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A 2-Aminobenzimidazole That Inhibits and Disperses Gram-Positive Biofilms through a Zinc-Dependent Mechanism

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Bacterial biofilms, which are defined as surface-attached communities of bacteria encased in an extracellular matrix of biomolecules, represent a significant hurdle for infectious disease control.[1] Within the biofilm state, bacteria are upward of 1000-fold more resistant to antibiotics and are inherently resistant to host immune responses.[2] The National Institutes of Health estimate that 75% of all infections are biofilm-based and that biofilms drive many hospital-acquired infections (such as those resulting from implanted medical devices).[3]

Because of the prominence of biofilms in the biomedical community, there has been a significant effort to identify molecules that target bacteria in the biofilm state.[4] To define additional motifs with antibiofilm properties, we became interested in assessing the activity of 2-aminobenzimidazole (2-ABI) derivatives. The decision to study the 2-ABI scaffold was based upon previous studies in our group that analyzed the antibiofilm properties of a number of small molecules based upon the natural product bromoageliferin (Figure 1).[5−7] One of the first derivatives studied was TAGE,[6] a bicyclic 2-aminoimidazole (2-AI) that represents the core architecture of bromoageliferin. The 2-ABI scaffold is a readily accessible, aromatized analogue of TAGE, which we hypothesized would provide unique and/or improved antibiofilm properties in comparison with 2-AIs.

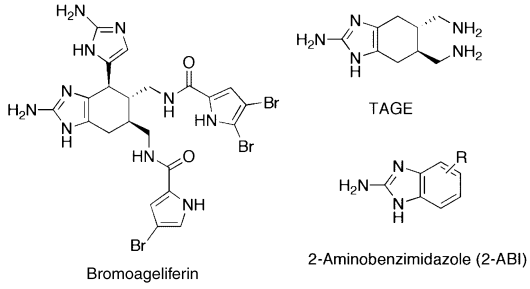
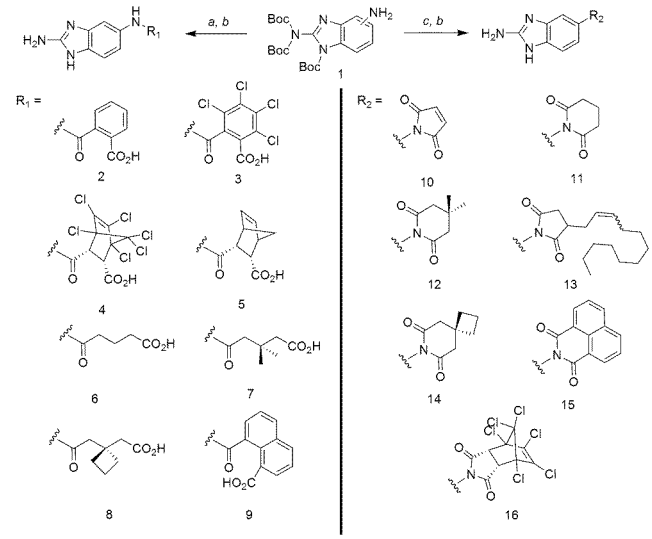


Figure 1

To explore this hypothesis, a preliminary library of 2-ABI analogues was synthesized for antibiofilm evaluation (Scheme 1). An isomeric mixture of tri-Boc-protected 5-amino-2-ABI (**1**)[8] was acylated with an array of cyclic anhydrides, and the products were subsequently deprotected to generate an initial set of isomerically pure 2-ABI derivatives. A related set of 2-ABI derivatives was also synthesized in which reaction of **1** with cyclic anhydrides under refluxing toluene followed by Boc deprotection delivered the target 2-ABI-imides.

