JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

COMMUNICATION

pubs.acs.org/JACS

Intercepting Bacterial Indole Signaling with Flustramine Derivatives

Cynthia A. Bunders,[†] Marine J. Minvielle,[†] Roberta J. Worthington,[†] Minoshka Ortiz,[†] John Cavanagh,[‡] and Christian Melander*[,†]

[†]Department of Chemistry, North Carolina State University, Raleigh, North Carolina 27695, United States

[‡]Department of Molecular and Structural Biochemistry, North Carolina State University, Raleigh, North Carolina 27695, United States

Supporting Information

ABSTRACT: Indole signaling is one of the putative universal signaling networks in bacteria. We have investigated the use of desformylflustrabromine (dFBr) derivatives for the inhibition of biofilm formation through modulation of the indole-signaling network in *Escherichia coli* and *Staphylococcus aureus*. We have found dFBr derivatives that are 10−1000 times more active than indole itself, demonstrating that the flustramine family of indolic natural products represent a privileged scaffold for the design of molecules to control pathogenic bacterial behavior.

The emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has become a significant global public health threat. In 2005, almost 95 000 people acquired methicillin-resistant *Staphylococcus aureus* (MRSA) infections in the United States, of which nearly 19 000 people died—more than die annually from medical complications of HIV/AIDS.[1] Without the development of innovative approaches to combat these multi-drug-resistant pathogens, many fields of medicine such as surgery and care of the critically ill will be severely affected. This situation is so dire that the Infectious Disease Society of America has recently issued a call to action for the medical community.[2]

One promising approach for controlling bacterial infections is to develop small molecules that attenuate bacterial behaviors that are detrimental to the human host.[3,4] Examples of these behaviors include the production of virulence factors and biofilm formation. Such an approach, which operates through a nonmicrobicidal mechanism, would be highly desirable, as this would not exert evolutionary pressure on the microorganisms to adapt and become resistant. Seminal approaches in this area include the use of acyl homoserine lactone derivatives,[5,6] brominated furanones,[7−9] and modulators of autoinducer-2 (AI-2)[10−14] (Figure 1).

Indole signaling is involved in the regulation of a number of bacterial behaviors.[15−17] Our group has recently become interested in harnessing this signaling pathway to control these behaviors through the design of small-molecule indole derivatives. We have chosen indole signaling because indole is a putative universal signal (along with AI-2) among diverse bacteria.[16] Eighty-five species of bacteria have been documented to produce indole, while both indole-positive and indole-negative strains of bacteria exhibit changes in behavior based upon the extracellular presence of indole. For example, indole modulates biofilm formation,[17] virulence,[18] antibiotic resistance,[19] and acid tolerance,[20] all key behaviors of pathogenic bacteria. Therefore, highly active indole derivates have the potential to modulate pathogenic behavior in wide swaths of bacteria (both Gram-positive and Gram-negative) and represent a potentially powerful approach for controlling pathogenic bacterial behavior in vivo. To the best of our knowledge, however, highly active modulators of indole signaling have not been developed, nor have molecular design principles been elucidated to augment the activity of indole in the context of bacterial indole signaling.

Our group has had success employing marine natural products as structural templates for the design of small molecules that control biofilm formation and antibiotic resistance.[21,22] To apply this approach to indole signaling, we became interested in investigating the potential of flustramine derivatives to control biofilm formation. The flustramines are a group of indole-derived natural products isolated from the North Sea bryozoans *Flustra foliacea*.[23−25] This family consists of 11 secondary metabolites: six pyrroloindoline and five indolic alkaloids (representative members are depicted in Figure 2). It was noted that these specific bryozoans contain no microbial settling on the distal part of the zooid, implying that they possess a chemical defense system geared toward controlling bacterial behavior.

We first investigated desformylflustrabromine (dFBr) as a potential modulator of bacterial behavior by assaying for its ability to inhibit biofilm formation. dFBr was synthesized as previously reported[26] and tested for the ability to modulate *Escherichia coli* and *S. aureus* biofilm formation (representative Gram-negative and Gram-positive strains) using the crystal violet reporter assay. dFBr inhibited *E. coli* and *S. aureus* biofilm formation with IC_{50} values of 174 and 70 μM, respectively. Follow-up growth curve analysis indicated that dFbr inhibits *E. coli* biofilm formation through a toxic mechanism, while early growth delay (4 h) was observed with *S. aureus* (bacterial density was equivalent to untreated control at 6, 8, and 24 h). Given the goal of identifying nontoxic modulators of bacterial behavior, we posited that we could modulate the structure of dFBr to develop indole derivatives that inhibit biofilm development in both bacterial strains through a nontoxic mechanism analogous to that for indole itself. To achieve this aim, we set out to probe systematically each of the four areas of highlighted diversity for its impact on the activity (Figure 3). The importance of the bromine (region A) was probed by synthesizing and assaying debromodesformylflustrabromine (Figure 4). This compound

