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Modulating the development of *E. coli* biofilms with 2-aminoimidazoles

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ABSTRACT

The synthesis of a 20 member 2-aminoimidazole/triazole pilot library is reported. Each member of the library was screened for its ability to inhibit or promote biofilm development of either *Escherichia coli* and *Acinetobacter baumannii*. From this screen, *E. coli*-selective 2-aminoimidazoles were discovered, with the best inhibitor inhibiting biofilm development with an IC_{50} of 13 μM. The most potent promoter of *E. coli* biofilm formation promoted biofilm development by 321% at 400 μM.

Bacterial biofilms, which are defined as a community of surface attached bacteria protected by an extracellular matrix of biomolecules, account for ca. 80% of the world's microbial mass at any given time.[1] When bacteria are embedded in a biofilm, they are significantly more resistant to eradication by microbicides and other environmental pressures that would lead to the elimination of their free-floating (planktonic) brethren.[2] Although biofilms are typically associated with infectious disease[3] (i.e., the NIH estimates that 3 in 4 bacterial infections are biofilm-based) and represent a tremendous hurdle for disease treatment[4] (biofilm bacteria are upwards of 1000-fold more resistant to antibiotics); there are a number of bacterial biofilm populations, such as gut flora[5] and commensal dental bacteria,[6] that provide significant benefits for their host. In this regard, when designing small molecules that modulate biofilm formation, it is desirable to have the ability to tune activity for specific bacterial strains through structural modification and have the ability to generate compounds that actually promote biofilm formation in a targeted bacterial population.

With the goal of identifying molecules that have selective activity against a particular bacterial species, we report the investigation of structural motifs based upon the 2-aminoimidazole (2-AI) scaffold. Previous studies in our group have established that 2-aminoimidazoles can be designed to inhibit and disperse biofilms from both gram-positive and gram-negative strains of bacteria.[7] Based upon these results, we wanted to design a new approach to rapidly generate diversity on the 2-AI scaffold, which could then be evaluated to identify small molecules that selectively inhibit biofilm formation against a target bacterial population. In addition, we were interested in determining if a subset of molecules from this new 2-AI library would promote biofilm formation. As a test case, we attempted to identify molecules with differential activity between two γ-proteobacteria: *Escherichia coli* and *Acinetobacter baumannii*.

The synthetic approach to this library is outlined in Scheme 1. We chose to synthetically elaborate chloroacetyl chloride into a diverse collection of azido amides due to their ease of preparation and potential for structural diversity. The azido amides were then planned to undergo a click reaction[8] with alkyne **1** to generate a diverse set of 2-AI small molecules.[9] Alkyne **1** was rapidly synthesized from 5-hexynoic acid by transformation into the α-bromoketone **2** followed by condensation with Boc-guanidine.[10] Each azido amide that was prepared (Scheme 1) was then subjected to the click reaction in the presence of **1**. Final deprotection and counterion exchange delivered 2-AIT conjugates **4a**–**p**.

Each 2-AIT conjugate was initially screened for the ability to modulate biofilm development of *E. coli* and *A. baumannii* at 400 μM. Biofilm development was monitored under static conditions using a crystal violet (CV) reporter assay.[11] Briefly, bacteria were incubated in the absence or presence of compound in 96-well microtiter plates. After 24 h, the media and planktonic bacteria were removed and the wells were washed with water. Each well was then treated with CV, which stains any biofilm bacteria that are attached to the surface of the well. Following the removal of CV and washing of the wells the remaining CV was then solubilized by ethanol and quantified spectrophotometrically (A_{540}). The results of this initial screen are summarized in Table 1.

From this initial assessment at 400 μM, we rapidly identified several compounds that not only selectively inhibited *E. coli* biofilm development (in comparison to *A. baumannii*), but we also identified a number of compounds that promote *E. coli* biofilm development. Interestingly, compounds **4k** and **4p** promoted biofilm formation by 277% and 321%, respectively, while compound **4b** inhibited biofilm development by 90.8%.

