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Effects of *N*-pyrrole substitution on the anti-biofilm activities of oroidin derivatives against *Acinetobacter baumannii*

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ABSTRACT

Bacteria of the genus *Acinetobacter* spp. are rapidly emerging as problematic pathogens in healthcare settings. This is exacerbated by the bacteria's ability to form robust biofilms. Marine natural products incorporating a 2-aminoimidazole (2-AI) motif, namely from the oroidin class of marine alkaloids, have served as a unique scaffold for developing molecules that have the ability to inhibit and disperse bacterial biofilms. Herein we present the anti-biofilm activity of a small library of second generation oroidin analogues against the bacterium *Acinetobacter baumannii*.

A bacterial biofilm is defined as a highly organized community of bacteria whose growth is generally thought of as a developmental process.[1–3] Industrially, biofilms are responsible for the microbe mediated corrosion of structures on land and underwater.[4–6] In the agricultural sector, biofilms are known to decimate crops and necessitate the use of toxic biocides as control measures.[7,8] Medically, it is estimated that upwards of 80% of infections in the human body are biofilm mediated.[9,10] Given the ubiquity of biofilms, recently there has been an intense effort to further understand their developmental processes in hopes of alleviating some of the problems they underpin.

One particular pathogen that has emerged as a threat to the medical community is the aerobic Gram-negative coccobacilli *Acinetobacter baumannii*. This bacterium, along with *Staphylococcus aureus* and *Pseudomonas aeruginosa*, consistently ranks among the highest in nosocomial infections.[11] Approximately 25% of all hospital swabs are positive for *A. baumannii* and recent outbreaks have occurred in both the US and Europe's healthcare system.[12–15] *A. baumannii* is known to form robust biofilms that allow the bacteria to survive for weeks on inanimate surfaces, making complete disinfection of hospital and medical instruments difficult.[16,17] Morbidity of patients suffering from *A. baumannii* infections has, in part, been correlated to the virulence associated with the formation of biofilms. More specifically, it has recently been disclosed that morbidity may arise from the virulence associated with outer membrane protein A (AbOmpA), the bacteria's most abundant surface protein, which has been shown to have the ability to induce apoptosis of epithelial cells through mitochondrial targeting.[18] Reports of anti-biotic resistance to carbapenems[19] and polymyxins[20] are becoming more frequent. It is believed that this is attributed to changes in porin proteins, development of efflux pumps, and production of β-lactamases in the bacteria.[19,21] Biofilms easily facilitate these adaptations for survival through the selection and reproduction of bacteria that can withstand harsh environmental pressures. Clearly, there lies a necessity to develop new therapies for *A. baumannii* remediation efforts.

Work in our group has focused on harnessing the innate biological activity of sponge-derived marine natural product analogues whose parent architectures belong to the oroidin class of marine alkaloids (Fig. 1).[22–24] These nitrogen dense molecules contain a 2-aminoimidazole (2-AI) subunit and provide the fundamental

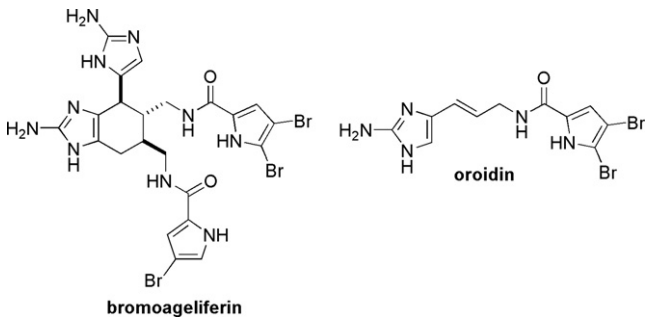

Figure 1. Molecular inspiration for anti-biofilm derivatives.

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basis for our research in hopes of discovering novel small molecules that inhibit and disperse medically relevant bacterial biofilms across order, class, and phylum.

