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Marine sponge alkaloids as a source of anti-bacterial adjuvants

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Abstract

Novel approaches that do not rely upon developing microbicidal compounds are sorely needed to combat multidrug resistant (MDR) bacteria. The potential of marine secondary metabolites to serve as a source of non-traditional anti-bacterial agents is demonstrated by showing that pyrrole-imidazole alkaloids inhibit biofilm formation and suppress antibiotic resistance.

Keywords

Marine sponge; Pyrrole-imidazole alkaloid; Biofilm; Antibiotic; Adjuvant

More than two million people are infected by multidrug resistant (MDR) bacterial pathogens each year in the United States, resulting in more than 23,000 deaths.¹ There is therefore an urgent need for new therapies to combat these infections. The traditional approach to identifying new antibacterial therapies is to screen for bacteriostatic or bactericidal compounds; however bacteria invariably develop resistance to such compounds, sometimes as early as one year after clinical deployment as in the case of daptomycin.^{2,3} In the face of rapid resistance acquisition and the dire state of our antibiotic pipeline (especially with respect to Gram-negative bacteria), our lab and others have been exploring the development of alternative approaches to combating MDR bacterial infections. One such approach is to identify compounds that target pathways responsible for resistance to clinically used antibiotics. Pathways include those involved in genotypic drug resistance, such as production of antibiotic modifying enzymes or efflux pumps, or those involved in phenotypic drug resistance such as biofilm formation, which can increase resistance upwards of 1000-fold.⁴ Such compounds should restore or enhance antibiotic activity and have the potential to preserve or revitalize established antibiotics that have shown outstanding clinical efficacy.^{5–7}

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmcl.2016.11.018>.

Marine invertebrates have long been a rich source of bioactive compounds with diverse applications.⁸ The sessile nature of marine sponges has necessitated the evolution of chemical defense systems to protect themselves from predation. This defense mechanism relies on the production of secondary metabolites that either kill or repel the predator, and means that sponges are one of the richest sources of chemicals in the marine environment.^{9,10} Numerous biological activities have been documented for compounds isolated from marine sponges including anticancer, antiinflammatory, antiviral, antibacterial, and anticoagulant activities.⁸

Many structurally diverse marine sponge secondary metabolites have been shown to exhibit antibiotic activities. For example, the sesterterpenes manoalide, secomanoalide, and (*E*)- and (*Z*)-neomanoalide from the sponge *Luffariella variabilis*, exhibit antibiotic activity against several Gram-positive bacteria including *Streptomyces pyogenes*, *Staphylococcus aureus* and *Bacillus subtilis*.¹¹ The bromotyrosine-derived natural product psammaphin A, from the sponges *Poecillastra* sp. and *Jaspis* sp.,¹² and the motualevic acids, unusual halogenated glyceryl-lipid conjugates,¹³ from the sponge *Siliquaria* sp. all inhibit *S. aureus* growth. However all of these natural products are inactive against Gram-negative bacteria.

Taking inspiration from another group of marine natural products, the pyrrole-imidazole alkaloids, the Melander lab has focused on studying how functionalized 2-aminoimidazole (2-AI) compounds affect bacterial behavior, first investigating their anti-biofilm properties and more recently their ability to suppress antibiotic resistance. These compounds were modeled upon the complex natural product bromoageliferin (compound **4**, Fig. 1), one member of the large family of pyrrole-imidazole alkaloids that are exclusively isolated from marine sponges.¹⁴ The most abundant members of this family are oroidin **1** and sceptrin **2**.

Oroidin **1**, which is a key intermediate in the biosynthesis of many of the more complex pyrrole-imidazole alkaloids, was first isolated from *Agelas oroides* in 1971,¹⁵ and subsequently isolated from several other sponges.¹⁶ The dimeric sceptrin was first identified from *Agelas sceptrum* in 1981, and was subsequently isolated from *Agelas conifera* along with its brominated analogue dibromosceptrin **3**, and related ageliferin compounds including bromoageliferin **4**.¹⁷ Sceptrin was also isolated from an unidentified Micronesian sponge in 1985 along with the tricyclic pyrrole-imidazole alkaloid stevensine **5**.¹⁸ Most recently, elegant total syntheses of these complex natural products have been completed by the Baran and Chen groups.^{19–21}