Received: October 19, 2011
Published: November 17, 2011

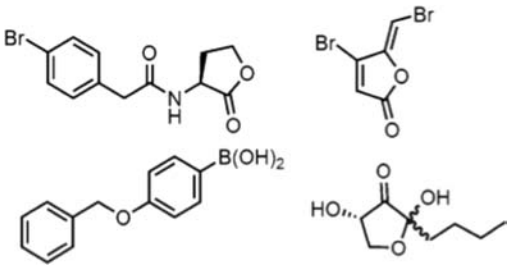

Figure 1. Synthetic small molecules that control bacterial behavior.

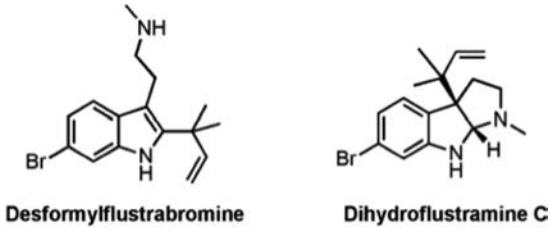

Figure 2. Representative members of the flustramine family.

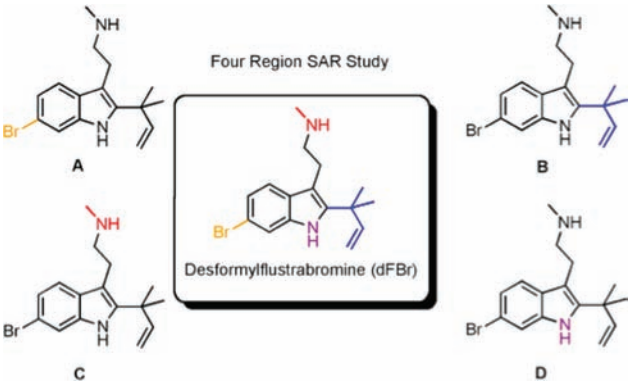

Figure 3. Four regions within the dFBr scaffold that could be rapidly modulated to deliver compounds that control bacterial behavior.

was found to be essentially devoid of activity. The reverse prenyl group (region B) was modulated by employing a Grandberg Fischer indolization to introduce C2 substituents, while substituents on the aliphatic nitrogen (region C) were introduced by alkylation of nosyl-protected indole **1** followed by installation of the reverse prenyl group, bromination, and deprotection. Finally, substituents on the indole nitrogen (region D) were introduced through protected tryptamine derivative **2** to probe the importance of the N−H bond and steric/electronic constraints (an inclusive list of all analogues screened is provided in the Supporting Information). Each derivative was then assayed for its ability to modulate *E. coli* and *S. aureus* biofilm formation.

The most potent analogue synthesized was derivative **3** (Figure 5), which recorded IC_{50} values of ca. 5.9 μM against *S. aureus* and 53 μM against our *E. coli* strain. Compound **4** was also found to be moderately effective, exhibiting IC_{50} values of 80 and 65 μM against *E. coli* and *S. aureus*, respectively. Follow-up growth curve and colony count analyses indicated that both compounds modulate biofilm development via a nonmicrobicidal mechanism.

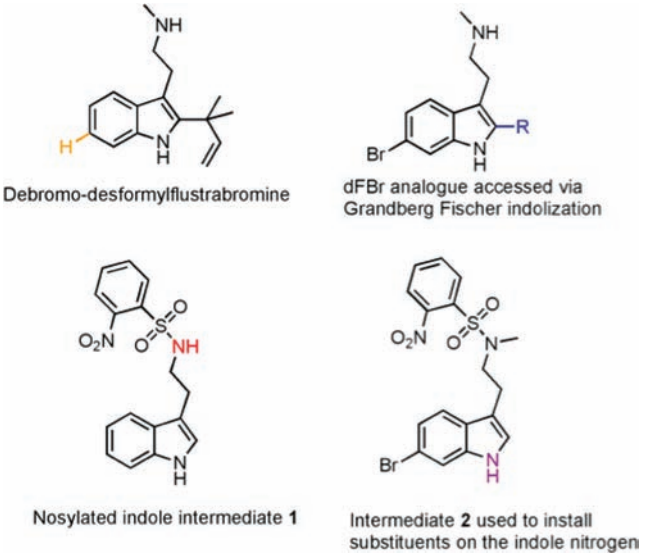

Figure 4. Derivatives and intermediates used to access dFBr analogues.

