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Identification of aryl 2-aminoimidazoles as biofilm inhibitors in Gram-negative bacteria

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ABSTRACT

The synthesis and biofilm inhibitory activity of a 30-member aryl amide 2-aminoimidazole library against the three biofilm forming Gram-negative bacteria *Escherichia coli*, *Psuedomonas aeruginosa*, and *Acinetobacter baumannii* is presented. The most active compound identified inhibits the formation of *E. coli* biofilms with an IC_{50} of 5.2 μM and was observed to be non-toxic to planktonic growth, demonstrating that analogues based on an aryl framework are viable options as biofilm inhibitors within the 2-aminoimidazole family.

Bacterial biofilms, which are defined as a surface-attached community of bacteria protected by an extracellular matrix of biomolecules,[1] are increasingly being recognized as an important pathogenic target.[2] Within a biofilm state, bacteria are upwards of 1000-fold more resistant to antibiotics and are generally resistant to the host immune response.[3] This presents a tremendous hurdle for the treatment of bacterial infections as the NIH estimates 75% of all bacterial infections are biofilm-based. Given this situation, there has been a significant effort to identify molecules that are capable of inhibiting and dispersing biofilms.[2]

Our research group has been studying the ability of simple analogues of the marine natural products bromoageliferin and oroidin (Fig. 1) to control biofilm development.[4–14] Bromoageliferin is a sponge-derived alkaloid that was reported to inhibit the formation of bacterial biofilms from marine sources, presumably as a defense mechanism against biofouling.[15] We reasoned that core structures derived from this complex molecule could be used as structural inspiration for the development of potent and synthetically accessible anti-biofilm agents. One of these scaffolds developed was based upon an aryl framework.[16] From a medicinal chemistry standpoint, this scaffold was deemed a promising platform to develop further functionalized 2-aminoimidazole (2-AI) derivatives as diversity could rapidly be introduced through manipulation of the benzene ring. Herein we describe the synthesis and anti-biofilm activity of these aryl 2-AI derivatives against three commonly studied Gram-negative bacteria.

We envisioned a straightforward synthesis to access aryl 2-AIs with variation in the location of structurally diverse amide appendages around a core phenyl ring obtained through an acylation reaction with an aniline intermediate. It was hoped that this might lend insight into what effect, if any, the position of the amide side group would have on biofilm inhibitory activity. Furthermore, this study would allow us to gauge whether an aryl ring could serve as a suitable surrogate for an aliphatic linker in the family of 2-aminoimidazole anti-biofilm agents. Previous studies examining the role of aliphatic linkers between the 2-AI head and tail of these compounds has indicated a correlation in activity.[6,12]

The first scaffold prepared bore the aniline functionality *para* to the 2-AI head (Scheme 1). This was achieved by employing similar methodology described in accessing aryl halide 2-AI substrates for palladium catalyzed Sonogoshira cross-couplings.[16] Synthesis commenced through activation of commercially available *p*-azidobenzoic acid with oxalyl chloride and subsequent reaction with diazomethane followed by quenching with concentrated HBr to deliver the α-bromoketone. Installation of the Boc-protected 2-aminoimidazole motif was affected through condensation of the α-bromoketone with Boc-guanidine to deliver azido intermediate

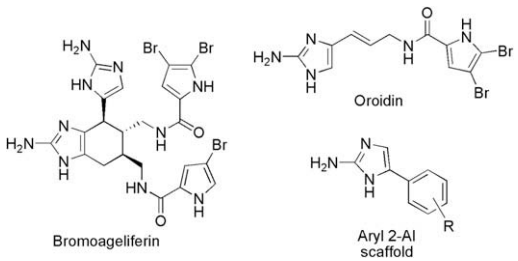

Figure 1. 2-Aminoimidazole-based anti-biofilm molecules.

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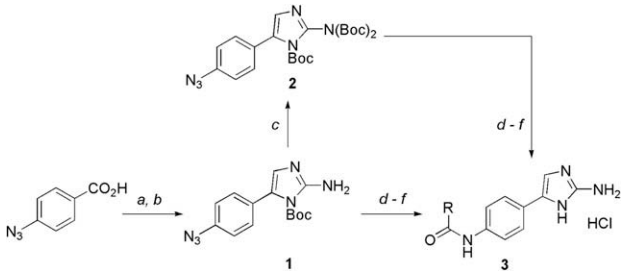
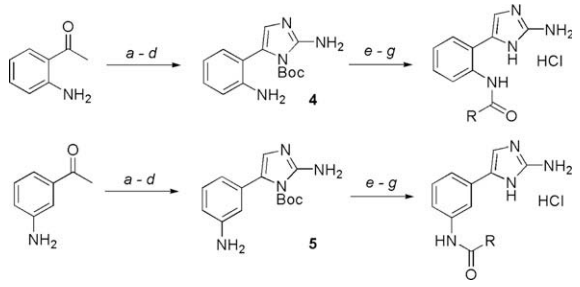


Scheme 1. Synthesis of *para* substituted acylated 2-AI. Reactions and conditions: (a) (i) $(COCl)_2$, DMF, CH_2Cl_2, rt; (ii) CH_2N_2 Et_2O, 0 °C; (iii) concd HBr; (b) Boc-guanidine, DMF, rt; (c) Boc_2O, Et_3N, THF, rt; (d) H_2 (1 atm), 10% Pd/C, THF, rt; (e) RCOCl, Et_3N, CH_2Cl_2, THF, −78 °C to rt; (f) (i) TFA, CH_2Cl_2, 0 °C; (ii) 2 M HCl in Et_2O.



