utilizing vast drug-like compound libraries have achieved minimal success.¹⁸ In light of these discovery programs, we developed a parallel strategy that employs whole cell screening of structurally diverse compound libraries inspired by natural products. Our pilot library consists of 120 polycyclic indolines with 26 unique core structures (Figure 1). Previous screening efforts led to the discovery of a tricyclic indoline, Of1, which does not have anti-proliferative activity against *S. aureus*, but selectively re-sensitizes MRSA to β -lactams (Figure 2A).¹⁹

Herein we report an *N*-benzyl tricyclic indoline, Of4, as a novel antibacterial agent. Of4 was discovered from a whole-cell antibacterial counter screen of our bio-inspired polycyclic indoline library in our previous report of Of1.¹⁹ The library was tested for antibacterial activity in methicillin-susceptible *S. aureus* (MSSA) ATCC 25923 and methicillin-resistant *S. aureus* (MRSA) ATCC BAA-44. Of4 showed moderate anti-proliferative activity against both MSSA and MRSA with minimum inhibitory concentrations (MICs) of 32 μ g/mL in the standard broth micro dilution assay.²⁰ Interestingly, the core structure of Of4 has the same carbocyclic architecture as Of1 (Figure 2A). However, Of4 does not potentiate β -lactam antibacterials in MRSA strains. This phenomenon is reminiscent of many classes of bioactive natural products, in particular isonitrile indole alkaloids isolated from cyanobacteria: hapalindole G and ambiguine A isonitrile (Figure 2B). These natural products share identical carbocyclic architecture and stereochemistry; however, hapalindole G differs from ambiguine A isonitrile by an alkyl group at C2-position of indole. Intriguingly, hapalindole G selectively inhibits the growth of *Mycobacterium tuberculosis*, while ambiguine A isonitrile is selective for *S. aureus*.^{21, 22} The difference in modifications of the tricyclic core of Of1 and Of4 is not as subtle as in isonitrile indole alkaloids; however, the isonitrile indole alkaloids only differ in selectivity of antibacterial activity while Of1 and Of4 have distinctly different activity in *S. aureus*. Namely, Of1 re-sensitizes MRSA to β -lactams without being anti-proliferative alone and Of4 acts directly on *S. aureus* as an antibacterial. The modification of a common core structure offers a biomimetic method to generate diverse bioactivity of synthetic antibacterials. In the context of this work, we explored the structure-activity relationship (SAR) of Of4 through different modification reactions to the common tricyclic indoline core.

Analogues **4a-k** were synthesized using our standard synthetic protocols (Figure 3).^{19, 23-25} In brief, alkynyl imines **1a-b** were used to prepare indoles **2a-d** using our modified one-pot

three-component indole synthesis conditions. Imines were activated by trifluoroacetic anhydride (TFAA) or benzyl chloroformate (CbzCl), in the presence of 4-dimethylaminopyridine (DMAP). The resulting activated imine was then treated with phenylhydrazine or 4-chlorophenylhydrazine hydrochloride and methanesulfonic acid (MsOH) followed by heating for 12 hours to give alkynyl indoles **2a-d**. Indoles **2a-d** were treated with 5 mol% of [bis(trifluoromethanesulfonyl)imidate](triphenylphosphine)gold(I) (2:1) toluene adduct to afford tetracyclic indolines **3a-d**.²⁶ One-pot sequential reductive-ring-opening-reductive-amination reactions with substituted benzaldehydes (e.g., 3, 5-dihydroxybenzaldehyde) in the presence of acetic acid and sodium cyanoborohydride provided *N*-benzyl tricyclic indolines **4a-i** and **4k**. In addition, the trifluoroacetyl (TFA) group was cleaved under basic conditions to give primary amine analogue **4j**.