Oroidin **1** and bromoageliferin **4** were originally reported to inhibit the growth of *Rhodospirillum salexigens* SCRC 113, a marine bacterium known to form biofilms.²² Following this observation, oroidin and bromoageliferin have been employed as templates for the design of numerous analogues that have been comprehensively studied for anti-biofilm activity.^{23–37} During these studies it was discovered that a subset of these synthetic derivatives suppress resistance of multiple antibiotic classes across a broad-spectrum of medically important bacteria.^{38–44} Although the approach of leveraging this scaffold to modulate pathogenic bacterial behavior has been successful in the context of anti-biofilm activity and antibiotic potentiation, with the exception of oroidin **1** (which exhibits modest anti-biofilm activity against laboratory strains of *Pseudomonas aeruginosa* with IC₅₀ values

of 190 and 166 μM against strains PAO1 and PA14 respectively),²⁶ the anti-biofilm activities of the more complex natural products themselves (bromoageliferin and the sceptrins) has not been established against commonly encountered human pathogens. Furthermore, to our knowledge, no study exists that establishes the antibiotic potentiation activity of any members of this family. This latter point is particularly relevant as it questions whether the parent natural products themselves have antibiotic potentiation activity, and, if so, suggests new directions for screening marine natural product extracts. Herein, we report that the pyrrole-imidazole alkaloids both inhibit biofilm formation and suppress antibiotic resistance against two representative human pathogens: the Gram-negative *Acinetobacter baumannii* and the commonly encountered Gram-positive methicillin-resistant *S. aureus* (MRSA), demonstrating that marine sponge alkaloids can potentially serve as a highly useful source of scaffolds for non-traditional anti-bacterial approaches.

We initiated this study by assembling representative monomeric and dimeric pyrrole-imidazole alkaloids. Oroidin **1** and stevensine **5** were purchased from Enzo Life Sciences and Santa Cruz Biotechnology respectively, while sceptrin **2**, dibromosceptrin **3**, and bromoageliferin **4** were isolated from an *Agleas* sp. collected in Palau (Supporting information). All compounds were first examined for their ability to inhibit both MRSA and *A. baumannii* biofilm formation (Table 1).

As with *P. aeruginosa*, oroidin exhibited modest activity against both strains (29% inhibition against *A. baumannii* at 100 μM , and an IC_{50} against MRSA of 77.3 μM), while the constrained analogue stevensine was essentially inactive. The dimeric alkaloids were universally more active than either monomer. Sceptrin **2** was slightly more active against *A. baumannii*, while both dibromosceptrin **3** and bromoageliferin **4** were more active against MRSA (Fig. 2). Bromoageliferin was the most potent compound studied, returning an IC_{50} value of 10.6 μM against MRSA.

The natural products were next tested for their ability to lower the minimum inhibitory concentrations (MICs) of β -lactam antibiotics against the same two bacterial species (Tables 2 and 3). The non-MDR 19606 strain was replaced with the *A. baumannii* strain AB5075,⁴⁵ which is an exceptionally virulent, MDR primary clinical isolate. Alkaloids **1**, **2** and **5** did not significantly lower (0–2-fold) MICs of meropenem against AB5075; however bromoageliferin **4** lowered the MIC by fourfold from 32 $\mu\text{g/mL}$ to 8 $\mu\text{g/mL}$ at a concentration of 10 μM . Interestingly, dibromosceptrin **3**, which was only moderately active in the biofilm inhibition assay, was equipotent to bromoageliferin, reducing the meropenem MIC to 8 $\mu\text{g/mL}$.

Next we evaluated the antibiotic repotential activity of each alkaloid against the USA-300 FPR3757 MRSA strain ATCC BAA-1556. This strain was chosen because it is the strain from which the NARSA (Network on Antimicrobial Resistance of *S. aureus*) strain library was built, allowing us to probe potential mechanistic pathways. Unlike *A. baumannii*, several alkaloids exhibited MIC lowering activity (Table 3). Oroidin lowered the MIC of oxacillin from 32 $\mu\text{g/mL}$ to 8 $\mu\text{g/mL}$ at 25 μM and to 2 $\mu\text{g/mL}$ and 0.5 $\mu\text{g/mL}$ at 40 μM and 50 μM , respectively. Dibromosceptrin and bromoageliferin also suppressed resistance, both

lowering the MIC to 2 µg/mL at a concentration of 10 µM, while dibromosceptrin retained activity at just 5 µM, lowering the MIC to 8 µg/mL.