To establish that the biofilm inhibition activity of the dFBr analogue **3** occurs via the indole signaling pathway, we compared the activity of **3** to that of indole against the *E. coli* strain BW25113, an isogenic *sdiA* knockout mutant, and an isogenic *tnaA* knockout mutant as a function of temperature (25 and 37 °C). Indole signaling pathways have been most widely studied in *E. coli*, and it has been shown that the transcriptional regulator SdiA is involved in the control of biofilm formation by indole in *E. coli* at 37 °C, although the exact mechanism by which this occurs has not yet been elucidated.[17,27] Indole signaling in *E. coli* has been observed primarily at temperatures lower than 37 °C, and it has been demonstrated that the addition of exogenous indole reduces biofilm formation to a greater extent at 25 °C than at 37 °C.

Analogous to previous reports, we found that biofilm formation by the wild-type strain was reduced in the presence of indole in a dose-dependent manner at 25 °C, while a lesser effect was observed at 37 °C (65% inhibition in the presence of 1 mM indole at 25 °C compared with 38% at 37 °C) (Figure 6). Similarly biofilm formation by the *tnaA* mutant, which lacks the ability to produce indole and should therefore exhibit a greater response to the addition of exogenous indole, exhibited a dose-dependent response that was amplified at 25 °C relative to 37 °C (74% inhibition in the presence of 1 mM indole at 25 °C compared with 25% at 37 °C). Against the *sdiA* mutant, indole addition resulted in a considerable response at 25 °C, reducing biofilm formation by 87% at 1 mM, while the response observed at 37 °C was reduced from that exhibited by the wild type (33% inhibition for the wild type compared with 9% for the *sdiA* mutant at 250 μM).

Compound **3** was much more active than indole as a biofilm inhibitor against all three strains, exhibiting activity at 10−100 μM comparable to that of indole at 250−1000 μM (Figure 7). As predicted, **3** displayed activity trends as a function of temperature similar to those for indole, affecting biofilm formation to a greater degree at 25 °C than at 37 °C for the mutant strains. Furthermore, the addition of **3** resulted a much greater effect on biofilm formation by the wild type than by the *sdiA* mutant at 37 °C (82% inhibition for the wild type compared with 49% for the *sdiA* mutant at 100 μM), indicating that, as for

dx.doi.org/10.1021/ja209836z |*J. Am. Chem. Soc.* 2011, 133, 20160–20163

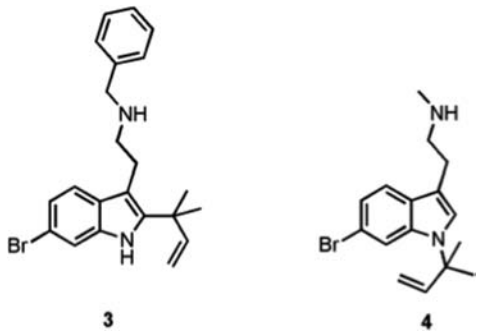

Figure 5. Lead dFBr analogues **3** and **4**.

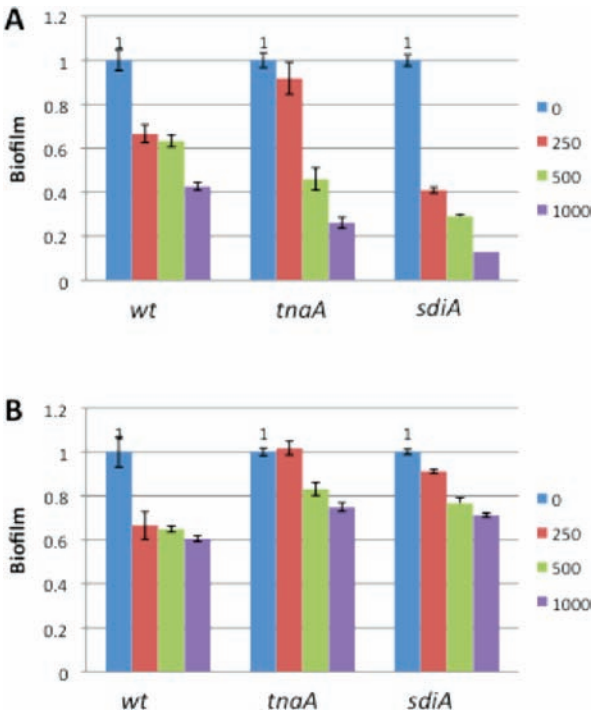

Figure 6. Biofilm inhibition activity of indole against wild-type and knockout *E. coli* strains: (A) 25 °C; (B) 37 °C. Concentrations in μM.