The MICs of all compounds were tested in the standard broth microdilution assays, and results are summarized in Table 1. Compound **4a** demonstrated the importance of indoline chlorine substitution on antibacterial activity. Elimination of the chlorine from Of4 resulted in a 4-fold loss in activity (entry 2, Table 1).²⁰ We hypothesized that hydrogen bonding held a prominent role for the 3,5-dihydroxybenzyl group in Of4 and synthesized compounds **4b-d** (entries 3-5, Table 1). However, these compounds did not display antibacterial activity within the range of the experiment. Furthermore, compound **4e** containing a trihydroxybenzyl group also had a comparable loss of activity as **4b-d**. Interestingly, different dihydroxybenzyl substitution patterns such as *ortho*, *meta*-substitution **4f**, and *ortho*, *para*-substitution **4g** (entries 7 and 8, Table 1), had equivalent activity to Of4. We suspect that the ability of the benzyl component to adopt different conformations could allow different phenol substitution patterns to retain activity.

Reducing the side chain length of Of4 by one methylene gave analogue **4h** (entry 9, Table 1), which displayed a 2-fold increase in activity over Of4. However, when substituting a more acidic hydrogen bond donor to the benzyl appendage, **4i** (entry 10, Table 1), activity was decreased in a similar manner as analogues **4b-e**. To investigate the role of the TFA group on the side chain, we removed TFA under basic conditions to reveal the primary amine, **4j** (entry 11, Table 1), which resulted in 2-fold loss of activity compared with Of4. Replacing TFA with benzyloxycarbonyl (Cbz) and reducing the side chain by one methylene gave **4k** (entry 12, Table 1), which showed an 8-fold increase in activity. Furthermore, **4k** showed a half growth inhibition concentration (GI₅₀) of 8 µg/mL in human cervical adenocarcinoma (HeLa) cells, 2 fold higher than its MIC (Supporting Information Figure 1).²⁰ This is an improved toxicity profile compared to Of4, whose GI₅₀ in HeLa cells is 3 fold greater than its MICs in MSSA and MRSA (Supporting Information Figure 2).²⁰

With a more potent analogue in hand, we next explored the scope of antibacterial activity in four different MRSA strains (Table 2). These strains were selected because of their diverse geographical origin, genetic background and resistance profiles. MRSA BAA-44 was originally isolated from a hospital in Portugal.²⁷ MRSA ATCC 33592 is a hospital-acquired clone from the United States.²⁸ ATCC BAA-1720 (a.k.a. MRSA 252) is a hospital-acquired strain from the United Kingdom.²⁹ NRS 100 (a.k.a. COL) is a community-acquired MRSA strain from the United Kingdom.³⁰ MRSA 252 is genetically diverse compared to other sequenced MRSA strains, particularly NRS 100. The most noteworthy aspect of these strains

is their known resistance to a wide range of clinically used antibacterials of different structural and mechanistic classes, such as: β -lactams (e.g., amoxicillin/clavulanic acid, cephalothin, and imipenem), aminoglycosides (e.g. gentamicin), tetracycline, macrolides (e.g. erythromycin), rifampicins (e.g. rifampin), quinolones (e.g. ciprofloxacin), and clindamycin. Despite these resistance profiles, analogue **4k** has the same MICs (i.e., 4 μ g/mL) for all four MRSA strains tested.

In summary, we have successfully improved the antibacterial activity and mammalian toxicity profile of a hit previously identified by a whole-cell antibacterial screen of our bio-inspired polycyclic indoline library. These *N*-benzyl tricyclic indolines are a novel class of anti-MRSA agents. Our versatile synthetic route allowed the efficient optimization of screening hit Of4 to give a more potent analogue, **4k**. This analogue has the same tricyclic indoline core structure as the β -lactam re-sensitizing agent Of1. From this study we found that both *N*-modification of the indoline core (i.e., dihydroxybenzyl) and side chain carbamate (i.e., Cbz) are crucial for its antibacterial activity in *S. aureus*. However, our previous SAR studies of Of1 showed that either modification of the tricyclic indoline core or side chain nitrogen abolished the resistance-modifying activity of Of1. This is reminiscent of many bioactive natural products with common carbocyclic architecture; and suggests that modifications of a common structural core can selectively alter biological function in tricyclic indolines. Further studies on the mode of action for *N*-benzyl tricyclic indoline antibacterial agents and more detailed SAR studies are ongoing and will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
A bio-inspired synthetic strategy of a polycyclic indoline library.

