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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

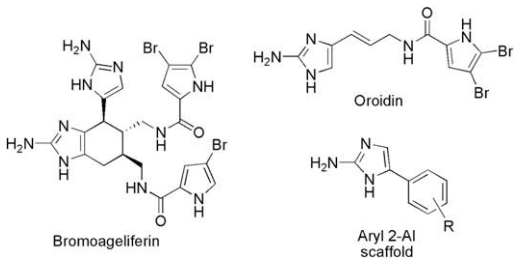

Figure 1. 2-Aminoimidazole-based anti-biofilm molecules.

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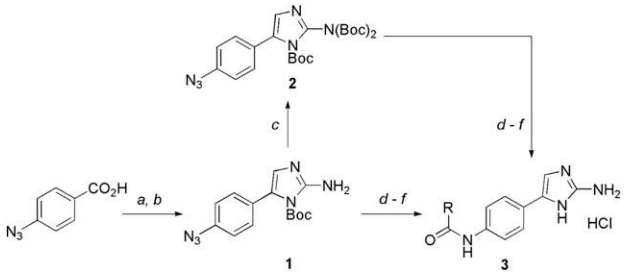
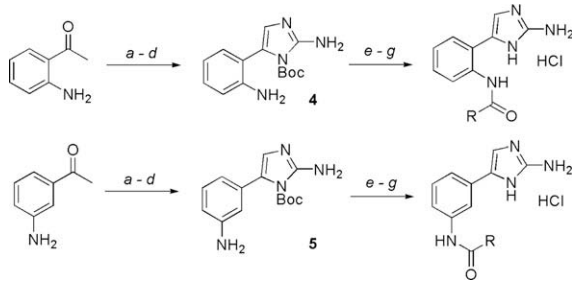


Scheme 1. Synthesis of *para* substituted acylated 2-AI. Reactions and conditions: (a) (i) $(COCl)_2$, DMF, CH_2Cl_2, rt; (ii) CH_2N_2 Et_2O, 0 °C; (iii) concd HBr; (b) Boc-guanidine, DMF, rt; (c) Boc_2O, Et_3N, THF, rt; (d) H_2 (1 atm), 10% Pd/C, THF, rt; (e) RCOCl, Et_3N, CH_2Cl_2, THF, −78 °C to rt;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

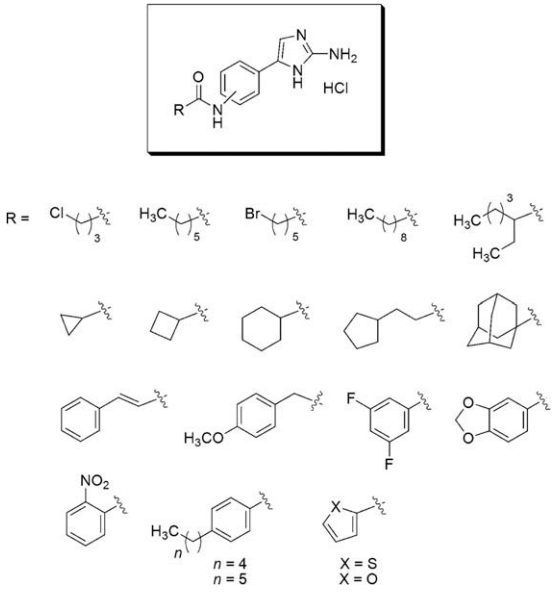
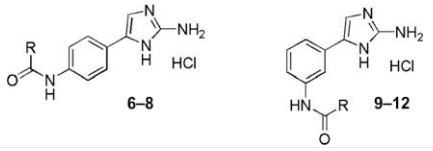
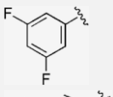
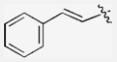
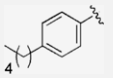
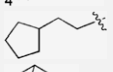
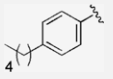


Figure 2. Side chains employed in the acylation reaction of the aryl 2-AI aniline scaffolds.