Previously we reported that the activity of a synthetic oroidin analogue that suppresses oxacillin resistance in MRSA is dependent on the *VraRS* two-component system (TCS).³⁹ *VraRS* dependence was determined by evaluating compound activity against strains from the NARSA transposon mutant library and looking for abrogation of MIC-lowering activity. In contrast to the synthetic analogue, oroidin potentiated oxacillin activity in the *vraR* mutant equally to the wild type strain, lowering the MIC by fourfold from 8 µg/mL to 2 µg/mL at 25 µM. This indicated that *VraRS* does not play the same role in the activity of oroidin as for the synthetic analogues.

In our earlier study, we identified strain NE481 that also exhibited a lower oxacillin MIC than the wild type. NE481 contains a disruption in the SAUSA300_0645 gene, which has since been reported to encode for the response regulator *GraR*.⁴⁶ The *GraRS* TCS has been reported to play a role in resistance to glycopeptide antibiotics and cationic antimicrobial peptides,^{47,48} and the reduced oxacillin MIC observed suggests that the *GraR* TCS may also play a role in β-lactam resistance in MRSA. Unlike for the *vraR* mutant, oroidin did not elicit the same magnitude of MIC-reduction against NE481 as against the wild type, indicating that the *GraRS* TCS may be involved in the mechanism of action; however further experiments are necessary to fully elucidate this dependence.

Finally, to determine whether resistance suppression activity was limited to β-lactam antibiotics, we investigated the ability of oroidin **1** to suppress glycopeptide resistance in the vancomycinintermediate *S. aureus* (VISA) strain ATCC 700699 (Table 4). Oroidin showed moderate suppression of vancomycin resistance in this strain, lowering the MIC from 8 µg/mL to 2 µg/mL at 50 µM, and to 4 µg/mL at 25 µM.

In conclusion, we have established that both the monomeric and dimeric pyrrole-imidazole alkaloids inhibit phenotypic and genotypic bacterial resistance mechanisms. To our knowledge, this is the first demonstration that these alkaloids suppress genotypic resistance and the first report of suppression of vancomycin resistance by any 2-AI-based adjuvant. As marine secondary metabolites encompass a vast, diverse source of bioactive scaffolds, and screening these natural products for antibiotic potentiation is rarely done, high throughput screening with combinations of antibiotics and marine natural products may identify compounds that can be used as adjuvants to restore the efficacy of FDA approved antibiotics and deliver desperately needed alternative approaches to combating MDR pathogenic bacteria.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
Structures of the pyrrole-imidazole alkaloids used in this study.

Fig. 2.
Inhibition of MRSA BAA-1685 biofilm formation by dibromosceptrin **3** (blue) and bromoageliferin **4** (red).

Table 1IC₅₀ values for biofilm inhibition.

Compound	<i>A. baumannii</i> 19606	MRSA BAA-1685
Oroidin 1	>100 (100 μ M, 29%) ^a	77.3 $\pm$ 6.9
Sceptrin 2	30.5 $\pm$ 0.13	37.6 $\pm$ 0.71
Dibromosceptrin 3	>50 (50 μ M, 48%)	15.3 $\pm$ 4.5
Bromoageliferin 4	28.3 $\pm$ 0.98	10.6 $\pm$ 1.8

^aPercent inhibition at designated concentration.

Table 2

Suppression of meropenem resistance in AB5075.

Compound	Concentration (μM)	Meropenem MIC ($\mu\text{g/mL}$)
No compound	–	32
Dibromosceptrin 3	10	8
Bromoageliferin 4	10	8

Table 3

Suppression of oxacillin resistance in MRSA BAA-1556.

Compound	Concentration (μM)	Oxacillin MIC ($\mu\text{g/mL}$)
No compound	–	32
Oroidin 1	50	0.5
	40	2
	25	8
Dibromosceptrin 3	10	2
	5	8
Bromoageliferin 4	10	2
	5	16

Table 4

Suppression of antibiotic resistance by oroidin.

Antibiotic	MIC ($\mu\text{g/mL}$)			
	Oroidin Conc. (μM)	MRSA BAA-1556	MRSA NE481	VISA 700699
Oxacillin	–	32	1	<i>n.t.</i> ^a
	25	8	0.5	<i>n.t.</i>
Vancomycin	–	1	0.5	8
	50	<i>n.t.</i>	<i>n.t.</i>	2
	25	<i>n.t.</i>	<i>n.t.</i>	4

^a
n.t. = not tested.