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Inhibition of biofilm formation by conformationally constrained indole-based analogues of the marine alkaloid oroidin

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ABSTRACT

Herein, we describe indole-based analogues of oroidin as a novel class of 2-aminoimidazole-based inhibitors of methicillin-resistant *Staphylococcus aureus* biofilm formation and, to the best of our knowledge, the first reported 2-aminoimidazole-based inhibitors of *Streptococcus mutans* biofilm formation. This study highlighted the indole moiety as a dibromopyrrole mimetic for obtaining inhibitors of *S. aureus* and *S. mutans* biofilm formation. The most potent compound in the series, 5-(trifluoromethoxy)indole-based analogue **4b** ($MBIC_{50}$ = 20 μM), emerged as a promising hit for further optimisation of novel inhibitors of *S. aureus* and *S. mutans* biofilms.

The growth of bacteria is faster on surfaces that present a protective and nutrient-rich environment compared to growth in water and in liquids with low nutrient contents.[1] The adhesion of bacterial cells to inert or living surfaces results in the formation of biofilms, that is, structured communities that are encased in a self-produced extracellular matrix of biomolecules.[1,2] The extracellular matrix, which consists of polysaccharides, proteins and nucleic acids, is the primary reason for profound protection from the host's immune system and for a nearly 1000-fold increase in biofilm resistance to conventional antibiotics when compared to planktonic bacteria.[3,4]

The robust resistance of biofilms to the hazards of their environment, for example, predators, antibiotics and host immune systems,[2,5,6] results not only in biofouling in the food and marine industries but also in various nosocomial and chronic infections in patients, which result in perpetual inflammation and tissue damage.[3,7] The formation of biofilms on inert surfaces includes indwelling medical devices, catheters and contact lenses, and infections of living surfaces most often include lung infections in cystic fibrosis patients, periodontitis, endocarditis and urinary tract infections.[4,8] Furthermore, an estimate by the National Institutes of Health states that 80% of microbial infections are biofilm-based.[9]

Facultative anaerobic cocci *Staphylococcus aureus* and *Streptococcus mutans* are among common Gram-positive bacteria that form infectious biofilms.[1,10] Nosocomial infections are frequently caused by omnipresent *S. aureus* bacteria, which are commonly found in the anterior nares in humans and can enter the circulatory system through an epithelial breach.[11,12] Osteomyelitis, indwelling medical device infections, chronic wound infections, endocarditis and periodontitis exemplify the most prevalent biofilm-based infections caused by *S. aureus*. While periodontitis is sometimes caused by *S. mutans*, its most common biofilm-based infection remains dental caries. *S. mutans* is considered to be one of the earliest colonisers of human teeth after their eruption, and its fermentation of dietary carbohydrates and consequential production of acids poses one of the most important factors for the formation of dental caries.[10]

Ubiquitous biofouling presents a considerable threat to immobile marine organisms such as sponges and seaweeds, which do not possess an immune system that can protect them against microbes. Hence, they have evolved to biosynthesise various secondary metabolites that function as a defence system against bacterial infections.[1,13] Among the most investigated anti-biofilm agents from marine sponges are the halogenated furanones from the seaweed *Delisea pulchra*,[14,15] terpenoids[16,17] and especially 2-aminoimidazole-based alkaloids.[18–20] The 2-aminoimidazole-based

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marine alkaloid oroidin (Fig. 1) and various series of its analogues were extensively studied as biofilm inhibitors, primarily by Melander and coworkers.[18,20–27]

Although numerous analogues of oroidin have been observed to control biofilm development,[18,20–27] to our knowledge, indole-based analogues of oroidin have not yet been studied. In contrast, various studies outlined the ability of indole to regulate the growth of various bacterial biofilms and therefore represents an important quorum sensing molecule.[28–30] In the present work, we first replaced the dibromopyrrole moiety of oroidin with the isosteric indole and 5-fluoroindole moieties, and evaluated the anti-biofilm activity of synthesised analogues **1**[31] and **2**[31] (Table 1) against two Gram-positive biofilm-forming strains of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Streptococcus mutans* and against a Gram-negative biofilm-forming strain of *Pseudomonas aeruginosa* (*cf.* Supplementary material). While indole-based analogue **1** did not exhibit any anti-biofilm activity against the tested strains, its 5-fluoroindole analogue **2** attenuated the formation of MRSA and *S. mutans* biofilms to 8.1% and 18.7%, respectively, at 100 μM (Table 1). Based on these encouraging anti-biofilm activities we further explored the structure–activity relationship of indole-based series of compounds by synthesising conformationally constrained analogues (Fig. 1) in which the central alkene region of oroidin was replaced with synthetically more favourable 1,3-phenylene (compounds **3a**–**14**) and 1,4-phenylene (compounds **15a**–**17b**) moieties. To study the structure–activity relationship of our small library of conformationally constrained indole-based analogues of oroidin we (*i*) evaluated the effect of the rigidification of analogues **1** and **2** on anti-biofilm activity, (*ii*) compared the 1,3-substituted series to the 1,4-substituted series of analogues and (*iii*) studied the influence of various substituents on indole and 2-aminoimidazole moieties on anti-biofilm activity (Fig. 1).

