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3-Hydroxy-1,5-dihydro-2*H*-pyrrol-2-ones as novel antibacterial scaffolds against methicillin-resistant *Staphylococcus aureus*

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Abstract

Herein, we report the synthesis and evaluation of 3-hydroxy-1,5-dihydro-2*H*-pyrrol-2-ones as antibacterial agents against methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant *S. epidermidis* (MRSE). Lead compound **38** showed minimum inhibitory concentrations (MICs) of 8 and 2 $\mu\text{g/mL}$ against MRSA and MRSE, respectively. Furthermore, compound **38** displayed a MIC of 8–16 $\mu\text{g/mL}$ against linezolid-resistant MRSA. These molecules, previously underexplored as antibacterial agents, serve as a new scaffold for antimicrobial development.

Graphical abstract

Keywords

Natural products; Antibiotics; Heterocycles; Cyclization; Medicinal chemistry

Arising from the acquisition of staphylococcal cassette chromosome *mec* (SCC*mec*) by *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA) displays an altered penicillin-binding protein (PBP2a) that renders β -lactam antibiotics (BLAs) ineffective as therapeutic agents.¹ As such, MRSA infections can be difficult to treat, and in some cases, life-threatening. While several classes of antibiotics have been developed to combat such infections, including oxazolidinones (linezolid)², resistance is becoming an increasing issue in the clinic. Therefore, the development of novel antibiotic scaffolds is crucial to our ability to effectively treat MRSA infections and natural products and their analogs are fruitful starting points for these explorations.³

Isolated in 1995 and 2005 from the marine fungus *Chromocleista* sp., phenopyrrozin⁴ (**1**) and *p*-hydroxyphenopyrrozin⁵ (**2**) are marine natural products containing the 2-hydroxy-5,6,7,7a-tetrahydro-3*H*-pyrrolizin-3-one core. While the isolation group reported no activity of **1** or **2** against *S. aureus* at tested concentrations⁴, a modest minimum inhibitory concentration (MIC) of 50–100 µg/mL was reported against the Gram-positive pathogen *Bacillus cereus*.⁶ Prompted by this initial report, as well as our recent synthesis of the natural products and a series of novel analogs (Scheme 1),^{7a–d} we aimed to explore the development of the scaffold as antibacterial agents against methicillin-resistant *S. aureus* and other Gram-positive pathogens.

Starting from the natural products **1** and **2**, we explored functionalization of the aryl side chain while maintaining the [3.3.0] bicyclic core (Figure 1). Building off of our previously reported cyclization/condensation reaction between cyclic imines and α-oxoesters, a library of phenopyrrozin analogs, along with the natural products, was prepared.^{7a} A variety of substitution patterns were investigated, including electron rich aryl (**3**), electron poor aryl (**4** – **6**), heteroaromatic (**7**), alkyl (**8**), and allyl (**9**) substituted derivatives. Initial antimicrobial testing of this series revealed a MIC of 128 µg/mL for compounds **4** and **5** against MRSA (ATCC 33591), while all other analogs showed no activity at the maximum tested concentration (128 µg/mL) (Table 1). Intrigued by the initial hits, several other analogs were prepared to further develop a structure-activity relationship (SAR). To explore whether the origin of the activity of **4** and **5** arises from the electron withdrawing nature of the substituents, or the steric bulk they impart, compounds **10** and **11** were synthesized (Figure 1). With a 4-fluorophenyl substituent, compound **10** electronically resembles **4** and **5**, yet is an isostere of the inactive natural product **1**. Compound **11**, with a 4-methylphenyl substituent, was designed as an electron-rich isostere of **4**. The 4-chlorophenyl analog **12** was also synthesized to complete the series of 4-haloaryl analogs. Compounds **10** and **12** showed no activity at tested concentrations, while **11** maintained the activity of **4** and **5** with MICs of 128 µg/mL.

We next aimed to investigate effects of varying the ring size of the core. Starting with a six-membered cyclic imine, a similar approach was employed to synthesize analogs with the [4.3.0] bicyclic core with varying substituents, including aryl, heteroaryl and electronically-varied aryl derivatives (Figure 2). A general trend of increased activity was observed (Table 1). Compounds **15** and **17**, the six-membered ring analogs of **4** and **5**, showed a 4× increase in activity with MICs of 32 µg/mL. Interestingly, the 2-thienyl substituted analog (**14**) was the most potent with a MIC of 16 µg/mL. The ring-expanded version of phenopyrrozin (**13**) remained inactive. Due to the metabolic liability of a thiophene, the 4-(trifluoromethyl)phenyl side chain of **15** was employed in further optimization studies.