Recently, we disclosed the synthesis and biological evaluation of a 50-compound analogue library based on the oroidin template.[25] An extensive structure–activity relationship (SAR) study was performed by systematically varying different regions of the template to tune biological potency within the context of anti-biofilm activity against the γ-proteobacterium *P. aeruginosa*. The most active member of the library, dihydrosventrin (DHS), was subsequently assayed for activity against other proteobacteria. DHS became the first non-toxic small molecule known to inhibit biofilm formation in a mucoid variant of *P. aeruginosa* while also crossing bacterial class to inhibit and disperse biofilms in the β-proteobacterium *Bordetella bronchiseptica*.[22] Additionally, DHS was shown to exhibit an IC_{50} value of 110 μM for inhibition of *A. baumannii* biofilms.

The SAR studies culminated with several observations about what features of the molecule were necessary for anti-biofilm activity. First, the 2-AI motif should remain intact. Second, the optimum chain length between the 2-AI head and pyrrole tail was three carbons and unsaturation was not necessary for activity. Third, methylation of the pyrrole ring nitrogen led to increased activity in conjunction with dibromination of the ring. To this end, we elected to synthesize an additional library of oroidin derivatives that focused on varying the pendant group located on the pyrrole nitrogen in hopes of further increasing anti-biofilm activity in relation to DHS (Fig. 2). It was then decided that *A. baumannii* would serve as the target bacterial strain for which the newly generated compounds would be assayed against for anti-biofilm activity.

Briefly, the Clausen-Kaas variant of the Paal-Knorr pyrrole synthesis was employed to synthesize six sterically and electronically different pyrroles (Scheme 1).[26] Installation of the trichloroacetyl ester group at the 2-position followed by dibromination of the ring with excess molecular bromine afforded the requisite pyrrole fragments for construction of the desired library members.[27,28] The final step employed in the synthesis was amide bond formation between the functionalized pyrroles and the 4-(3-aminopropyl)-2-aminoimidazole scaffold.[29] While varying the alkylation on the pyrrole ring was of primary concern, we also sought to delineate the importance of the pyrrole moiety overall in eliciting a biological response. A single atom replacement in the 3′-position on the pyrrole ring was envisioned to afford the imidazole based isostere **6**. This molecule could then be directly compared to the activity of DHS. Access to this derivative was achieved by utilizing identical chemistry as used in the pyrrole functionalization. All final targets were then converted into their corresponding hydrochloride salts before biological testing.

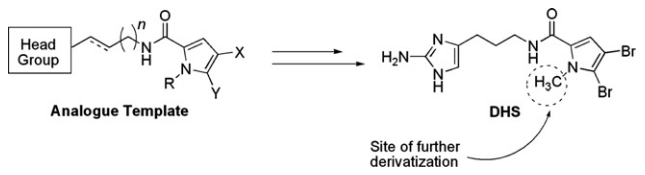
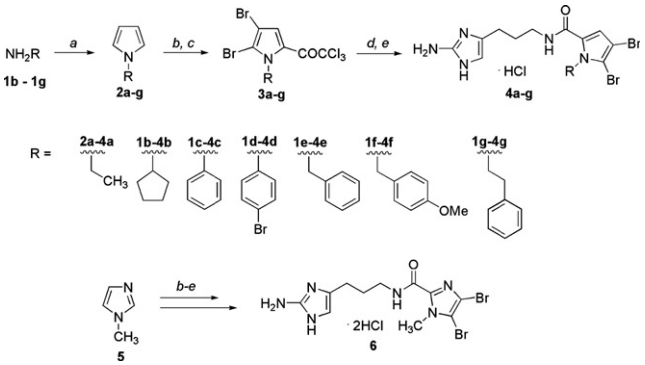


Figure 2. Template mediated derivatization of the oroidin scaffold.



Scheme 1. Synthesis of second generation oroidin library. Reactions and conditions: (a) 2,5-dimethoxytetrahydrofuran, HOAc, reflux; (b) trichloroacetyl chloride, $C_2H_4Cl_2$, reflux; (c) Br_2, $CHCl_3$, −10 °C to rt; (d) 4-(3-aminopropyl)-2-aminoimidazole, Na_2CO_3, DMAP (cat.), DMF, 50 °C; (e) 2 M HCl in Et_2O.

