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A facile synthesis of 1,5-disubstituted-2-aminoimidazoles: Antibiotic activity of a first generation library

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ABSTRACT

An efficient synthetic route to 1,5-disubstituted 2-aminoimidazoles from readily available amino acids and aldehydes has been developed. A library of simple analogues was synthesized and several compounds were shown to exhibit notable antibiotic activity against a variety of bacterial strains including multi-drug resistant isolates.

The number of antibiotic-resistant infections encountered globally continues to rise and the current supply of anti-bacterial drugs is becoming increasingly ineffective. This has lead to a resurgence in research into anti-infective strategies.[1] Bacterial strains resistant to many, or even all, currently available antibiotics are increasingly common, while at the same time fewer new antibiotic classes are being developed. Resistance is mediated by many different mechanisms including inactivation by enzymes, bypass of antibiotic targets and efflux of antibiotics from the bacterial cell.[2] In addition to the search for novel antibiotics, other approaches to the control of bacterial infections will also prove valuable. One such approach is the control and maintenance of bacteria within the biofilm state.[3]

Biofilms are microbially derived sessile communities, characterized by cells that are irreversibly attached to a substratum or interface or to each other, and are embedded in a matrix of extracellular polymeric substances that they have produced. They exhibit an altered phenotype with respect to growth rate and gene transcription compared to their planktonic counterparts.[4] Biofilms are increasingly recognized as being significant in human disease, accounting for over 80% of microbial infections in the body.[5] Diseases associated with bacterial biofilms include, lung infections of cystic fibrosis (CF), colitis, urethritis, conjunctivitis, otitis, endocarditis and periodontitis.[4,5] Bacteria within biofilms are inherently insensitive to antiseptics and host immune responses, and residing within the biofilm state confers resistance to conventional antibiotics upwards of 1000 times that of planktonic bacteria.[3] Biofilm infections are therefore rarely resolved by host defense mechanisms and while antibiotic therapy generally reverses the symptoms caused by planktonic cells released from the biofilm, it fails to kill bacteria residing within the biofilm. For this reason biofilm infections typically show recurring symptoms, even after cycles of antibiotic therapy.[6] One mechanism of biofilm resistance to antibiotics is the inability of the antibiotic to penetrate the full depth of the biofilm, as the polymeric matrix of a biofilm is known to retard the diffusion of antibiotics. A second hypothesis is the fact that some cells within a biofilm experience nutrient limitation and therefore exist in a slow-growing or starved state making them less susceptible to many antimicrobial agents. This heterogeneity of biofilms is an important survival strategy as at least some of the cells are almost certain to survive any metabolically directed attack.[6]

We have developed several diverse libraries of compounds with anti-biofilm activity against a variety of bacterial strains, both Gram-positive and Gram-negative, in addition to compounds with fungal anti-biofilm activity.[7–25] These compounds are based on the structures of natural products with demonstrated anti-biofilm activity. The majority are based on oroidin **1** and bromoageliferin **2** (Fig. 1), secondary metabolites of the marine sponge family *Agelasidae*, which were initially reported to possess anti-biofouling activity against the Gram-negative marine α-proteobacterium *Rhodospirillum salexigens*,[26] and contain a key 2-aminoimidazole moiety. Many of these anti-biofilm compounds inhibit the formation of a bacterial biofilm without exhibiting microbicidal activity towards planktonic bacteria. There is significant potential for molecules that possess the ability to inhibit and/or disperse bacterial biofilms via a non-toxic mechanism in infectious disease therapy,