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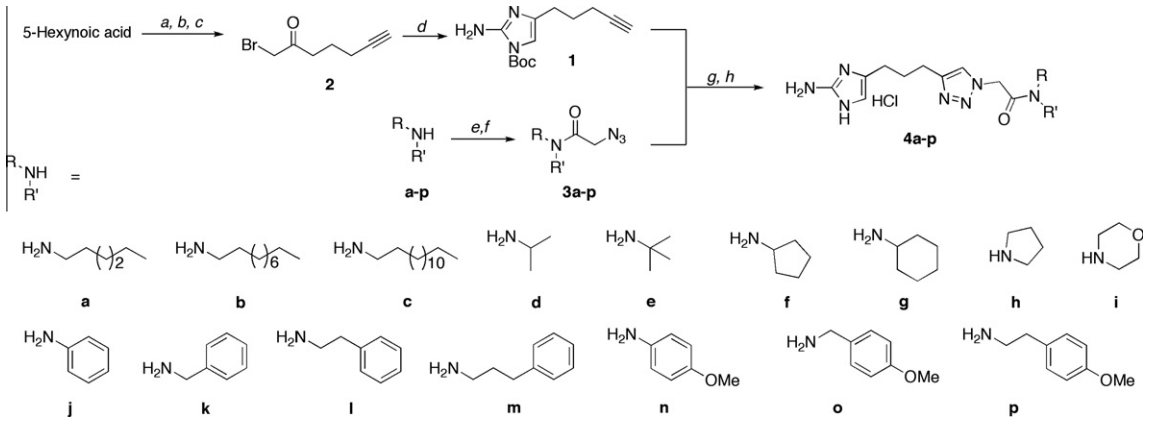
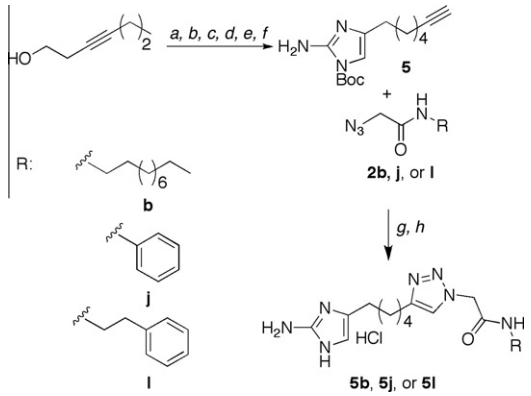


Scheme 1. Synthesis of 2-AIT library. Reagents and conditions: (a) $(COCl)2$, CH_2Cl_2, cat. DMF; (b) CH_2N_2, Et_2O; (c) HBr; (d) Boc-guanidine, DMF; (e) chloroacetyl chloride, Et_3N, CH_2Cl_2; (f) NaN_3, DMF; (g) sodium ascorbate, $CuSO_4{\cdot}5H_2O$, CH_2Cl_2, EtOH, H_2O; (h) TFA, CH_2Cl_2; then MeOH/HCl.

Table 1
Initial % biofilm inhibition for compounds **5a**–**5u** at 400 μM in comparison to untreated controls

Compound	*E. coli* biofilm [a]	*A. baumannii* biofilm [a]
4a	−30.3 ± 7.50	31.4 ± 6.5
4b	90.8 ± 0.52	28.3 ± 6.8
4c	57.8 ± 2.42	36.4 ± 6.1
4d	−127 ± 18.7	13.6 ± 3.5
4e	17.6 ± 4.73	21.2 ± 7.5
4f	−47.8 ± 8.49	15.9 ± 8.0
4g	−169 ± 22.1	17.7 ± 3.3
4h	2.6 ± 5.6	6.2 ± 8.9
4i	−183 ± 61.2	30.3 ± 10.8
4j	−142 ± 19.9	23.0 ± 3.2
4k	−278 ± 31.05	19.5 ± 3.3
4l	−41.8 ± 30.7	20.8 ± 12.3
4m	−176 ± 22.7	23.2 ± 3.1
4n	−169 ± 22.1	13.9 ± 3.5
4o	−19.9 ± 6.89	17.5 ± 7.8
4p	−321 ± 34.6	8.5 ± 3.7

[a] Values are means of three experiments.



Scheme 2. Reagents and conditions: (a) ethylene diamine, NaH; (b) CrO_3, H_2SO_4; (c) $(COCl)_2$, CH_2Cl_2, cat. DMF; (d) CH_2N_2, Et_2O; (e) HBr; (f) Boc-guanidine, DMF; (g) sodium ascorbate, $CuSO_4{\cdot}5H_2O$, CH_2Cl_2, EtOH, H_2O; (h) TFA, CH_2Cl_2, MeOH/HCl.

With preliminary information, we performed a brief structure–activity relationship (SAR) study in attempt to understand the structural components necessary to elicit control of biofilm development on this 2-AIT scaffold. First, we wanted to study the impact that the number of methylene units between the 2-AI head and the triazole had upon biofilm activity. A previous study from our group has demonstrated that anti-biofilm activity is dependent upon the linker distance between the 2-AI head and triazole subunit.[9] Specifically, we demonstrated that activity increased as a function of linker length from 1 to 5 methylene units, at which point any further increase in linker length led to decreased activity. This library employed a 3-methylene unit spacer and, given the observation that 5 methylene units was optimal in our previous library, we were curious as to the impact that altering the linker to 5 methylene units had upon anti-biofilm activity of our lead compounds from this library.

The synthetic approach to these second generation analogs is outlined in Scheme 2. 3-Octyn-1-ol was first isomerized[12] to 7-octyn-1-ol followed by Jones oxidation resulting in 7-octynoic acid. Installation of the Boc-protected 2-aminoimidazole was identical to that of alkyne **1** to deliver **5**. We then subjected **5** to the click reaction with azido amides **2b**, **2j**, and **2l** generating 2-AITs **5b**, **5j**, and **5l**, respectively. Compound **2b** was selected due to their interesting biofilm inhibiting activity from the initial study (**4b**), while **2j** and **2l** were chosen as two representative tail groups that promoted biofilm formation (**4j** and **4l**).