Scheme 2. Synthesis of *ortho* and *meta* acylated 2-AI. Reactions and conditions: (a) CbzCl, $NaHCO_3$, H_2O, acetone, 0 °C to rt; (b) Br_2, $AlCl_3$ (cat.), 0 °C; (c) Boc-guanidine, DMF, rt; (d) H_2 (35 psi), 10% Pd/C, EtOH, rt; (e) RCOCl, Et_3N, CH_2Cl_2, THF, −78 °C to rt; (f) (i) TFA, CH_2Cl_2, 0 °C; (ii) 2 M HCl in Et_2O.

1. Exhaustive Boc protection of the exocyclic amino functionality and reduction of the azide under mild hydrogenation conditions followed by in situ acylation of the aniline with various acid chlorides delivered the Boc-protected targets. However, analogous to our report utilizing a similar reductive acylation strategy,[11] the direct use of intermediate **1** in the acylation sequence afforded good yields of mono-acylated product thereby allowing for circumvention of the extra synthetic step. Acidic removal of the Boc group with TFA followed by counterion exchange (chloride for trifluoroacetate) gave the target *para* oriented 2-AI analogues. These compounds were synthesized by employing the corresponding acid chlorides of the groups depicted in Figure 2 (Supplementary data for complete list of synthesized compounds).

Due to the commercial unavailability of the corresponding *ortho* and *meta* oriented azido benzoic acids, each remaining 2-AI aniline scaffold was accessed through its readily available aminoacetophenone building block (Scheme 2). These starting materials were first protected as their benzyl carbamates.[17] This would allow for their selective removal under neutral conditions later on in the synthesis once the Boc-protected 2-AI residues had been installed into the scaffolds. The methyl ketones were mono-brominated under the action of Br_2 and catalytic $AlCl_3$ before again undergoing cyclization with Boc-guanidine. Removal of the Cbz groups required elevated pressure (35 psi), yet in each case delivered the desired 2-AI aniline products. Unlike the in situ acylation reaction applied to the synthesis of the *para* derivatives, scaffolds **4** and **5** could not be directly carried onto the acylation step after reduction as each intermediate required purification and isolation. Regardless, acylation and deprotection proceeded smoothly to yield the requisite derivatives through implementing the various acid chlorides available listed in Figure 2 (Supplementary data for complete list of synthesized compounds).

One aspect worth noting is in reference to the compatibility of the acylation reaction with acylating reagents other than acid chlorides. Unlike the previous reductive acylation approach we disclosed involving an aliphatic-based amine intermediate[6] (compatible with acid chlorides, anhydrides, trichloromethylketones, and succinate esters), each aniline scaffold was unable to react with anything other than acid chlorides even when trying to modify the reaction conditions through the addition of acyl transfer catalysts (such as DMAP) or the application of heat.

Escherichia coli bacterial infections account for significant medical cost and morbidity worldwide.[18] Surgical site infection, Pneumonia, Meningitis, and Urinary tract infections are just a few health problems caused by *E. Coli* bacteria. For anti-biofilm assessment, each compound in the library was initially screened at 100 μM for its ability to inhibit the formation of *E. coli* biofilms using a crystal violet reporter assay.[19] Compounds that demonstrated >95% inhibition were then subjected to dose response studies to determine IC_{50} values. Under this criteria, compounds **6–11**

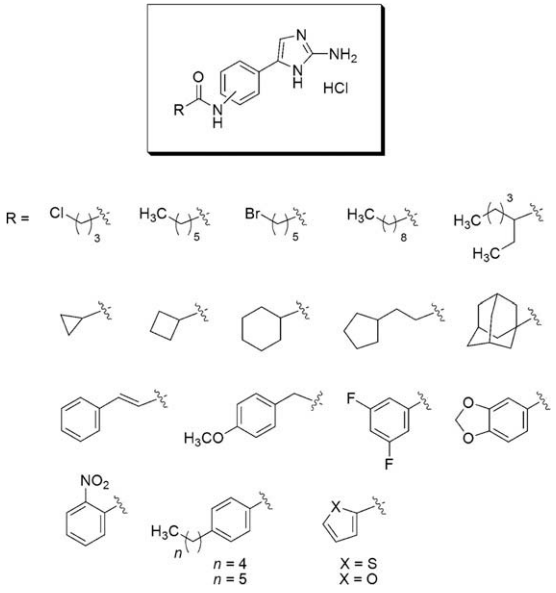
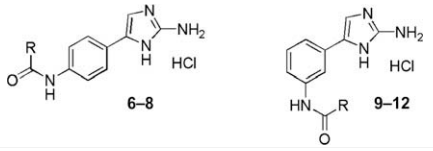
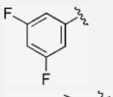
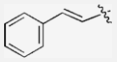
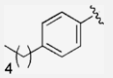
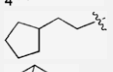
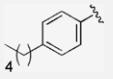


Figure 2. Side chains employed in the acylation reaction of the aryl 2-AI aniline scaffolds.

Table 1
Hits identified from the *E. coli* biofilm inhibition assays



Compound	R=	*E. coli* biofilm IC_{50} (μM)
6		5.2 ± 1.1
7		16.3 ± 3.7
8		39.4 ± 1.5
9		47.0 ± 1.0
10		43.4 ± 1.3
11		16.5 ± 1.0