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# Expanded Structure−Activity Studies of Lipoxazolidinone Antibiotics

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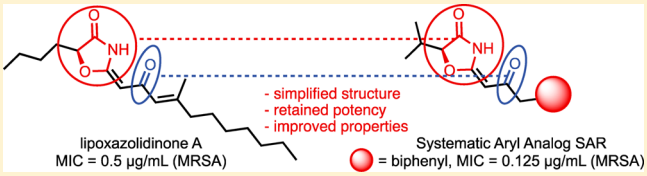
Supporting Information

**ABSTRACT:** The lipoxazolidinone family of marine natural products, which contains an unusual 4-oxazolidinone core, was found to possess potent antimicrobial activity against methicillin resistant *Staphylococcus aureus* (MRSA). Herein, we expanded our previous synthetic efforts by preparing selected aryl derivatives of the lipoxazolidinones and further evaluating the potential to expand the activity of this class of molecules to Gram-negative pathogens. With these analogs, we explored the effect of varying the substitution pattern around the aromatic ring, increasing the chain length between the oxazolidinone core and the aryl system, and how altering the position of more polar functional groups affected the antimicrobial activity. Finally, we utilized a TolC knockout strain of *E. coli* to demonstrate that our compounds are subject to efflux in Gram-negative pathogens, and activity is restored in these knockouts. Together these results provide additional data for the further development of 4-oxazolidinone analogs **5**, **20**, and **21** for the treatment of infectious disease.




**KEYWORDS:** *Oxazolidinones, antibiotics, MRSA, heterocycles*



Multidrug resistant (MDR) bacteria pose a significant threat to human and animal health, and there is a critical need for antibiotics with activity against MDR strains.[1] The marine environment provides a plethora of novel compounds with unique chemical scaffolds and biological activities, which can serve as valuable starting points for antibiotic development.[2−5] Previously, our group developed the first total synthesis of lipoxazolidinone A (**1**), a 4-oxazolidinone containing antimicrobial natural product isolated from marine sediment off the coast of Guam (Figure 1A).[6,7] The lipoxazolidinone family of natural products contains an unusual 4-oxazolidinone moiety at its core, and this heterocycle is only found in two other families of natural products, the synoxazolidinones, which have demonstrated antibiofouling activity, and 2,2-dimethyl-2-(4-hydroxyphenyl)-4-oxazolidinone.[8−13] Both the lipoxazolidinones and synoxazolidinones are structurally related to the 2-oxazolidinone antibiotic linezolid, and its derivatives yet possess quite distinct three-dimensional structures (Figure 1B). Linezolid was FDA approved in 2000 for the treatment of methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Enterococcus* (VRE), two multidrug resistant organisms of high clinical relevance.[14] Like all antibiotics, growing resistance threatens the long-term longevity of these compounds in the clinic.[15]

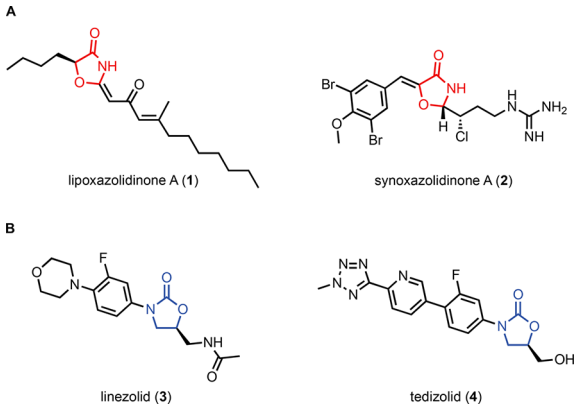



**Figure 1.** (A) Biologically active 4-oxazolidinones. (B) Clinically relevant 2-oxazolidinones.

Previously, our group has synthesized analogs of the lipoxazolidinones that are potent against methicillin susceptible *S. aureus* (MSSA) and MRSA; however, these analogs have potential liabilities, including high lipophilicity.[16,17] Since little was known regarding the SAR of the right-hand domain of the lipoxazolidinones, we drew inspiration from one of the analogs that we previously synthesized (**6**, Figure 2), which had similar antimicrobial activity against MSSA and MRSA to that of the natural product, and a cLogP of 3.84, compared to a cLogP of 6.66 for **1** and 5.26 for simplified analog **5** (Figure 2).[16]

Inspired to further explore this initial result, the goal of this study was 2-fold: first, we planned to synthesize a panel of aryl derivatives aiming to maintain high levels of antimicrobial activity while exploring the effect of aryl substitution patterns; second, we sought to vary chain lengths and install more polar

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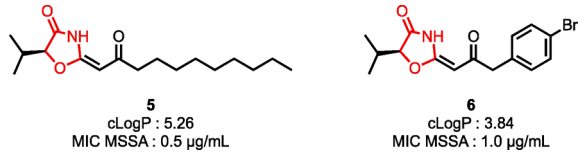


**Figure 2.** Comparison of the cLogP and MIC values for previously synthesized 4-oxazolidinone analogs.

functionality around the aromatic ring to further elucidate the structure−activity relationship for these aryl derivatives and the oxazolidinones more broadly.