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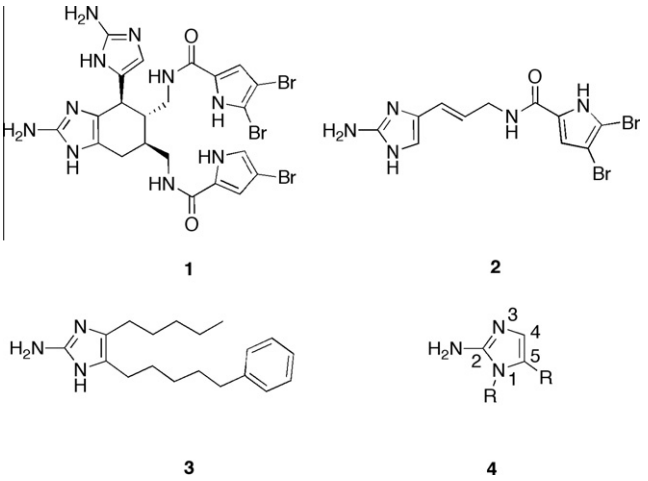


Figure 1. Natural products possessing anti-biofilm activity, bromoageliferin **1** and oroidin **2**, lead 4,5-disubstituted-2-aminoimidazole **3** and the general structure of 1,5-disubstituted-2-aminoimidazoles **4**.

as if a molecule does not directly kill bacteria there is a reduced likelihood of the bacteria acquiring resistance to the molecule. Additionally we have shown that these anti-biofilm compounds can be used in combination with traditional antibiotics to re-sensitize bacteria to the antibiotic.[25]

We recently reported the synthesis of a pilot library of 4,5-disubstituted-2-aminoimidazoles via a nitroenolate approach.[24] Many of these compounds displayed promising biological activity, with the lead compound, **3**, shown in Figure 1. Methods to access 2-aminoimidazoles with other pre-defined substitution patterns will allow examination of the effect of specific substitution patterns on the antibiotic and antibacterial activity of 2-aminoimidazole derivatives. We have therefore developed a facile route to 1,5-disubstituted-2-aminoimidazoles **4**, in which a substituent is placed at one of the endocyclic nitrogen atoms (Fig. 1).

Reported methods for the preparation of 1,5-disubstituted-2-aminoimidazoles do not allow for the introduction of a diverse array of C-5 substituents. Guanylation of 1-amidino-3-trityl-thioureas followed by reaction with α-bromo ketones allows the preparation of 2-aminoimidazoles with an acyl group at the 5-position.[27] The preparation of 5-aryl 1,5-disubstituted-2-aminoimidazoles has been reported via the formation of imidazo (1,2-α)pyrimidinium salts from substituted 2-aminopyrimidines and α-bromocarbonyl compounds, followed by opening of the pyrimidine ring with hydrazine.[28] A library of N-1-substituted 4(5)-phenyl-2-aminoimidazoles was recently reported to exhibit biofilm inhibitory activity against *Salmonella typhimurium* and *Pseudomonas aeruginosa*.[29] In order to rapidly assemble a library for antibiotic and anti-biofilm screening we desired a method that utilized readily available building blocks and allowed for the introduction of diversity at both positions. Such a method would be of use in many areas of medicinal chemistry as, in addition to possessing anti-bacterial activity, substituted 2-aminoimidazoles have been identified in many pharmacologically active substances including compounds with anti-cancer activity,[30,31] anti-fungal compounds,[31] and nitric oxide synthase inhibitors.[32]

To this end, we envisioned that the desired 1,5-disubstituted-2-aminoimidazoles derivatives could be readily prepared from the condensation of N-substituted α-aminoaldehydes with cyanamide. In turn, these N-substituted α-aminoaldehydes could be prepared from a reductive amination reaction between commercially available aldehydes and readily available amino acids, followed by reduction of the carboxylic acid to the aldehyde (Fig. 2).