At this stage we investigated the role of the enolic proton in the activity of the scaffold by converting **15** to vinyl methyl ether **21** (Figure 3). Treatment of **15** with K₂CO₃ and iodomethane in acetone yielded the desired compound **21** with only *O*-methylation observed. Interestingly, **21** displayed no activity at tested concentrations. As such, we postulate the ability to hydrogen bond or to tautomerize between the enolketo- form is critical to the general activity of the scaffold.

Given the importance of ring size in the structure-activity relationship, we next aimed to explore the synthesis of a further ring expanded analog. To achieve the synthesis of the [5.4.0] bicyclic motif with our established methods, a seven-membered imine would be required. The instability of the imine, however, requires an *in-situ* generation, followed by trapping with the corresponding α -oxoester. To accomplish this, hexamethyleneimine was treated with NCS in Et₂O to yield the corresponding *N*-chloroamine, which was then subjected to KOH in ethanol to generate the seven-membered cyclic imine.⁸ The resulting imine was subsequently trapped with an α -oxoester to provide **22** (Scheme 2). **22** had comparable activity to that of the six-membered analog **15**, with a MIC of 32 μ g/mL (Table 1).

The substitution on the six-membered ring of **15** was next explored. The respective *p*-methyl six-membered cyclic imine was prepared and yielded **23** as a 4:1 mixture of diastereomers (Figure 3). As the diastereomers were inseparable by column chromatography, the 4:1 mixture was tested against MRSA to provide a MIC of 128 μ g/mL (Table 1).

In parallel to exploring various ring sizes of cyclic imine substrates, we continued our investigations to include the monocyclic 3-hydroxy-1,5-dihydro-2H-pyrrol-2-one scaffold. Analogous to the preparation of the bicyclic compounds, 3-hydroxy-pyrrol-2-ones were accessed via the condensation of acyclic imines with α -oxoesters. Our recent advances in the synthesis of such motifs allow for their facile preparation in a one-pot multicomponent reaction (Scheme 1b).^{7b}

Employing this approach, methylamine, propanal, and the corresponding α -oxoester yielded **24** (Figure 3). While **24** presented only modest activity, with a MIC of 64 μ g/mL (Table 1), the scaffold provides a novel avenue for optimization in which both side chains may be independently varied. As such, an initial screening of substituents on nitrogen was carried out. Analogs **25**, **26** and **27**, with hydrogen, benzyl, and *n*-hexyl substituents, respectively, were prepared (Figure 4). Compound **25** revealed that an unsubstituted nitrogen results in a MIC of 128 μ g/mL, maintaining only one half the potency of **24**. Analogs **26** and **27**, however, proved to be significantly more active, with MICs of 8 and 2 μ g/mL. Given the substantial difference in the chemical nature of the *n*-hexyl and benzyl substituents, we opted to continue optimization of each analog independently.

Starting from **26**, we sought to vary the electronic nature of the benzyl substituent by installing the electron rich 2,4-dimethoxybenzyl group (**28**), along with the electron poor 4-(trifluoromethyl)phenyl group (**29**); however, both analogs were 4 $\times$ less active than **26** (MICs = 32 μ g/mL). Subsequently, compounds **30** and **31** were synthesized from 4-(aminomethyl)pyridine and 4-phenylbenzyl amine, respectively. Neither compound maintained the activity of **26**. Both **30** and **31** presented MICs of 64 μ g/mL (Table 1). Additionally, we explored methyl substitution of the α carbon on the benzyl group with compound **32**. The multi-component reaction of α -methylbenzyl amine, propanal, and the corresponding α -oxoester yielded a 2:1 mixture of inseparable diastereomers. The compound was tested as a mixture, resulting in a MIC of 32 μ g/mL.

Compound **33** was synthesized employing 4-aminobutanol as the amine component in the previously described multi-component reaction. The 1-(4-hydroxybutyl) substituent on nitrogen maintains the nature of the medium length alkyl chain of **27**, while introducing a polar, H-bonding group in the terminal position. This modification to the chain resulted in complete loss of activity (MIC >128 µg/mL). A shorter, branched isobutyl substituent resulted in **34** being eight times less active than **27**, with MIC = 16 µg/mL. Finally, analogs **35** and **36**, with allyl and propargyl groups, displayed MICs of 32 µg/mL (Table 1).

Optimization was continued by exchanging the *n*-hexyl chain of **27**, for a cyclohexyl substituent (**37**). Moderate activity was observed for **37** (MIC = 8 µg/mL), albeit still reduced with respect to **27**. Building off of **37**, compound **38**, with a phenyl group on nitrogen, was then prepared from aniline. The unsaturated phenyl ring yielded similar activity to the fully saturated cyclohexyl group, with a MIC of 8 µg/mL.

We then opted to explore variation of the 5- position of the 3-hydroxy-pyrrol-2-one (Figure 5). As such, three additional analogs were prepared from benzaldehyde, hydrocinnamaldehyde, and valeraldehyde to yield compounds **39**, **40**, and **41**. None of the analogs with the further functionalized 5- position were comparable in activity to **27**, displaying MICs of 32–64 µg/mL (Table 1).