Utilizing a synthetic approach that was previously developed and optimized to a one-pot procedure by our group, we were able to synthesize a panel of 26 oxazolidinone analogs (Figure 3).[9] In short, the TBS-protected α-hydroxyamides were

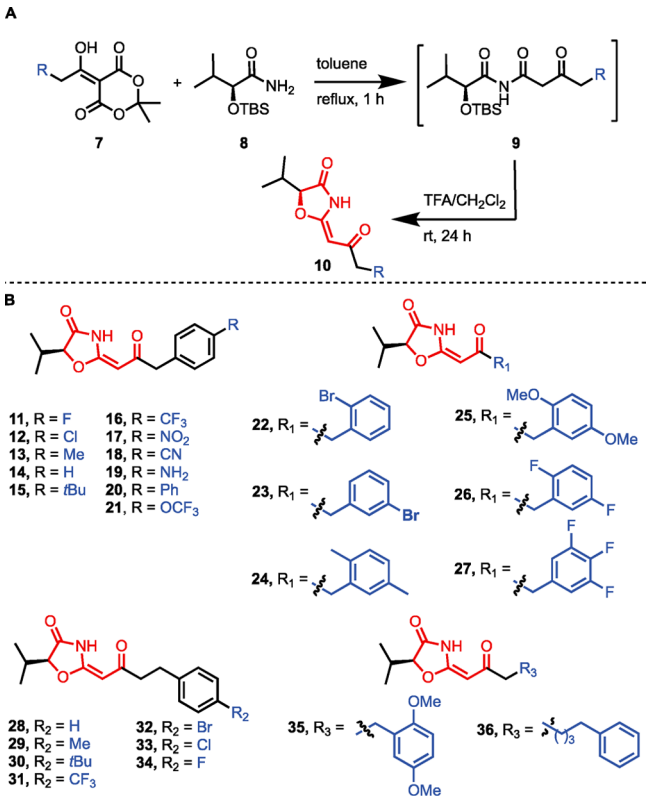


**Figure 3.** (A) One-pot strategy to synthesize 4-oxazolidinones. (B) Novel aryl analogs of lipoxazolidinone A.

synthesized via known procedures and then heated to reflux with acylated Meldrum's acid derivatives in toluene for 1 h. After removal of the solvent the mixture was resuspended in dichloromethane, and trifluoroacetic acid was added to induce cyclization/dehydration over 24 h. Through this procedure an array of electron-rich and electron-poor aryl derivatives were prepared in moderate to good yields (see the Supporting Information for full experimental protocols and characterization data/spectra).

The lipoxazolidinone derivatives were then tested in MIC assays against strains of MSSA and MRSA to compare their activity (Table 1). In addition, select analogs were tested against *A. baumannii* to determine if they possessed any activity against this Gram-negative pathogen. All of the analogs tested were found to have good to moderate activity against MSSA and MRSA. Analogs with weak electron-withdrawing (**11**, **12**) or weak electron-donating groups (**13**−**15**, **20**) at the 4-position were found to have the most potent antimicrobial activity against MSSA and MRSA, while analogs with strong electron-withdrawing (**17**) or strong electron-donating substituents (**19**) at the 4-position had significantly less activity. Relocation of the electron-withdrawing substituent to the 2- or 3-position (**22**, **23**) or incorporating di- and trisubstitution (**24**−**27**) around the aryl ring also resulted in a decrease in activity. Similarly, lengthening the chain by an additional methylene resulted in analogs following a similar antimicrobial trend, with those having electron-withdrawing substituents (**31**−**33**) possessing an increase in antimicrobial activity and disubstitution (**35**) resulting in a decrease in activity. One of the most promising analogs was biphenyl compound **20** which possessed potent activity against MSSA and MRSA.

