# Inhibition and dispersion of proteobacterial biofilms†

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**A small molecule derived from a marine natural product with the ability to inhibit biofilm formation and also disperse established proteobacterial biofilms is presented.**

A bacterial biofilm is a community of surface-attached bacteria protected by an extracellular matrix of biomolecules.[1] The formation of biofilms is a developmental process, in which planktonic bacteria adhere to a solid surface and initiate the formation of a complex sessile microcolony.[1,2] Bacteria within biofilms are inherently insensitive to antiseptics, microbicides and cells derived from the host. They are also upwards of 1000-fold more resistant to conventional antibiotics.[3] Since biofilms account for between 50–80% of microbial infections in the body, this is a considerable impediment to antimicrobial therapy.[4,5] In particular, persistent infections of indwelling medical devices remain a serious problem, since eradication of these infections is virtually impossible.[2,6] Other diseases in which biofilms are of notable importance include endocarditis, otitis media, chronic prostatitis, periodontal disease, chronic urinary tract infections, osteomyelitis and cystic fibrosis (CF).[4,7]

We recently reported the synthesis of TAGE and CAGE,[8] two derivatives of the marine natural product bromoageliferin (Fig. 1). Both compounds were shown to be effective inhibitors of *Pseudomonas aeruginosa* biofilm formation. *P. aeruginosa* is an opportunistic γ-proteobacterium[9] that is a threat to immunocompromised patients, and is responsible for mortality in cystic fibrosis patients.[10,11] It is also one of the most well studied models in terms of biofilm formation and quorum sensing (QS).[12] Embedded within bromoageliferin is dihydrooroidin (**4**), a compound related to the natural product oroidin (**5**). Based upon the success of the TAGE and CAGE analogues,[8] we elected to synthesize and screen a 50-member focused library of derivatives based upon the dihydrooroidin template. The ultimate goal was to delineate whether structurally-simpler motifs derived from the bromoageliferin skeleton could also serve as unique small molecules that inhibit biofilm development and disperse established biofilms. Herein we

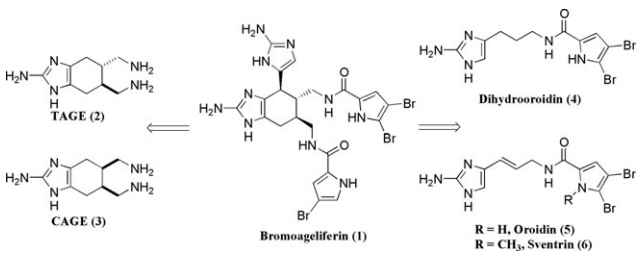


**Fig. 1** Natural product derivatives as anti-biofilm molecules.

detail both the inhibition and dispersion profile of the most active member of this library, dihydrosventrin (DHS, **7**), against both γ- and β-proteobacterial biofilms.

The dihydrooroidin library (see ESI†) was assembled by solution-based synthetic methods, and each member of the library characterized (by $^{1}$H NMR, $^{13}$C NMR and HRMS). The analogue library was then screened in a 96-well format using a crystal violet reporter assay to assess each analogue's ability to inhibit the formation of *P. aeruginosa* biofilms.[13] From this initial screen, the previously unreported and yet to be isolated derivative **7** (Fig. 2) was discovered to be the most potent member of the library, and was subsequently selected for further evaluation.

Follow-up dose response experiments revealed that DHS had $IC_{50}$ values of 51 μM against PAO1 and 111 μM against PA14 (Table 1), indicating that it was approximately 2-fold more active than both TAGE and CAGE. It is highly noteworthy that DHS displayed greater activity than both oroidin (**5**) ($IC_{50}$ = 190 μM PAO1, $IC_{50}$ = 166 μM PA14) and its unsaturated parent sventrin (**6**) ($IC_{50}$ = 75 μM PAO1, $IC_{50}$ = 115 μM PA14).[14] Dihydroorodin (**4**) showed only marginal activity (<70%) at 500 μM. Comparison of both growth curves and colony counts for PAO1 and PA14 grown in the absence or presence of **5**, **6** and **7** indicated that the inhibition of biofilm formation was *not* due to a bactericidal effect.

Given that DHS displayed exceptional activity in inhibiting the formation of PAO1 and PA14 biofilms, we decided to determine whether it would also inhibit the formation of a mucoid variant of *P. aeruginosa*. No small molecules have been documented as inhibiting the formation of mucoid

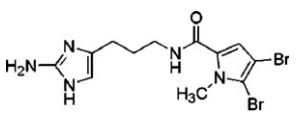


**Fig. 2** Dihydrosventrin (DHS).

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† Electronic supplementary information (ESI) available: Synthesis and characterization data for DHS, representative $^{1}$H spectra, representative dose–response curves, planktonic growth curves for all strains in the presence and absence of DHS, and colony counts for PAO1, PA14 and *A. baumannii* in the presence and absence of DHS. See DOI: 10.1039/b719802g

 

**Table 1** $IC_{50}$ values for DHS across selected proteobacteria[a]

| Proteobacterial strain | $IC_{50}$ value for DHS/μM |
|---|---|
| *P. aeruginosa* (PAO1) | 51 |
| *P. aeruginosa* (PA14) | 111 |
| *P. aeruginosa* (PDO300) | 115 |
| *A. baumannii* (Actb) | 110 |
| *B. bronchiseptica* (RB50) | 238 |

[a] All assays performed in triplicate.

