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COMMUNICATIONS

Published on Web 05/15/2007

Inhibition of *Pseudomonas aeruginosa* Biofilm Formation with Bromoageliferin Analogues

Robert W. Huigens III,[†] Justin J. Richards,[†] Gina Parise,[‡,§] T. Eric Ballard,[†] Wei Zeng,[†] Rajendar Deora,[‡,§] and Christian Melander*[,†]

Department of Chemistry, North Carolina State University, Raleigh, North Carolina 27695, and Program in Molecular Genetics and Department of Microbiology and Immunology, Wake Forest University Health Sciences, Winston-Salem, North Carolina 27157

Received December 16, 2006; E-mail: christian_melander@ncsu.edu

Biofilms play a significant role in infectious disease.[1] Biofilms are formed when planktonic bacteria adhere to a surface and initiate the formation of a microcolony that exists as a community encased in a protective extracellular matrix.[1,2] It is estimated that biofilms account for up to 80% of microbial infections in the body.[3,4] Biofilms also underlie importunate infections of implanted medical devices.[2] Within a biofilm, bacteria display differential gene expression[4] and are upward of 1000-times more resistant to conventional antibiotic treatment.[5]

Even though there is a pressing medical need for strategies to control biofilm formation, there is a paucity of small molecules that have been reported to inhibit the formation of terrestrial-based bacterial biofilms.[1] Current efforts toward small molecule-based strategies to control biofilm formation have focused almost exclusively on inhibiting quorum sensing (QS), a signaling cascade that is critical for bacterial communication[6,7]

To provide alternative molecular scaffolds that inhibit the formation of terrestrial-based bacterial biofilms, we have turned to anti-microfouling marine natural products as a source for structural insight to guide molecular design. Microfouling is the first step of biofouling that entails the formation of a marine-based bacterial biofilm on the surface of a submerged object.[8] Previous studies in this field have focused on the use of brominated furanones, simple marine natural products that accelerate the turnover of LuxR-type proteins and disrupt QS.[9] We posited that simple structural motifs that are prevalent in the more complex, anti-microfouling natural products would provide novel chemical architecture for inhibitors of terrestrial-based bacterial biofilms.

The first structural motif we have investigated is based upon the marine natural product bromoageliferin (Figure 1). Bromoageliferin has been reported to possess anti-biofilm activity against the marine α-proteobacteria *R. salexigens*[10] and is a member of the oroidin class of biologically active natural products that are characterized by a 2-aminoimidazole (2-AI) subunit.[11] The prevalence of the 2-AI subunit led us to hypothesize that this structural motif, in tandem with the bicyclic core of bromoageliferin, was the key pharmacophore that imparts biological activity. To test our hypothesis, we synthesized and assayed two bromoageliferin analogues, *trans*-bromoageliferin analogue **1** (TAGE) and the *cis*-bromoageliferin analogue **2** (CAGE) (Figure 1) for anti-biofilm activity. We also synthesized 4-(3-aminopropyl)-2-aminoimidazole as a control.

4-(3-Aminopropyl)-2-aminoimidazole was synthesized as previously described.[12] The synthesis of TAGE (**1**) is outlined in Scheme 1. The known diol **3**[13] was bismesylated and then reacted with sodium azide to yield the diazide **4** in 90% yield. Diazide **4** was epoxidized with *m*-CPBA, and the epoxide ring was subsequently opened with sodium azide to yield the triazidoalcohol **5**. Triazidoalcohol **5** was hydrogenated in the presence of Boc_2O, and then oxidized with PDC to generate the tri-Boc protected α-aminoketone **6**. Quantitative removal of the Boc-groups were affected with 1:4/TFA/CH_2Cl_2, and the resulting TFA salt was treated with a saturated solution of HCl in methanol to give the HCl salt of the α-aminoketone. Finally, condensation with cyanamide[12] generated TAGE (**1**) in 24% overall yield in eight steps from diol **3**. Application of a similar synthetic sequence to the known cis-diol **7**[14] generated CAGE (**2**) in 18% overall yield.