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

showed the highest activities and, after dose response studies, were determined to exhibit IC_{50}'s of 5.2 μM, 16.3 μM, 39.4 μM, 47.0 μM, 43.4 μM, and 16.5 μM, respectively (Table 1). Follow-up growth curve analysis of each analogue at its respective IC_{50} concentration indicated that only compound **6**, the most active compound, was eliciting activity through a non-microbicidal mechanism. All other compounds showed varying degrees of *E. coli* growth inhibition (Supplementary data).

Once we had demonstrated that the aryl 2-AI scaffold provided a basis for the discovery of active anti-biofilm compounds, we assayed the derivatives for activity in preventing the formation of *Pseudomonas aeruginosa* PA14 and *Acinetobacter baumannii* biofilms. Both *P. aeruginosa* and *A. baumannii* are opportunistic Gram-negative bacteria that place a severe burden on the global health care system. *P. aeruginosa* is known for driving morbidity and mortality of cystic fibrosis patients[20] while *A. baumannii* is known for causing closures of intensive care wards and infecting wounded soldiers in the Middle East.[21] As with the *E. coli* experiments, each compound was initially screened at 100 μM and IC_{50} values were determined for compounds that demonstrated >95% inhibition (Fig. 3, all data summarized in Table 2). Against *P. aeruginosa*, the three compounds **6**, **12**, and **13** revealed IC_{50}'s of 27.6 μM, 16.8 μM, and 39.4 μM, respectively. Against *A. baumannii*, only compound **14** displayed noteworthy activity (IC_{50} = 43.0 μM). Follow-up growth curve analysis at each compounds respective IC_{50} concentration, however, indicated that these compounds elicited reduction in bacterial growth in comparison to untreated controls.

Analysis of the structure–activity relationship (SAR) profile of the aryl 2-AI compound set indicated a few interesting features with respect to biofilm inhibition. First, a number of these derivatives demonstrated a toxic effect to the growth of planktonic *E. coli*, *P. aeruginosa*, and *A. baumannii*. The exception was compound **6**, which was observed to be non-toxic to *E. coli*. Moreover, the position of the difluorobenzyl appendage on the aryl 2-AI scaffold was critical for eliciting maximum activity (Fig. 4). When this group was *para* to the 2-AI head (**6**), the analogue exhibited an *E. coli* IC_{50} of 5.2 μM. Relocation of this group to either the *meta* (**15**), or *ortho* (**16**) positions on the ring severely compromised *E. coli* activity. A modest 64% inhibition at 100 μM was observed in the case of **15** while **16** essentially demonstrated no activity within the concentration range analyzed (<5% biofilm inhibition at 100 μM). This trend was also observed against *P. aeruginosa*. Compound **6** exhibited an IC_{50} of 27.6 μM, while compounds **15** and **16** both had <5% biofilm inhibition at 100 μM.

From a medical standpoint, molecules that inhibit the formation of a bacterial biofilm could be used as a prophylactic measure; however, molecules that disperse pre-formed biofilms could be used to treat established biofilm infections. Therefore, molecules that disperse established biofilms presumably have more therapeutic potential than molecules that simply inhibit their formation. Our active hits were assayed for the ability to disperse existing biofilms from all three strains of bacteria employed in the current study. Biofilms were first allowed to form in the absence of any compound. Media and planktonic bacteria were then washed away and either media alone or media containing compound was added to the residual biofilm. After 24 h, media and planktonic bacteria were again washed away and the remaining biofilm was stained with crystal violet. Only compounds **6** and **7** were able to disperse established *E. coli* biofilms (51% and 67%, respectively at 50 μM), while compound **14** was able to disperse 40% of *A. baumannii* biofilms at 100 μM. None of the compounds were observed to possess the ability to disperse pre-formed *P. aeruginosa* biofilms.

Finally, given the success of previous ventures into studying the anti-biofilm activities of 2-aminoimidazole/triazole conjugates,[10,12] we elected to functionalize intermediate **1** with a triazole isostere to determine if potency could be improved within the aryl 2-AI framework (Scheme 3). Facile preparation of **17** through treatment of **1** with LiHMDS and one equivalent of Boc anhydride[22] followed by subjection to standard conditions for the copper mediated [3+2] cycloaddition reaction employing 1-ethynyl-3,5-difluorobenzene as the alkyne quickly afforded target **18** after deprotection. Unfortunately, preliminary inhibition screens