*P. aeruginosa* biofilms. After a CF patient is colonized by *P. aeruginosa*, the bacterium undergoes a phenotypic shift from a non-mucoid to a mucoid form.[15,16] Numerous studies have correlated the appearance of mucoid *P. aeruginosa* with a decline in the pulmonary clinical status of CF patients.[15,17,18] PDO300 was employed to assay if DHS would inhibit the formation of mucoid biofilms. PDO300 is a well-characterized mucoid strain of *P. aeruginosa* that is genotypically identical to PAO1, except for the *mucA* mutation that converts the bacterium to the mucoid phenotype.[19] We determined that DHS has an $IC_{50}$ of 115 μM against PDO300 (Table 1). Growth curves again indicated that DHS lacked significant bactericidal activity against PDO300.

We also assessed the ability of DHS to inhibit the formation of *Acinetobacter baumannii* biofilms. *A. baumannii* is an opportunistic γ-proteobacterium that has become a severe threat over the last decade due to its multi-drug resistance.[20] Approximately 25% of all hospital swabs are positive for *A. baumannii*.[21] *A. baumannii* survives for weeks on dry surfaces due to its ability to form robust biofilms.[22] Clearly, this is a serious impediment to control strategies, and small molecules that inhibit *A. baumannii* biofilms may be particularly valuable for *A. baumannii* remediation efforts. There are currently no known small molecules documented that inhibit *A. baumannii* biofilm formation. DHS was slightly more potent against *A. baumannii* than it was against PA14, revealing an $IC_{50}$ value of 110 μM (Table 1). Both growth curves and colony counts of *A. baumannii* grown in the absence or presence of DHS indicated this compound has no microbicidal effects.

Next, we determined if DHS had the ability to inhibit the formation of biofilms across bacterial order. To the best of our knowledge, no small molecule has been documented with this activity. The *Bordetella bronchiseptica* strain RB50, a β-proteobacterium, was chosen for evaluation. Bacteria of the genus *Bordetellae* are frequently isolated from mammalian respiratory tracts.[23] *B. bronchiseptica* shares many of the same virulence factors as *Bordetella pertussis*, a β-proteobacterium that causes whooping cough and is responsible for 300 000 fatalities per year.[24] DHS was found to have an $IC_{50}$ of 238 μM against RB50 (Table 1).

Perhaps the more significant activity associated with small molecule modulation of biofilm development is the ability to disperse an established biofilm. From a clinical perspective, this is highly significant because a physician is typically faced with treating an established biofilm infection. Therefore, molecules that simply inhibit biofilm formation will not be effective toward treating diseases caused by established biofilms. DHS was assessed for its ability to disperse existing biofilms of *P. aeruginosa*, *A. baumannii* and *B. bronchiseptica*.

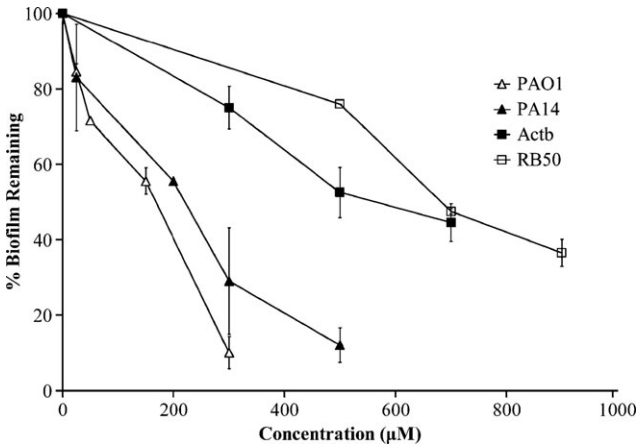


**Fig. 3** Dispersion of proteobacterial biofilms with DHS.

Each bacterial strain was allowed to form biofilms for 24 hours in the absence of compound. At the end of 24 hours, the media and planktonic bacteria were removed, and the remaining biofilm was treated either with media alone or media containing DHS. DHS was able to successfully disperse each biofilm across a range of concentrations (Fig. 3).

In conclusion, we have identified a simple derivative of a marine alkaloid that is active in inhibiting and dispersing proteobacterial biofilms. The mechanism of action of these compounds is currently unknown, but is actively under investigation. The performance of DHS is unprecedented because it seems to inhibit *and* disperse the formation of bacterial biofilms across bacterial order. Given this activity, the 2-aminoimidazole sub-unit, which is present in all members of the oroidin alkaloids, may have the potential to provide a scaffold for the development of therapeutics directed towards controlling and eliminating biofilm infections. Current research efforts are being directed toward the synthesis of other novel chemical libraries containing the 2-aminoimidazole motif for future anti-biofilm studies. The remaining characterization and activity data of the entire dihydrooroidin library will be disclosed in due course.[25]

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