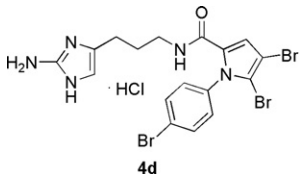
Biofilm inhibition assays were performed with the second generation oroidin library against *A. baumannii* utilizing a crystal violet reporter assay (Table 1).[30] Surprisingly, ethyl derivative **4a** was almost completely inactive, only being able to inhibit *A. baumannii* biofilm formation approximately 10% at 500 μM. Compounds **4b**–**4g** were initially insoluble during the preliminary 500 μM screen. At lower concentrations (<200 μM), solubility was not observed to have a limiting effect. These six analogues were subsequently assayed for IC_{50} values and all were determined to be more potent than the previous lead compound DHS (Actb IC_{50} = 110 μM). Of the derivatives with an unsubstituted phenyl ring (**4c**, **4e**, and **4g**), a single methylene spacer between the pyrrole nitrogen and the phenyl ring (**4e**) resulted in a compound approximately 2.5× more active than no spacer at all (**4c**). An additional methylene unit (**4g**) seemed to elicit only a small effect by slightly lowering the determined IC_{50} value. Interestingly, addition of a methoxy group to the phenyl ring also resulted in a derivative (**4f**) that was active. Toleration of the single atom replacement in the pyrrole ring of **6** was not evident as a significant drop in activity was seen against *A. baumannii* (∼50% inhibition at 500 μM) in relation to DHS. The most active compound identified from the inhibition screens was compound **4d** (Actb IC_{50} = 26.8 ± 2.28 μM) which contained an additional bromine atom in the para position on the phenyl appendage. Growth curve experiments performed in either the presence or absence of this compound at its determined IC_{50} value over a 24-h time period validated that like its predecessors, **4d** was not acting as a microbicide and inducing bacterial cell death before biofilm initiation had begun. Also, analogous to the observations made in our previous study,[25] modification of this compound through removal of both bromine atoms on the pyrrole moiety while keeping the newly introduced bromine on the phenyl ring led to a sharp decrease in activity (20% inhibition at 500 μM, data not shown).

From a medical standpoint, the ability to disperse an existing biofilm is more important than being able to prevent its formation. Once a biofilm forms, it is known to be upwards of 1000-times more resistant to conventional anti-biotics.[31] Bacteria within biofilms also are more resistant to microbicides, antiseptics, and basic host immune responses. Therefore, compounds that only inhibit biofilm formation will be less effective in treating a disease caused by an established biofilm. The most active analogue from the

Table 1
Biological evaluation of second generation oroidin analogues against *Acinetobacter baumannii*

Compound	*A. baumannii* (Actb) biofilm inhibition at 500 μM (%)[a]	IC_{50}[b] (μM)
DHS	>99	110
4a	10.3 ± 4.61	—
4b	—	40.5 ± 3.80
4c	—	97.7 ± 10.5
4d	—	26.8 ± 2.28
4e	—	35.4 ± 1.69
4f	—	42.8 ± 4.12
4g	—	40.7 ± 3.61
6	50.7 ± 2.64	—

[a] Assays performed in a minimum of triplicate.
[b] See Supplemental information for dose–response curves.



A. baumannii biofilm inhibition IC_{50} = 26.8 ± 2.28 μM, biofilm dispersion EC_{50} = 41.3 ± 4.50 μM

Figure 3. Anti-biofilm activity of analogue **4d**.

inhibition assays, **4d** (Fig. 3), was therefore assayed for the ability to disperse existing *A. baumannii* biofilms. Gratifyingly, **4d** displayed significant dispersion activity with an EC_{50} value of 41.3 ± 4.50 μM, making it one of the most potent known compound to date that disperses existing *A. baumannii* biofilms.[22,32,33]

In conclusion, a second generation oroidin library was synthesized and assayed for anti-biofilm activity against the emerging pathogen *A. baumannii*. Numerous derivatives of the library showed greater potency than the previous first generation lead compound DHS. Specifically, these derivatives are highlighted by incorporation of an alkyl-based phenyl chain off the pyrrole ring nitrogen or an *N*-arylated dibromopyrrole derivative. The most active analogue, **4d**, was also shown to have exceptional activity in dispersing preformed *A. baumannii* biofilms, making it a noteworthy addition to the limited number of compounds which share this ability.[22,32,33] Future work in this area includes mechanistic studies on how these compounds elicit their anti-biofilm effects and the identification and development of other unique biologically active scaffolds that incorporate a 2-aminoimidazole motif.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.06.089.

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