Each compound was screened at 100 μM for its ability to inhibit the formation of *Pseudomonas aeruginosa* PAO1 and multidrug-resistant *Acinetobacter baumannii* (MDRAB) biofilms. As opposed to the 2-AI class of antibiofilm agents, none of the 2-ABI derivatives were able to inhibit formation of biofilms of either γ-proteobacteria. Next, each compound was screened for its ability to inhibit biofilm development in three Gram-positive bacterial strains that are prominent in human medicine. The three strains chosen were MRSA, vancomycin-resistant *Enterococcus faecium* (VRE), and *Staphylococcus epidermidis*. Again, all of the 2-ABI derivatives were initially screened at 100 μM. Each compound was able to inhibit biofilm development of at least two of the bacterial strains. A dose−response study was then initiated to determine both the IC_{50} and EC_{50} values of each compound toward the bacterial strains (Table 1). Here, IC_{50} is defined as the concentration of compound that inhibits biofilm development by 50%, while EC_{50} is defined as the concentration of compound that disperses 50% of a preformed biofilm. Of these compounds, 2-ABI derivative **3** had the best activity profile, with IC_{50} and EC_{50} values of 890 nM and 2.9 μM (MRSA), 1.4 μM and 75 μM (VRE), and 570 nM and 7.3 μM (*S. epidermidis*), respectively. Growth-curve and colony-count analyses demonstrated that each compound was nonmicrobicidal at the IC_{50} value.

Scheme 1. Synthesis of the 2-Aminobenzimidazole Library[a]



[a] Reaction conditions: (a) cyclic anhydride, CH_2Cl_2; (b) HCl, H_2O, THF; (c) cyclic anhydride, toluene, 110 °C.

Next, the mechanistic basis of how **3** was able to inhibit and disperse bacterial biofilms was investigated. Iron levels are known to effect Gram-positive biofilm development[9] and were deemed a plausible driver of the observed antibiofilm activity. To examine Fe(II)-related antibiofilm behavior, a dose-dependent study was performed against each Gram-positive bacteria in which the ability of **3** to inhibit biofilm development was measured under increasing Fe(II) concentration. It was noted that the activity of **3** was not affected by increasing the Fe(II) concentration, thus indicating iron homeostasis is not involved in 2-ABI antibiofilm activity.

Next, Zn(II) homeostasis was examined. Zn(II) has been implicated in the pathogenesis of Gram-positive bacterial infections

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Table 1. Biofilm Inhibition and Dispersion Data

	IC_{50} (μM); EC_{50} (μM)		
compound	MRSA	VRE	*S. epidermidis*
2	5.9 ± 1.3; 35 ± 2.8	21 ± 2.9; 75 ± 6.7	32 ± 3.8; 44 ± 7.9
3	0.89 ± 0.01; 2.9 ± 0.4	1.4 ± 0.4; 75 ± 2.1	0.57 ± 0.2; 7.3 ± 0.2
4	1.5 ± 0.4; 6.6 ± 0.2	210 ± 35; 280 ± 8.3	1.9 ± 0.4; 19 ± 5.8
5	16 ± 2.9; 63 ± 6.9	63 ± 9.1; 107 ± 11	4.1 ± 2.0; 45 ± 6.1
6	>300; >300	22 ± 8.3; 88 ± 5.2	1.9 ± 0.9; 13 ± 6.1
7	69 ± 2.7; 205 ± 15	0.9 ± 0.3; 94 ± 10	240 ± 20; 260 ± 6.7
8	103 ± 6.3; 250 ± 19	2.4 ± 0.7; >300	73 ± 8.2; 175 ± 8.5
9	>300; >300	0.9 ± 0.4; 6.3 ± 1.9	6.2 ± 1.2; 30 ± 5.7
10	2.8 ± 0.8; 36 ± 1.7	7.7 ± 0.8; 150 ± 8.4	180 ± 17; 266 ± 7.3
11	17 ± 1.9; 25 ± 2.0	1.6 ± 0.9; 280 ± 4.8	12 ± 1.0; 137 ± 15
12	48 ± 4; 101 ± 6	16 ± 4.7; 203 ± 5.5	0.9 ± 0.3; 17 ± 4.0
13	9.6 ± 0.8; 14 ± 2.9	6.9 ± 2.2; 30 ± 8.5	15 ± 2.7; 70 ± 4.2
14	42 ± 2.4; 48 ± 2.1	1.9 ± 0.5; 230 ± 9.6	170 ± 14; 180 ± 13
15	>300; >300	69 ± 10; 110 ± 2.9	24 ± 6.3; 81 ± 4.9
16	5.7 ± 0.5; 9.5 ± 2.5	7.4 ± 1.7; 31 ± 7.8	1.5 ± 0.5; 33 ± 3.7