Syntheses of oroidin and compounds **1**–**5**, **7**, **8**, **9** and **11**–**15b** were performed as previously described.[31,32] Compounds **10a** (*cf.* Supplementary material), **16a** and **17a** were synthesised using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU)-promoted coupling of *tert*-butyl 2-amino-4-(3-aminophenyl)-1*H*-imidazole-1-carboxylate[31] or *tert*-butyl 2-amino-4-(4-aminophenyl)-1*H*-imidazole-1-carboxylate[31] with the corresponding indole-2-carboxylic acids.[33] Subsequent treatment of compounds **10a**, **16a** and **17a** with gaseous hydrogen chloride gave compounds **10**, **16b** and **17b**. Compound **6a** was synthesised from **10a**, using

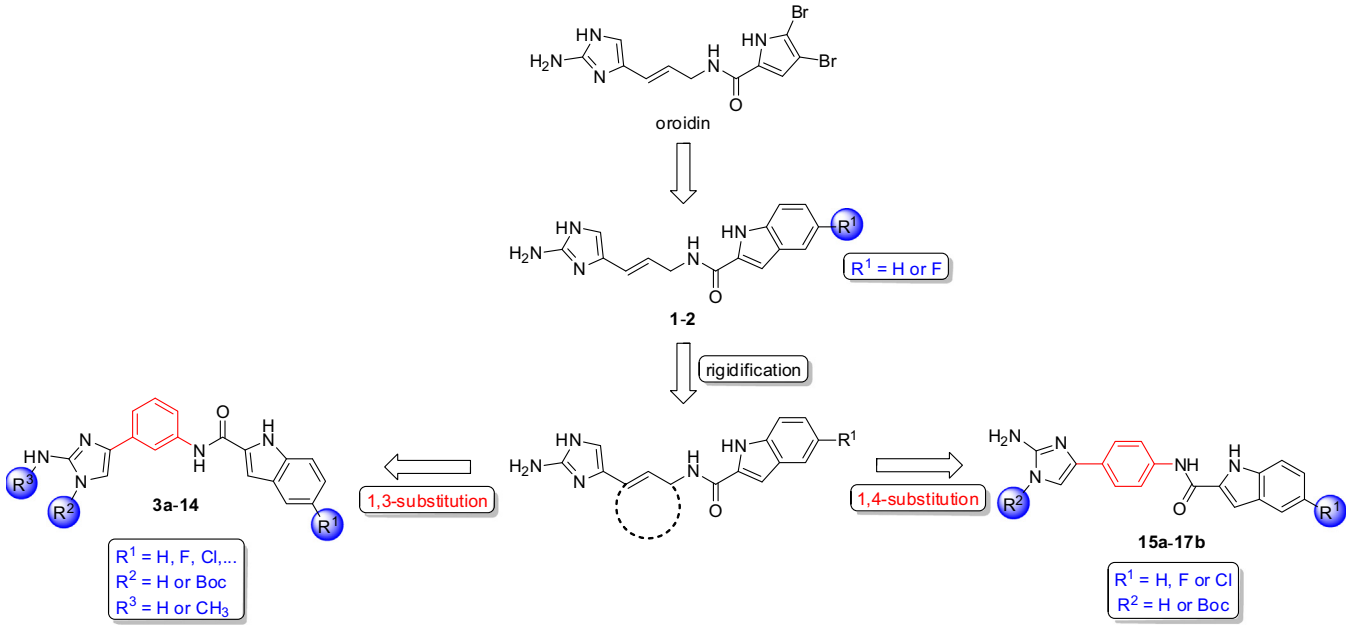
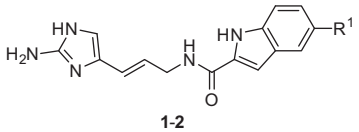


Figure 1. Design of conformationally constrained indole-based analogues of oroidin with potential anti-biofilm activity.

Table 1
Anti-biofilm activities of oroidin and its indole-based analogues



Compounds	R^1	*Staphylococcus aureus*		*Streptococcus mutans*	
		% Of biofilm formation[a]	$MBIC_{50}$[b] (μM)	% Of biofilm formation[a]	$MBIC_{50}$[b] (μM)
Oroidin	—	n.a.	—	18.0 ± 8.6	—
1	H	n.a.	—	n.a.	—
2	F	8.1 ± 0.7	60	18.7 ± 9.7	60

[a] Percentage of biofilm formation compared to the negative control. Compounds were tested at 100 μM. Each value is the mean of three independent experiments. n.a. = not active.
[b] The concentration of compound that inhibits biofilm formation by at least 50%. Each $MBIC_{50}$ value is the mean of three independent experiments.