Lead compounds **26**, **27**, **37**, and **38** were then tested against a panel of MRSA and methicillin-resistant *S. epidermidis* (MRSE) strains (Table 2). While analog **27** was the most potent against the ATCC 33591 (MRSA) strain in our initial screen, the activity varied greatly by strain. MICs of 64 µg/mL were observed against BAA-44, BAA-1685, and ATCC43300. Only NR 45904 yielded a similar MIC value to that of ATCC 33591 at 4 µg/mL. Poor potency of **27** was also observed against linezolid-resistant MRSA (NR 45924, NR 45926) with MICs ranging from 32 to 64 µg/mL; however, **27** remained active against MRSE (ATCC 51625, NR 46386) displaying MIC values of 4 to 8 µg/mL. Interestingly, compounds **37** and **38** displayed more consistent MIC values against the library of strains. Analog **38** yielded MICs of 8 µg/mL across several strains of MRSA, including linezolid-resistant MRSA. Similar values were recorded for MRSE, with MICs ranging from 4 to 8 µg/mL. Compounds **37** and **38** were, however, significantly less active against ATCC 43300 (MRSA) with MICs of 64 µg/mL.

In conclusion, we have reported the synthesis and biological evaluation of a series of 2-hydroxy-5,6,7,8a-tetrahydro-3*H*-pyrrolizin-3-ones, 2-hydroxy-6,7,8,8a-tetrahydroindolizin-3(5*H*)-ones, and 3-hydroxy-1,5-dihydro-2*H*-pyrrol-2-ones as antimicrobial agents for Gram-positive MRSA and MRSE pathogens. From determining a SAR through molecular optimization, our resulting lead compound **38** presented MICs of 8 and 2 µg/mL against MRSA and MRSE, respectively. Furthermore, **38** displayed MICs ranging from 8 to 16 µg/mL against linezolid-resistant MRSA, representing an interesting new scaffold for further optimization. Future efforts are focused on studying the mechanism of action and improving the potency of these compounds to clinically-relevant levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Synthesis of 2-hydroxy-5,6,7,7a-tetrahydro-3H-pyrrolizin-3-one core analogs.

Figure 2.
Synthesis of 2-hydroxy-6,7,8,8a-tetrahydroindolizin-3(5*H*)-one core analogs.

Figure 3.
Expanded SAR via structural modifications to compound 15.

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Figure 4.

Synthesis of 3-hydroxy-1,5-dihydro-1*H*-pyrrol-2-one analogs via a multi-component reaction with various amines.

Figure 5.
Variation of the 5-position of the 3-hydroxy-1,5-dihydro-2H-pyrrol-2-one core.

Scheme 1.

Our previously reported approaches to 2-hydroxy-5,6,7,7a-tetrahydro-3*H*-pyrrolizin-3-ones (a) and 3-hydroxy-1,5-dihydro-2*H*-pyrrol-2-one scaffolds (b).

Scheme 2.

In-situ generation and trapping of seven-membered cyclic imine to **22**.

Table 1

MIC values of compounds against ATCC 33591 (MRSA).

Compound	MIC (µg/mL)	Compound	MIC (µg/mL)
1	>128	22	32
2	>128	23	128
3	>128	24	64
4	128	25	128
5	128	26	8
6	>128	27	2
7	>128	28	32
8	>128	29	32
9	>128	30	64
10	>128	31	64
11	128	32	32
12	>128	33	>128
13	>128	34	16
14	16	35	32
15	32	36	32
16	>128	37	8
17	32	38	8
18	>128	39	32
19	>128	40	64
20	>128	41	32
21	>128	Linezolid	1

Table 2

MIC values (µg/mL) against various MRSA and MRSE strains.

Strain/Compound	26	27	37	38
<i>S. aureus</i> ATCC 33591 ^a	8	2	8	8
<i>S. aureus</i> NR 45898 ^a	16	32	8	8
<i>S. aureus</i> NR 45913 ^a	16	32	8	8
<i>S. aureus</i> NR 45924 ^b	32	64	32	16
<i>S. aureus</i> NR 45926 ^b	16	32	32	8
<i>S. aureus</i> NR 45906 ^a	8	4	2	8
<i>S. aureus</i> BAA-44 ^a	64	64	32	32
<i>S. aureus</i> BAA-1685 ^a	32	64	32	32
<i>S. aureus</i> ATCC 43300 ^a	32	64	64	64
<i>S. epidermidis</i> NR 46386 ^c	8	8	2	4
<i>S. epidermidis</i> ATCC 51625 ^c	8	4	16	8

^aMRSA.^bLinezolid resistant MRSA.^cMRSE. Box indicates lead compound.