indole, the biofilm inhibitory activity of **3** is partially dependent upon SdiA at elevated temperatures.

In conclusion, we have employed desformylflustrabromine (dFBr) as a structural template to design indolic derivatives that are nonmicrobicidal inhibitors of biofilm formation. Lead compound **3** is 10−1000 times more active than indole. Mechanistic studies in wild-type and knockout *E. coli* strains demonstrated that, as for indole itself, the activity of lead compound **3** is dependent on temperature, SdiA, and TnaA, thus suggesting that the antibiofilm activity of **3** may result from modulation of indole-based signaling pathways. The fact that **3**, as for indole, retains some activity against the *sdiA* mutant suggests that factors other than SdiA are involved in biofilm regulation by indole in *E. coli*. Indeed, indole has been shown to affect the expression of 59 genes in biofilm bacteria at 37 °C, including *yceK*, which was shown to affect biofilm formation.[27] Since indole is a putative universal signal among diverse bacteria, **3** can be employed as a probe to investigate further the effects of manipulating indole signaling pathways in vitro and in vivo, as a

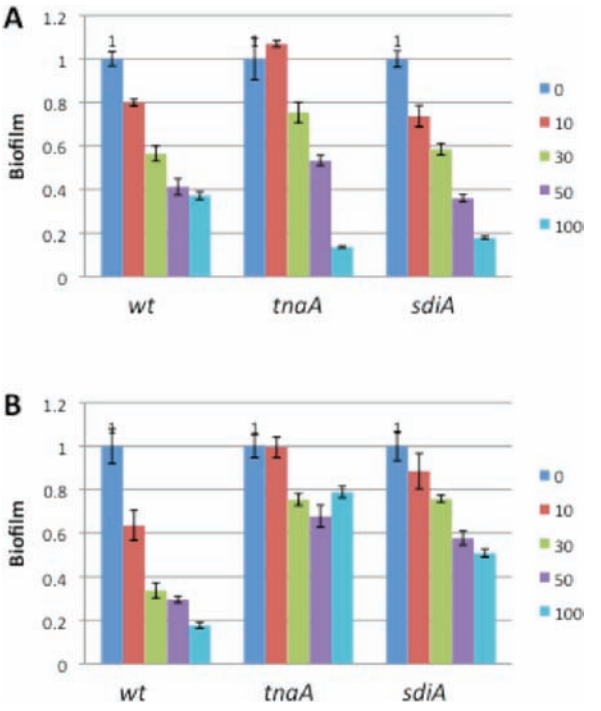

Figure 7. Biofilm inhibition activity of compound **3** against wild-type and knockout *E. coli* strains: (A) 25 °C; (B) 37 °C. Concentrations in μM.

mechanistic probe to deconvolute indole signaling in both indole-positive and indole-negative bacteria, and ultimately as a model to determine the therapeutic potential of controlling pathogenic bacterial behavior in vivo.

■ ASSOCIATED CONTENT

Supporting Information. Synthetic methods and compound characterization for all new compounds, representative ^{1}H NMR and ^{13}C NMR spectra, protocols for biofilm inhibition assays, and representative dose−response curves and growth curves for lead compounds against all bacterial strains studied. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
ccmeland@ncsu.edu

■ ACKNOWLEDGMENT

The authors thank the National Institutes of Health (GM055769 to J.C. and C.M.) for support of this work.

■ REFERENCES

(1) Klein, E.; Smith, D. L.; Laxminarayan, R. *Emerging Infect. Dis.* **2007**, *13*, 1840–1846.
(2) Spellberg, B.; Guidos, R.; Gilbert, D.; Bradley, J.; Boucher, H. W.; Scheld, W. M.; Bartlett, J. G.; Edwards, J., Jr. *Clin. Infect. Dis.* **2008**, *46*, 155–164.
(3) Bjarnsholt, T.; Givskov, M. *Philos. Trans. R. Soc., B* **2007**, *362*, 1213–1222.
(4) Rasmussen, T. B.; Givskov, M. *Int. J. Med. Microbiol.* **2006**, *296*, 149–161.