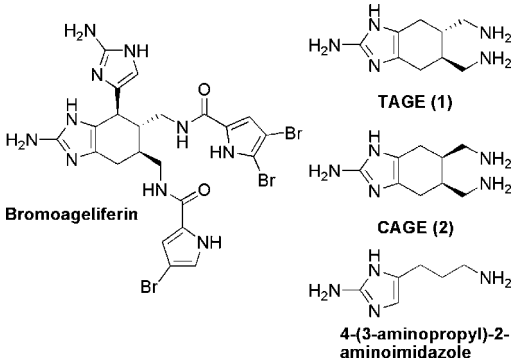
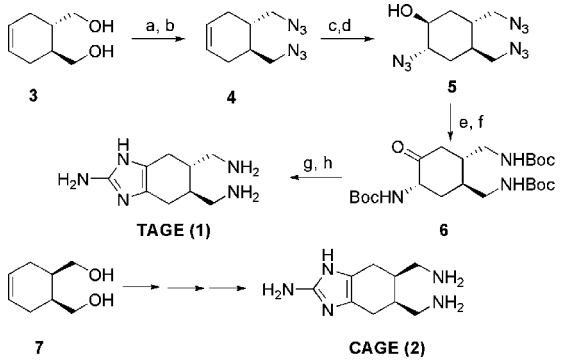


Figure 1. Bromoageliferin and bromoageliferin analogues.

Scheme 1. Synthesis of TAGE (**1**) and CAGE (**2**)[a]



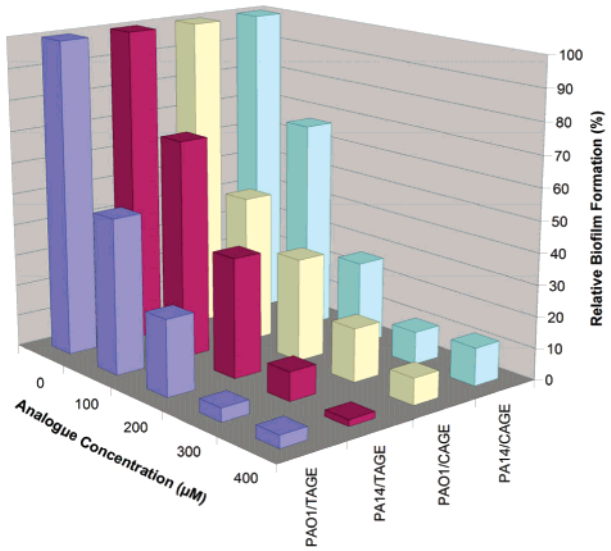
[a] Reaction conditions (TAGE): (a) MsCl, TEA, CH_2Cl_2, −78 °C −25 °C; (b) NaN_3, DMF, 100 °C, 90%; (c) *m*-CPBA, CH_2Cl_2, 88%; (d) NaN_3, DMF, 120 °C, 84%; (e) H_2 (1 atm), 10% Pd/C, Boc_2O, DMF, 71%; (f) PDC, DMF, 70%; (g) TFA/CH_2Cl_2, then HCl/MeOH; (h) NH_2CN, H_2O/pH = 4.3, 95 °C, 74%.

With both 2-aminoimidazole derivatives in hand, we tested the ability of these simplified bromoageliferin scaffolds to inhibit the formation of *Pseudomonas aeruginosa* biofilms. *P. aeruginosa* is a γ-protoeobacteria that is in the same phylum as *R. salexigens*

[†] North Carolina State University.
[‡] Program in Molecular Genetics, Wake Forest University Health Sciences.
[§] Department of Microbiology and Immunology, Wake Forest University Health Sciences.

10.1021/ja069017t CCC: $37.00

Chart 1. Relative Biofilm Formation of PAO1 and PA14 in the Presence of TAGE (**1**) or CAGE (**2**) after 24 h.



and is a serious threat to cystic fibrosis (CF) patients.[15,16] As CF patients age, *P. aeruginosa* becomes the predominant pulmonary pathogen and is present in ca. 85% of cultures isolated from patients with advanced disease.[15,16] Despite significant progress in the management of CF symptoms, virtually all CF patients succumb to chronic pulmonary infections.