and is an important regulator of biofilm formation.[10] Furthermore, generic Zn(II) chelators such as diethylenetriaminepentaacetic acid (DTPA) are known to inhibit the formation of *Staphylococcus* spp. biofilms at midmicromolar concentrations.[10] As opposed to the Fe(II) study, it was noted that Zn(II), in a dose-dependent manner against each Gram-positive bacteria, suppressed the ability of **3** to inhibit biofilm development. When supplemented with 200 μM $ZnCl_2$, **3** was unable to inhibit biofilm formation.

In view of this Zn(II) dependence, the ability of **3** to bind zinc directly was examined to ascertain whether the biofilm inhibition was potentially occurring via a Zn(II)-binding mechanism. To answer this question, an ^{1}H NMR binding experiment was performed in which the chemical shifts of **3** were measured in the presence of 0, 0.5, and 1.0 equiv of $ZnCl_2$ (Figure 2). Comparison of the aromatic peaks clearly indicates peak broadening with increasing amounts of $ZnCl_2$, indicating that **3** directly binds $ZnCl_2$. As a control, the same experiment was performed with $FeSO_4$. No change in the NMR signal of **3** was observed with 0, 0.5, or 1.0 equiv of $FeSO_4$.

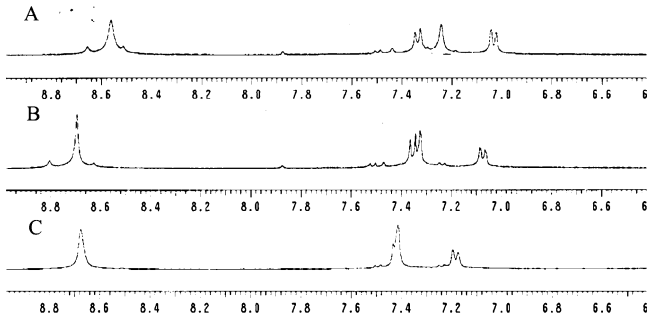
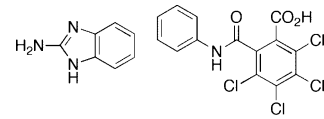


Figure 2. NMR spectra of 228 mM **3** with (A) 0.0, (B) 0.5, and (C) 1.0 equiv of $ZnCl_2$.

Finally, two control compounds were tested (**17** and **18**; Figure 3) to probe whether substructures within **3** were responsible for the antibiofilm properties of the compound. Neither compound was able to inhibit MRSA, VRE, or *S. epidermidis* biofilm formation at 100 μM (the highest concentration tested). Furthermore, no change was noted in either ^{1}H NMR spectrum in the presence of $ZnCl_2$.



17 **18**

Figure 3. Control compounds.

In conclusion, a novel inhibitor and disperser of Gram-positive biofilms that is based upon a 2-ABI scaffold and operates via a Zn(II)-dependent mechanism has been identified. Preliminary NMR studies indicated that this compound binds zinc directly. These 2-ABI molecules are unique in that they are some of the most potent antibiofilm agents identified to date that do not operate through a microbicidal mechanism. Furthermore, molecules that bind Zn(II) have recently been shown to be efficacious in animal models of disease;[11,12] therefore, appropriately designed 2-ABI molecules may provide a basis for remediating biofilm-based infections.

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Supporting Information Available: Experimental procedures and characterization data for all new compounds; planktonic growth curves for MRSA, VRE, and *S. epidermidis* in the presence and absence of active 2-ABIs; dose−response curves of **3** in the absence and presence $ZnCl_2$ and $FeSO_4$; and dose−response curves and NMR binding studies for **17** and **18**. This material is available free of charge via the Internet at http://pubs.acs.org.

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