The first step of our route to 1,5-substituted 2-aminoimidazoles involved the formation of N-substituted α-amino acids. We tested several conditions for the reductive amination of L-phenylalanine with benzaldehyde and determined the best method to be a two-step procedure using sodium borohydride as the reducing agent, which, after Boc-protection, afforded the desired N-substituted phenylalanine 5a in 70% yield. We then turned our attention to the conversion of the α-amino acid to its corresponding α-amino aldehyde. Several methods were investigated including thiol ester formation followed by reduction with triethylsilane[33] and sodium amalgam (Akabori) reduction[15] of the methyl ester of compound **5a**. It was decided that the most efficient route, and that which allowed for the greatest introduction of diversity, was via the formation of the *N*-methoxy-*N*-methylamide (Weinreb amide) and subsequent reduction.[33] Conversion of the Boc protected amino acid **5a** to the Weinreb amide **6a** was achieved using (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) as the coupling reagent. Reduction of the Weinreb amide **10a** to the aldehyde was carried out using diisobutylaluminum hydride (DIBAL-H) followed by in situ Boc-deprotection with HCl, and cyclization with cyanamide to afford the 1,5-disubstituted-2-aminoimidazole **15a** (Scheme 1). It is worth noting that the use of α-amino Weinreb amides also has the advantage of allowing for the future preparation of 1,4,5-substituted 2-aminoimidazoles via α-amino ketone formation from Grignard addition to these α-amino Weinreb amides.

Following these test reactions, an initial 20-member library was generated, possessing a variety of substituents including, straight chain and branched alkyl groups, phenyl and alkyl phenyl groups and a more polar carbamate group (Scheme 1). Briefly, imine formation between equimolar amounts of the commercially available amino acids and aldehydes in methanol in the presence of lithium hydroxide was followed by reduction with 2 equiv of sodium borohydride. The crude secondary amines were Boc protected, using Boc anhydride in acetonitrile with tetramethylammonium hydroxide as base, to furnish the N-substituted Boc amino acids **5–9a–d**. Amide coupling of these protected amino acids with *N,O*-dimethylhydroxylamine, using BOP as the coupling reagent in CH_2Cl_2 in the presence of triethylamine, afforded the desired Weinreb amides **10–14a–d**. The Weinreb amides were then reduced with DIBAL-H in THF and after quenching of the reaction, the crude aldehydes were extracted with diethyl ether then treated with diethyl ether/aqueous HCl or TFA/CH_2Cl_2 to remove the Boc group. Following solvent removal the crude amino aldehydes were dissolved in a 1:1 mixture of ethanol/water, the pH adjusted to 4.3, and allowed to react with cyanamide at 95 °C. Following purification the desired 1,5-disubstituted-2-aminoimidazoles **15–19a–d** were converted to their HCl salts for biological testing.

Following the discovery that our 4,5-dialkylated 2-aminoimidazoles[24] exhibited significant microbicidal activity, we opted to initially investigate the antibiotic activity of this 1,5-disubstituted-2-

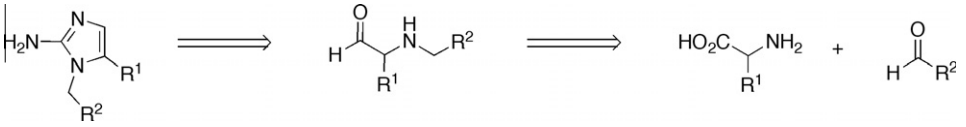
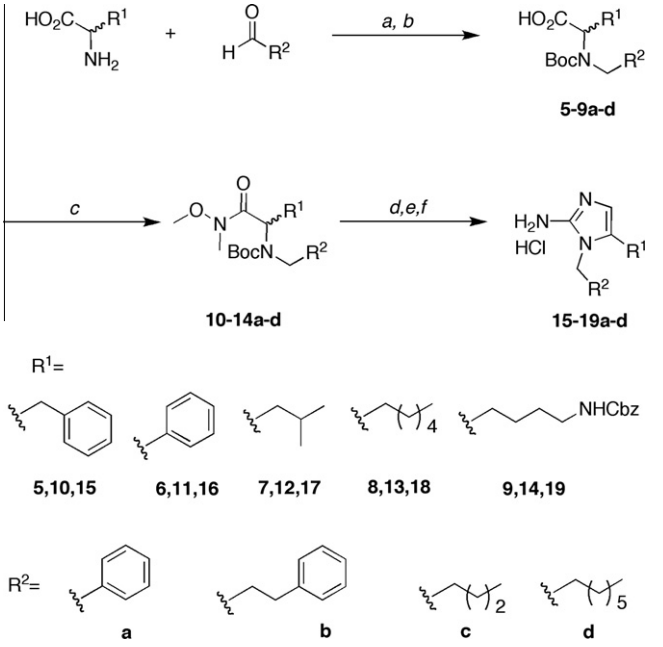
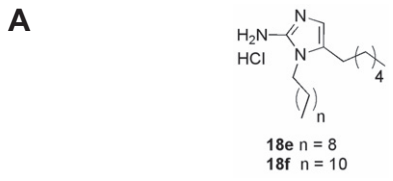