(5) Geske, G. D.; Wezeman, R. J.; Siegel, A. P.; Blackwell, H. E. *J. Am. Chem. Soc.* **2005**, *127*, 12762–12763.
(6) Smith, K. M.; Bu, Y. G.; Suga, H. *Chem. Biol.* **2003**, *10*, 81–89.
(7) Hentzer, M.; Riedel, K.; Rasmussen, T. B.; Heydorn, A.; Andersen, J. B.; Parsek, M. R.; Rice, S. A.; Eberl, L.; Molin, S.; Hoiby, N.; Kjelleberg, S.; Givskov, M. *Microbiology* **2002**, *148*, 87–102.
(8) Wu, H.; Song, Z.; Hentzer, M.; Andersen, J. B.; Molin, S.; Givskov, M.; Hoiby, N. *J. Antimicrob. Agents Chemother.* **2004**, *53*, 1054–1061.
(9) Hjelmgaard, T.; Persson, T.; Rasmussen, T. B.; Givskov, M.; Nielsen, J. *Bioorg. Med. Chem.* **2003**, *11*, 3261–3271.
(10) Wang, B. H.; Ni, N. T.; Choudhary, G.; Peng, H. J.; Li, M. Y.; Chou, H. T.; Lu, C. D.; Gilbert, E. S. *Chem. Biol. Drug Des.* **2009**, *74*, 51–56.
(11) Janda, K. D.; Lowery, C. A.; Abe, T.; Park, J.; Eubanks, L. M.; Sawada, D.; Kaufmann, G. F. *J. Am. Chem. Soc.* **2009**, *131*, 15584–15585.
(12) Janda, K. D.; Lowery, C. A.; Park, J.; Kaufmann, G. F. *J. Am. Chem. Soc.* **2008**, *130*, 9200–9201.
(13) Meijler, M. M.; McKenzie, K. M.; Lowery, C. A.; Qi, L. W.; Janda, K. D. *Abstr. Pap.—Am. Chem. Soc.* **2005**, *229*, U218.
(14) Sintim, H. O.; Smith, J. A. I.; Wang, J. X.; Nguyen-Mau, S. M.; Lee, V. *Chem. Commun.* **2009**, 7033–7035.
(15) Wood, T. K.; Hong, S. H.; Ma, Q. *Trends Biotechnol.* **2011**, *29*, 87–94.
(16) Lee, J.; Lee, J. H. *FEMS Microbiol. Rev.* **2010**, *34*, 426–444.
(17) Lee, J. T.; Wood, T. K.; Jayaraman, A. *BMC Microbiol.* **2007**, *7* No. 42.
(18) Bartlett, D. H.; Mueller, R. S.; Beyhan, S.; Saini, S. G.; Yildiz, F. H. *J. Bacteriol.* **2009**, *191*, 3504–3516.
(19) Nishino, K.; Nikaido, E.; Yamaguchi, A. *J. Bacteriol.* **2007**, *189*, 9066–9075.
(20) Peti, W.; Lee, J.; Page, R.; Garcia-Contreras, R.; Palermino, J. M.; Zhang, X. S.; Doshi, O.; Wood, T. K. *J. Mol. Biol.* **2007**, *373*, 11–26.
(21) Richards, J. J.; Melander, C. *Antiinfect. Agents Med. Chem.* **2009**, *8*, 295–314.
(22) Rogers, S. A.; Huigens, R. W.; Cavanagh, J.; Melander, C. *Antimicrob. Agents Chemother.* **2010**, *54*, 2112–2118.
(23) Carle, J. S.; Christophersen, C. *J. Org. Chem.* **1981**, *46*, 3440–3443.
(24) Carle, J. S.; Christophersen, C. *J. Org. Chem.* **1980**, *45*, 1586–1589.
(25) Carle, J. S.; Christophersen, C. *J. Am. Chem. Soc.* **1979**, *101*, 4012–4013.
(26) Lindel, T.; Brauchle, L.; Golz, G.; Bohrer, P. *Org. Lett.* **2007**, *9*, 283–286.
(27) Wood, T. K.; Lee, J.; Zhang, X. S.; Hegde, M.; Bentley, W. E.; Jayaraman, A. *ISME J.* **2008**, *2*, 1007–1023.