The *P. aeruginosa* bacterial strains PAO1 or PA14 were allowed to form biofilms in a 96-well plate in the absence or presence of TAGE (**1**) or CAGE (**2**). After 24 h,[17] the media and planktonic bacteria were removed, the wells were washed vigorously, and crystal violet was added. Crystal violet stains the bacterial biofilm that forms on the inside wall of the well, which, following ethanol solubilization, can be quantitated by spectrophotometry (A_{540}).[21] Chart 1 summarizes the results of this assay. We determined IC_{50} values of biofilm inhibition for TAGE (**1**) against PAO1 (100 μM) and PA14 (190 μM) and IC_{50} values for CAGE (**2**) against PAO1 (100 μM) and PA14 (180 μM). 4-(3-Aminopropyl)-2-aminoimidazole displayed only marginal activity at 500 μM (20% against PAO1 and 15% against PA14) and did not inhibit biofilm development at 100, 200, 300, or 400 μM (data not shown).

A fundamental question underlying the inhibition assays was the effect each compound had on planktonic growth. Growth curves were determined for both PAO1 and PA14 in the absence and presence of TAGE (**1**) or CAGE (**2**) (Supporting Information). TAGE (**1**) showed no effect on the growth of either PAO1 or PA14 over 24 h at 100, 200, or 300 μM. At 400 μM TAGE (**1**), we observed a modest reduction in bacterial growth (ca. 25%), for both PAO1 and PA14, while we observed significant reduction in planktonic growth (>50%) at 500 μM TAGE (**1**). For CAGE (**2**), we observed no effect on bacterial growth (for either PAO1 or PA14) at 100 or 200 μM, modest reduction at 300 μM (≤25%), and significant reduction (>50%) at both 400 and 500 μM. Colony counts were also performed for both PAO1 and PA14 grown in the absence or presence of TAGE (**1**) and CAGE (**2**) that verified that no reduction in the growth curve correlated with no reduction in viable colonies (Supporting Information).

In conclusion, we have identified two analogues of the marine natural product bromoageliferin that have biological activity against the formation of *P. aeruginosa* biofilms. Although both compounds inhibit the formation of *P. aeruginosa* biofilms, we note differential toxicity between TAGE (**1**) and CAGE (**2**) toward planktonic *P. aeruginosa* bacteria. This activity is driven by the inversion of one stereocenter. At the higher concentrations of CAGE (**2**) (400 and 500 μM) where we observe significant antimicrobial activity, the observed reduction of biofilm mass may be due to a combination of bactericidal and biofilm inhibition activity. Further experiments are necessary to decouple these two effects. Both compounds have no structural homology to any of the known inhibitors of *P. aeruginosa* biofilm formation[1] and may be operating by a unique mechanism that can be exploited for future drug development efforts. We are currently establishing the mechanism of action for both these compounds and will report our findings in due course.

Acknowledgment. Financial support is gratefully acknowledged from NCSU, Burroughs-Wellcome (predoctoral fellowship to J.J.R.), and GSK (predoctoral fellowship to T.E.B.). Research in the laboratory of R.D. is supported by funds from WFU Health Sciences and by grants from the USDA (CSREES-USDA Grant No. 2006-35604-16874) and the NIH (Grant R21 AI071054). Mass spectra were obtained at the Mass Spectrometry Laboratory for Biotechnology at NCSU. We thank Professor Daniel Wozniak (WFU) for providing PAO1 and PA14 and for helpful discussions. We also thank Professor Reza Ghiladi (NCSU) for spectroscopic assistance.

Supporting Information Available: Experimental procedures and characterization data for all new compounds; planktonic growth curves and colony counts for PAO1 and PA14 in the presence and absence of TAGE and CAGE. This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) We determined that both PAO1 and PA14 reached maximum cellular density (in the absence of TAGE or CAGE) after 24 hours of growth (see Supporting Information).

JA069017T