**Table 1. Initial Evaluation of Antimicrobial Activity of Aryl Lipoxazolidinone Analogs**[a]

| compd | MSSA[b] | MRSA[c] | *A. baumannii*[d] |
|---|---|---|---|
| **6** | 1 | 0.5 | 64 |
| **11** | 4 | 2 | nt |
| **12** | 2 | 0.5 | 64 |
| **13** | 4 | 2 | 128 |
| **14** | 4 | 2 | nt |
| **15** | 4 | 2 | nt |
| **16** | 4 | 2 | 128 |
| **17** | 64 | 8 | nt |
| **18** | 8 | 4 | nt |
| **19** | >128 | 64 | nt |
| **20** | 0.25 | 0.125 | >128 |
| **21** | 2 | 0.5 | nt |
| **22** | 16 | 8 | nt |
| **23** | 16 | 2 | nt |
| **24** | 16 | 8 | nt |
| **25** | 16 | 4 | nt |
| **26** | 16 | 8 | nt |
| **27** | 8 | 4 | nt |
| **28** | 8 | 4 | 128 |
| **29** | 16 | 8 | nt |
| **30** | 2 | 0.25 | nt |
| **31** | 2 | 0.5 | nt |
| **32** | 2 | 1 | 128 |
| **33** | 2 | 0.5 | 128 |
| **34** | 16 | 8 | nt |
| **35** | 4 | 8 | >128 |
| **36** | 4 | 2 | 128 |
| linezolid | 1 | 0.5 | 64 |

[a]nt = not tested. All MIC values in μg/mL. [b]ATCC 29213. [c]ATCC 33591. [d]ATCC 19606.

Additional derivatives with extended alkyl chains between the exocyclic ketone and the aryl ring (**28**, **36**) were also synthesized to determine if the length of the alkyl chain would affect the antimicrobial activity. With these compounds in hand it was demonstrated that extending the alkyl chain by two to four methylene units did not result in a significant decrease in antimicrobial activity.

A few of the analogs displayed modest activity against *A. baumannii*, suggesting that the structures could be modified to further increase activity against Gram-negative organisms. Specifically, the bromo- and chloro-substituted derivatives (**6** and **12**, respectively) had MICs of 64 μg/mL against *A. baumannii*, demonstrating that modifications could potentially


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be made to further increase activity against additional bacteria. To further explore the potential for Gram-negative bacteria to be subject to the 4-oxazolidinone antibiotics, we employed the use of a series of *E. coli* knockouts to evaluate the role of efflux and influx on the activity of our lead compounds (Table 2).[18−20] These experiments revealed that the knockout of the

**Table 2. Initial Evaluation of Antimicrobial Activity of Lead Oxazolidinones against *E. coli* Mutants[a]s**

| compd | SQ110 | SQ110 DTC | SQ110 LPTD |
|---|---|---|---|
| **5** | >128 | 1−2 | 32 |
| **14** | >128 | 2 | 32 |
| **20** | >128 | 0.25 | 32 |
| **21** | >128 | 0.06 | 32 |
| linezolid | >128 | 2 | 32 |

[a]All MIC values in μg/mL.

TolC efflux mechanism sensitized the *E. coli* to **5**, **14**, **20**, and **21**, suggesting that efflux, and not simply influx, is likely responsible for the diminished activity in Gram-negative organisms.[21] We are optimistic that further chemical modification of the antibiotic scaffold can further overcome these efflux mechanisms.[22−24]

Finally, we evaluated the toxicity of compounds **5** and **21** in red blood cell hemolysis assays and also A549 cell toxicity assays. We observed <1% hemolysis at up to 40 μM concentrations for both **5** and **21**. In the mammalian cellular toxicity assay, compound **5** was shown to have $IC_{50}$ against A549 cells of 10 μg/mL, and for **21**, an $IC_{50}$ of 14 μg/mL (see Table S1 in the Supporting Information for details) after an extended 72 h exposure. These compounds are classified as mildly toxic, and current studies are underway to further increase the therapeutic window of these oxazolidinones in the next phase of optimization.

In summary, we have utilized a previously developed synthetic route to access aryl-substituted 4-oxazolidinones. These new analogs allowed us to further probe the structure−activity relationship of the right-hand side of the lip-oxazolidinones, as well as indicating whether an increase in chain length between the core pharmacophore and the aryl system affected biological activity. Further, we have gained additional insight into the mechanism of resistance in Gram-negative organisms. Taken together, these results in conjunction with ongoing mechanism of action studies will be utilized to further drive structure−activity relationship studies toward analogs with additional heteroatoms and/or heterocycles in hopes of expanding biological activity to additional high-priority pathogens.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.9b00015.

Experimental procedures and analytical data ($^1$H and $^{13}$C NMR) for all new compounds and bioassay procedures (PDF)

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### Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.


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### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS

MDR, multidrug resistant; MIC, minimum inhibitory concentration; MRSA, methicillin resistant *Staphylococcus aureus*; MSSA, methicillin susceptible *Staphylococcus aureus*

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