Figure 2. Retrosynthetic analysis of 1,5-disubstituted-2-aminoimidazoles.



Scheme 1. Synthesis and structures of 1,5-disubstituted 2-aminoimidazoles **15**–**19a**–**d**. Reagents and conditions: (a) (i) $LiOH{\cdot}H_2O$, CH_3OH, rt, 18 h, (ii) $NaBH_4$, rt, 1 h; (b) Boc_2O, TMAH, CH_3CN, rt, 18 h; (c) $HN(OCH_3)CH_3$, Et_3N, BOP, DCM, rt 18 h; (d) DIBAL-H/hexanes, THF, −78 °C to rt, 1 h; (e) 4 M HCl(aq)/Et_2O or (1:10) rt, 2 h; (f) EtOH/H_2O, pH 4.3, H_2NCN, 95 °C 2.5 h.



Bacterial strain	18e	18f
E. coli	1	2
A. baumannii	8	64
MDRAB	8	>128
K. pneumoniae	32	>128
S. epidermidis	0.25	0.5
MRSE	1	1
MSSA	1	2
MRSA	1	2

Figure 3. (A) Structures of compounds **18e** and **18f**. (B) MIC values (μg/mL) for compounds **18e** and **18f**.

aminoimidazole library. This was carried out by measurement of the minimum inhibitory concentration (MIC) of each derivative against a variety of representative pathogenic bacterial strains, both Gram-negative and Gram-positive, using a standard broth microdilution protocol.[34] We tested activity against: *Escherichia coli*, *Acinetobacter baumannii*, multi-drug resistant *A. baumannii* (MDRAB), *Staphylococcus epidermidis*, methicillin resistant *S. epidermidis* (MRSE), methicillin susceptible *Staphylococcus aureus* (MSSA), methicillin resistant *S. aureus* (MRSA) and a carbapenem resistant strain of *Klebsiella pneumoniae* which produces the recently reported New Delhi metallo-β-lactamase (NDM-1).[35] The results of this screen are outlined in Table 1. It can be seen that compound **18d** is the most active antibiotic, with MIC values of 2, 8, 8, 32, 0.125, 2, 2 and 2 μg/mL against *E. coli*, *A. baumannii*, MDRAB, *K. pneumoniae*, *S. epidermidis*, MRSE, MSSA and MRSA, respectively. This compound demonstrated greater antibiotic activity against the Gram-positive strains tested than against the Gram-negative strains, and has particularly notable activity against the opportunistic bacterium *S. epidermidis*. For compounds **18c** and **18d**, it can be seen that increasing the chain length of the N-1 substituent leads to an increase in activity from 8- or 16-fold for the Gram-negative strains up to 64-fold for *S. epidermidis*. We therefore decided to synthesize two more compounds with the same C-5 substituent and increasing alkyl chain lengths of the N-1 substituent to see whether this trend would be continued. These compounds (**18e** and **18f**, Fig. 3) were synthesized in the same manner as the initial library and were tested for antibiotic activity against the same eight bacterial strains (Fig. 3). Unfortunately, a significant increase in antibiotic activity from compound **18d** was not observed, though these compounds still possess considerable antibiotic activity against *S. epidermidis*, MRSE, MSSA, MRSA and *E. coli*.