Each of the new 2-AIT derivatives were screened at 400 μM for their ability to modulate *E. coli* biofilm formation. Results from these experiments are summarized in Table 2. In terms of biofilm inhibition, **5b** displayed a significant reduction in anti-biofilm activity (58.9%) in comparison to **4b** (90.8%), while the biofilm promotion activity of **5j** was essentially identical to that of **4j** (143% vs 142%). Compound **5l**, however, demonstrated a significant increase in activity in comparison to **4l** (174% vs 41.8%). Therefore, it appears that a 5-methylene unit spacer is not optimal for all 2-AIT conjugates and must be tuned based on the application (inhibition or promotion) and target bacterium.

Given this observation, we elected to probe whether the activity of 2-AIT conjugate was governed by overall molecular length. We have previously observed this phenomenon with our reverse amide series of 2-aminoimidazole conjugates.[13] To answer this question, compound **6** was synthesized and assayed for its ability to inhibit *E. coli* biofilm developmental 400 μM (Figure 1). At this concentration, compound **6** inhibited the formation of *E. coli*

Table 2
SAR biofilm inhibition results for compounds **5b**, **5j**, and **5l**

Compound	*E. coli* biofilm inhibition at 400 μM (%)[a]
5b	58.9 ± 8.8
5j	−142.9 ± 52.5
5l	−174.1 ± 59.3

[a] Values are means of three experiments.

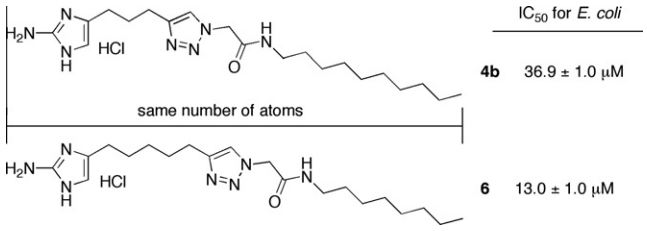


Figure 1. Structure/activity comparison of 4b and **6**.

Table 3
Summary of biological data for **4b**, **4k**, **4p**, and **6**

Compound	*E. coli* IC_{50}[a] (μM)	*E. coli* EC_{50}[a] (μM)	HD_{50}[a] (μM)
4b	36.9 ± 1.0	120.4 ± 12.7	487 ± 87.0
6	13.0 ± 1.0	NA	675 ± 21.6
4k	—	—	>800
4p	—	—	>800

[a] Values are means of three experiments.

biofilms by 95%. To compare the activity of compounds **4b** and **6**, we then performed a dose-response study to determine the IC_{50} value for each compound. For 2-AIT **4b**, an IC_{50} value of 36.9 μM was observed, while for **6** we observed an IC_{50} of 13.0 μM (ca. three-fold increase in activity). Therefore, precise placement of the triazole and amide within the structure of the 2-AIT conjugate can significantly impact activity.

Next, we analyzed the ability of **4b** and **6** to disperse preformed *E. coli* biofilms. Compounds that have the ability to disperse preformed biofilms are significant because they have the potential to remediate established biofilms.[14] Compound **4b** was able to disperse pre-established *E. coli* biofilms with an EC_{50} of 120 μM, where we define EC_{50} as the concentration of compound necessary to elicit 50% biofilm dispersion. Compound **6** showed no dispersal activity against *E. coli*, paralleling our previous results with reverse amide 2-AI anti-biofilm agents.[13] Specifically, biofilm dispersion is dependent on tail composition.

Finally, red blood cell hemolysis of **4b**, **6**, **4k**, and **4p** was performed.[15] Compounds **4b** and **6** were chosen because these compounds had the greatest inhibition activity against *E. coli*. On the other hand, compounds **4k** and **4p** exhibited the greatest biofilm promotion of *E. coli*. The HD_{50} (the dose that lyses 50% of the red blood cells) for **4b** and **6** was found to be 391 μM and 675 μM, respectively, while compounds **4k** and **4p** had HD_{50}'s >800 μM (highest concentration tested). The biological data for all three of these compounds are summarized in Table 3.

In summary, we have developed an approach to the introduction of diversity on the 2-aminoimidazole/triazole scaffold through the use of chloroacetyl chloride as a key building block for structural diversification. Furthermore, we have shown that 2-aminoimidazole-derived anti-biofilm agents can be designed to be selective for a target bacterial population, and that both inhibitors and promoters of biofilm formation can be identified. Based upon these studies, compounds **4b**, **6**, **4k**, and **4p** represent promising small molecule probes to study the role of *E. coli* biofilms in *E. coli* pathogenesis.

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Supplementary data

Supplementary data associated with this article, including representative examples of experimental procedures, characterization data, inhibition and dispersion assay protocols, and bacterial growth curves can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.075.

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