Table 2
Summary of biofilm inhibition results for compounds **6**–**14** against three Gram-negative strains of bacteria

Compound	*E. coli* Biofilm inhibition IC_{50} values[a]	*P. aeruginosa*[b] *A. baumannii*[c] Biofilm inhibition IC_{50} values[a]
6	5.2 ± 1.1	27.6 ± 2.0[b]
7	16.3 ± 3.7	nd
8	39.4 ± 1.5	nd
9	47.0 ± 1.0	nd
10	43.4 ± 1.3	nd
11	16.5 ± 1.0	nd
12	nd	16.8 ± 1.7[b]
13	nd	39.4 ± 1.4[b]
14	nd	43.0 ± 1.9[c]

All values are reported at μM concentrations.
nd = not determined.
[a] Values are means of three or more experiments.
[b] *P. aeruginosa.*
[c] *A. baumannii.*

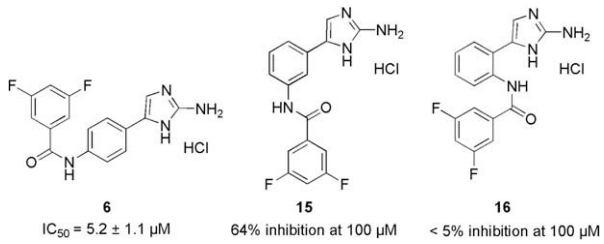


Figure 4. Observed activity of difluorobenzyl analogues on biofilm formation in *E. coli*.

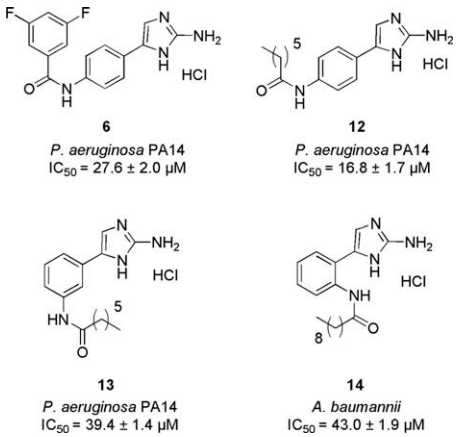
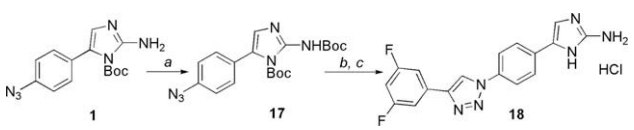


Figure 3. Aryl 2-aminoimidazoles found to inhibit biofilm formation in either *P. aeruginosa* (**6**, **12**, and **13**) or *A. baumannii* (**14**).



Scheme 3. Synthesis of triazole isostere **18**. Reactions and conditions; (a) LiHMDS, Boc_2O, THF, 0 °C; (b) $CuSO_4{\cdot}5H_2O$, sodium ascorbate, 1-ethynyl-3,5-difluorobenzene, CH_2Cl_2, EtOH, H_2O, rt; (c) (i) TFA, CH_2Cl_2, 0 °C; (ii) 2 M HCl in Et_2O.

at 100 μM against all strains revealed very weak activity against all strains (35% inhibition against *E. coli*, <5% biofilm inhibition against *P. aeruginosa*, 21% inhibition against *A. baumannii*). Therefore, it appears that within the aryl 2-AI scaffold, substitution of a triazole is detrimental to anti-biofilm activity when **18** is compared to the most potent analogue **6** (*E. coli* IC_{50} = 5.2 μM).

In conclusion, we have developed a synthetic route to access *ortho*, *meta*, and *para*-substituted aryl amide 2-aminoimidazoles. Using this approach, a set of 30 unique small molecules with varying substitution patterns were screened as potential inhibitors and dispersal agents against biofilms formed by the medically relevant Gram-negative bacteria *E. coli*, *P. aeruginosa*, and *A. baumannii* (Table 2). Compound **6** was identified as a low micromolar *E. coli* biofilm inhibitor and was also found to be non-toxic to the bacterium despite other analogues in the compound set demonstrating poorer activity and greater toxicity towards bacterial growth in all strains. Overall, the data suggests the feasibility of aryl derived 2-aminoimidazoles for development as effective leads for the discovery and further optimization of unique chemical probes for determining the importance of *E. coli* biofilm formation in a variety of *E. coli* driven infections.