We then screened the 1,5-disubstituted 2-aminoimidazole library for the ability to inhibit bacterial biofilm formation. For this investigation five biofilm forming bacterial strains were selected,

Table 1
MIC values (μg/mL) for compounds **15**–**19a**–**d** against several bacterial strains

	E. coli	*A. baumannii*	MDRAB	*K. pneumoniae*	*S. epidermidis*	MRSE	MSSA	MRSA
15a	128	>128	>128	>128	128	128	>128	>128
15b	32	32	32	128	16	16	32	32
15c	64	64	64	128	32	64	64	128
15d	128	128	128	>128	128	128	128	128
16a	8	128	16	128	2	8	8	4
16b	32	32	32	128	8	32	32	32
16c	128	64	64	>128	16	128	64	64
16d	64	16	64	128	16	64	64	64
17a	128	128	128	128	16	128	128	128
17b	16	32	32	128	4	32	64	32
17c	64	64	64	128	32	128	>128	128
17d	2	16	16	64	2	8	16	8
18a	32	16	16	64	16	16	16	32
18b	8	16	32	64	4	16	8	8
18c	32	64	64	64	8	32	32	32
18d	2	8	8	32	0.125	2	2	2
19a	64	64	64	128	32	32	32	32
19b	64	32	32	128	16	16	16	16
19c	32	128	128	>128	16	64	32	128
19d	8	64	64	128	2	16	16	16

Table 2
Percentage biofilm inhibition at 200 μM

	E. coli	*A. baumannii*	MDRAB	MSSA	MRSA
15b	97 ± 2	96 ± 2	94 ± 4	98 ± 2	90 ± 5
16a	89 ± 1	96 ± 2	97 ± 3	93 ± 6	95 ± 2
16b	94 ± 0.1	97 ± 0.5	97 ± 1	95 ± 4	94 ± 5
17b	92 ± 1	96 ± 0.3	97 ± 2	87 ± 9	96 ± 3
17d	88 ± 1	97 ± 0.6	96 ± 0.3	93 ± 5	97 ± 3
18b	94 ± 2	96 ± 3	98 ± 0.6	98 ± 2	91 ± 6
18c	97 ± 1	97 ± 0.1	Not active	92 ± 0.3	98 ± 1
18e	59 ± 3	91 ± 3	97 ± 2	73 ± 2	83 ± 10
19d	90 ± 1	93 ± 1	91 ± 3	91 ± 0.4	84 ± 1

E. coli, *A. baumannii*, MDRAB, MSSA and MRSA. Compounds were initially screened at 200 μM using a crystal violet reporter assay.[36] Several compounds exhibited biofilm inhibition activity against all five tested strains (Table 2). Compounds exhibiting greater than 90% inhibition were subject to a dose response assay in hopes of determining the IC_{50} value for biofilm inhibition activity. However, upon performing the dose response assay, we noticed a rapid drop in activity over a narrow concentration range, indicative of activity via a microbicidal mechanism. IC_{50} values for biofilm inhibition activity for this library of compounds were therefore not determined.

In conclusion, we have developed a facile route to 1,5-disubstituted-2-aminoimidazoles that allows rapid assembly of a diverse array of 2-aminoimidazole derivatives from readily available starting materials. The lead compounds identified in this study show significant antibiotic activity against a wide variety of bacterial strains. Several of the simple analogues developed in this study demonstrated the ability to inhibit biofilm formation, albeit through a microbicidal mechanism. This chemistry can now be used to access 1,5-substituted derivatives of our lead anti-biofilm compounds, in which the 5-position substituent has been finely tuned through numerous cycles of analogue synthesis and testing. These compounds are currently being developed in our lab and will be tested to determine whether this substitution pattern leads to enhanced anti-biofilm activity.

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Supplementary data

Supplementary data (experimental protocols for the synthesis and biological assays, and characterization of new compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.05.123.

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