Figure 2.

A) Structures of antibacterial hit Of4 and β -lactam re-sensitizing agent Of1. Of1 does not have antibacterial activity.; **B)** Antibacterial indole alkaloid natural products hapalindole G and ambigua A.

Figure 3.

Synthesis of Of4 and analogues **4a-k**.

Reagents and conditions: a) TFAA or CbzCl, DMAP, 2 hr, 0-25 °C, then MsOH, 0-25 °C, then *p*-ClPhNHNH₂·HCl or PhNHNH₂, 80 or 120 °C, 12 hr, DMF, 20-30%; b) 5 mol% [bis(trifluoromethanesulfonyl)imide](triphenylphosphine)gold(I) (2:1) toluene adduct, 2-12 hr, 50°C, toluene; c) substituted benzaldehyde, NaCNBH₃, AcOH, 2-12 hr, 0-25 °C, MeOH, 30-70 % for two steps; d) Of4, LiOH·H₂O, 24 hr, 25 °C, MeOH, 99%.

Table 1

Structures and MIC values of Of4 analogues against MSSA.

Entry	Compound	Z ¹	Z ²	R ¹	R ²	R ³	R ⁴	Y	MIC ^{a,b}
1	Of4	TFA ^c	Cl	OH	H	OH	H	(CH ₂) ₂	32
2	4a	TFA ^c	H	OH	H	OH	H	(CH ₂) ₂	>128
3	4b	TFA ^c	Cl	OMe	H	OMe	H	(CH ₂) ₂	>128
4	4c	TFA ^c	Cl	F	H	F	H	(CH ₂) ₂	>128
5	4d	TFA ^c	Cl	H	H	H	H	(CH ₂) ₂	>128
6	4e	TFA ^c	Cl	OH	OH	OH	H	(CH ₂) ₂	>128
7	4f	TFA ^c	Cl	H	H	OH	OH	(CH ₂) ₂	32
8	4g	TFA ^c	Cl	H	OH	H	OH	(CH ₂) ₂	32
9	4h	TFA ^c	Cl	OH	H	OH	H	CH ₂	16
10	4i	TFA ^c	Cl	H	COOH	H	OH	CH ₂	>128
11	4j	H	Cl	OH	H	OH	H	(CH ₂) ₂	64
12	4k	Cbz ^d	Cl	OH	H	OH	H	CH ₂	4

^a All MIC values are reported in µg/mL;^b MSSA strain ATCC 25923;^c trifluoroacetyl;^d benzyloxycarbonyl

Table 2

Evaluation of the MIC values of **4k** in a panel of MRSA strains and MSSA.^a

Antibacterial	ATCC BAA-44 ^b	ATCC 33592 ^c	ATCC BAA-1720 ^d	NRS 100 ^e	MSSA ^f
4k	4	4	4	4	4
Of4	32	N.T. ^g	N.T. ^g	N.T. ^g	32
methicillin	>128	128	>128	>128	2

^a All MIC values are reported in µg/mL; 20

^b An Iberian clone resistant to methicillin, imipenem, cephalothin, erythromycin, ciprofloxacin, doxycycline, clindamycin, rifampin;

^c A hospital-acquired strain isolated in the United States clone resistant to methicillin and gentamicin;

^d A hospital-acquired strain, isolated in the United Kingdom, a.k.a. MRSA 252;

^e A community-acquired strain isolated in the United Kingdom, resistant to methicillin, a.k.a. COL;

^f MSSA strain ATCC 25923;

^g Not tested.