Acknowledgments

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Supplementary data

Supplementary data (experimental procedures, characterization data, inhibition and dispersion assay protocols, and bacterial growth curves) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.04.042.

References and notes

1. Donlan, R. M.; Costerton, J. W. *Clin. Microbiol. Rev.* **2002**, *15*, 167.
2. Richards, J. J.; Melander, C. *Anti-Infect. Agents Med. Chem.* **2009**, *8*, 295.
3. Davies, D. *Nat. Rev. Drug Disc.* **2003**, *2*, 114.
4. Richards, J. J.; Huigens, R. W.; Ballard, T. E.; Basso, A.; Cavanagh, J.; Melander, C. *Chem. Commun.* **2008**, *14*, 1698.
5. Richards, J. J.; Ballard, T. E.; Melander, C. *Org. Biomol. Chem.* **2008**, *6*, 1356.
6. Richards, J. J.; Ballard, T. E.; Huigens, R. W.; Melander, C. *ChemBioChem* **2008**, *9*, 1267.
7. Huigens, R. W.; Ma, L.; Gambino, C.; Basso, A.; Moeller, P. D. R.; Cavanagh, J.; Wozniak, D. J.; Melander, C. *Mol. Biosyst.* **2008**, *4*, 614.
8. Ballard, T. E.; Richards, J. J.; Wolfe, A. L.; Melander, C. *Chem. Eur. J.* **2008**, *14*, 10745.
9. Huigens, R. W.; Richards, J. J.; Parise, G.; Ballard, T. E.; Zeng, W.; Deora, R.; Melander, C. *J. Am. Chem. Soc.* **2007**, *129*, 6966.
10. Huigens, R. W.; Rogers, S. A.; Steinhauer, A. T.; Melander, C. *Org. Biomol. Chem.* **2009**, *7*, 794.
11. Ballard, T. E.; Richards, J. J.; Aquino, A.; Reed, C. S.; Melander, C. *J. Org. Chem.* **2009**, *74*, 1755.
12. Rogers, S. A.; Melander, C. *Angew. Chem., Int. Ed.* **2008**, *47*, 5229.
13. Richards, J. J.; Reed, C. S.; Melander, C. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4325.
14. Richards, J. J.; Reyes, S.; Stowe, S. D.; Tucker, A. T.; Ballard, T. E.; Mathies, L. D.; Cavanagh, J.; Melander, C. *J. Med. Chem.* **2009**, *52*, 4582.
15. Yamada, A.; Kitamura, H.; Yamaguchi, K.; Fukuzawa, S.; Kamijima, C.; Yazawa, K.; Kuramoto, M.; Wang, G. Y. S.; Fujitani, Y.; Uemura, D. *Bull. Chem. Soc. Jpn.* **1997**, *70*, 3061.
16. Richards, J. J.; Melander, C. *J. Org. Chem.* **2008**, *73*, 5191.
17. Ali, A.; Kiran, G. S.; Cao, R.; Anjum, S. G.; Nalam, M. L.; Schiffer, C. A.; Rana, T. M. *J. Med. Chem.* **2006**, *49*, 7342.
18. Croxen, M. A.; Finlay, B. B. *Nat. Rev. Microbiol.* **2010**, *8*, 26.
19. O'Toole, G. A.; Kolter, R. *Mol. Microbiol.* **1998**, *30*, 295.
20. Moreau-Marquis, S.; Stanton, B. A.; O'Toole, G. A. *Pulm. Pharmacol. Ther.* **2008**, *21*, 595.
21. Hujer, K. M.; Hujer, A. M.; Hulten, E. A.; Bajaksouzian, S.; Adams, J. M.; Donskey, C. J.; Ecker, D. J.; Massire, C.; Eshoo, M. W.; Sampath, R.; Thomson, J. M.; Rather, P. N.; Craft, D. W.; Fishbain, J. T.; Ewell, A. J.; Jacobs, M. R.; Paterson, D. L.; Bonomo, R. A. *Antimicrob. Agents Chemother.* **2006**, *50*, 4114.
22. Ando, N.; Terashima, S. *Synlett* **2006**, 2836.