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Structural effects on persister control by brominated furanones

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ABSTRACT

Bacterial persister cells are a small population of dormant cells that are tolerant to essentially all antibiotics. Recently, we reported that a quorum sensing (QS) inhibitor, (*Z*)-4-bromo-5-(bromomethylene)-3-methylfuran-2(5*H*)-one (BF8), can revert antibiotic tolerance of *Pseudomonas aeruginosa* persister cells. To better understand this phenomenon, several synthetic brominated furanones with similar structures were compared for their activities in persister control and inhibition of acyl-homoserine lactone (AHL) mediated QS. The results show that some other furanones in addition to BF8 are also AHL QS inhibitors and can revert antibiotic tolerance of *P. aeruginosa* PAO1 persister cells. However, not all QS inhibiting BFs can revert persistence at growth non-inhibitory concentrations, suggesting that QS inhibition itself is not sufficient for persister control.

Persister cells are phenotypic variants that can be found in virtually any bacterial culture and are tolerant to different antibiotics.[1] It is believed that this subpopulation can survive from aggressive antibiotic treatments, leading to chronic infections with reoccurring symptoms. For example, bacterial strains with high-level persistence have been isolated from patients with tuberculosis[2] and cystic fibrosis associated infections.[3] Since antibiotics are not effective against persister cells, it is important to develop better control methods.

Recently, we reported that (*Z*)-4-bromo-5-(bromomethylene)-3-methylfuran-2(5*H*)-one (BF8, Fig. 1A) at 2 μg/mL can sensitize *Pseudomonas aeruginosa* PAO1 persister cells to antibiotics and BF8 alone at this concentration does not affect the viability of these persister cells.[4] In addition, BF8 was found to reduce persistence during the growth of *P. aeruginosa* PAO1[4] and the mucoid strain *P. aeruginosa* PDO300.[5] BF8 is a known inhibitor of quorum sensing (QS),[4,6,7] a bacterial system of gene regulation in response to cell population density, which regulates different phenotypes[8] such as biofilm formation[9] and bioluminescence.[10] For example, at 10 μg/mL, BF8 was found to inhibit acyl-homoserine lactone (AHL)-mediated QS in *Vibrio harveyi* BB886 without affecting the viability of this reporter strain.[4] Some other BF compounds have also been shown as inhibitors of AHL and autoinducer-2 (AI-2) mediated QS;[6,7] and consistently, some BFs have been shown to interact with the regulator R protein in AHL-mediated QS systems[11] and covalently modify LuxS, the enzyme for AI-2 production.[12] It is worth noticing that, although this QS inhibitor was found to control *P. aeruginosa* persister cells,[4] the role of QS in persister control is still elusive. The QS signal 3-oxo-C_{12}-homoserine lactone (HSL) has been shown to promote persister formation in exponential cultures of *P. aeruginosa*.[13] Interestingly, we found that 3-oxo-C_{12}-HSL can also sensitize PAO1 persisters (isolated from stationary cultures) to ciprofloxacin when treated in 0.85% NaCl.[4]

To better understand if QS inhibition plays a role in persister control and to design better antagonists, we conducted this study to compare a group of BFs with similar structures (Fig. 1B) for their effects on *P. aeruginosa* persistence and AHL-mediated QS. Two non-brominated furanones (NFs) were also involved as controls to understand if the presence of Br is important.

Effects of BFs on AHL-mediated QS: To characterize the effects of BFs on AHL-mediated QS, the method described by Surette et al.[14] was followed with slight modifications (see Supplementary data for more details). In this assay, the reporter strain *V. harveyi* BB886 (BAA-1118 from ATCC) produces bioluminescence in response to extracellular AHL.[14] Thus, by measuring the relative bioluminescence (luminescence normalized by the number of live *V. harveyi* BB886 cells) after incubation in the presence or absence of each BF or NF, the effects of BFs and NFs on AHL-mediated QS can be determined. The BF molecules were synthesized as described previously[15] and the two NF compounds were purchased from Sigma (St. Louis, MO, USA). Concentrations of 0, 0.1, 0.5, 1,

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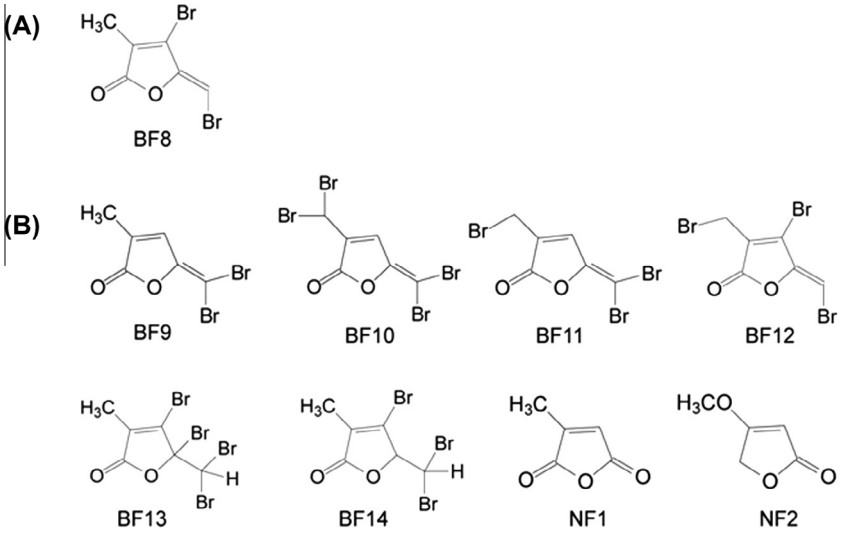

Figure 1. Structures of brominated furanones and non-brominated furanones. (A) Structure of BF8. (B) Structures of BFs and NFs used in this study.

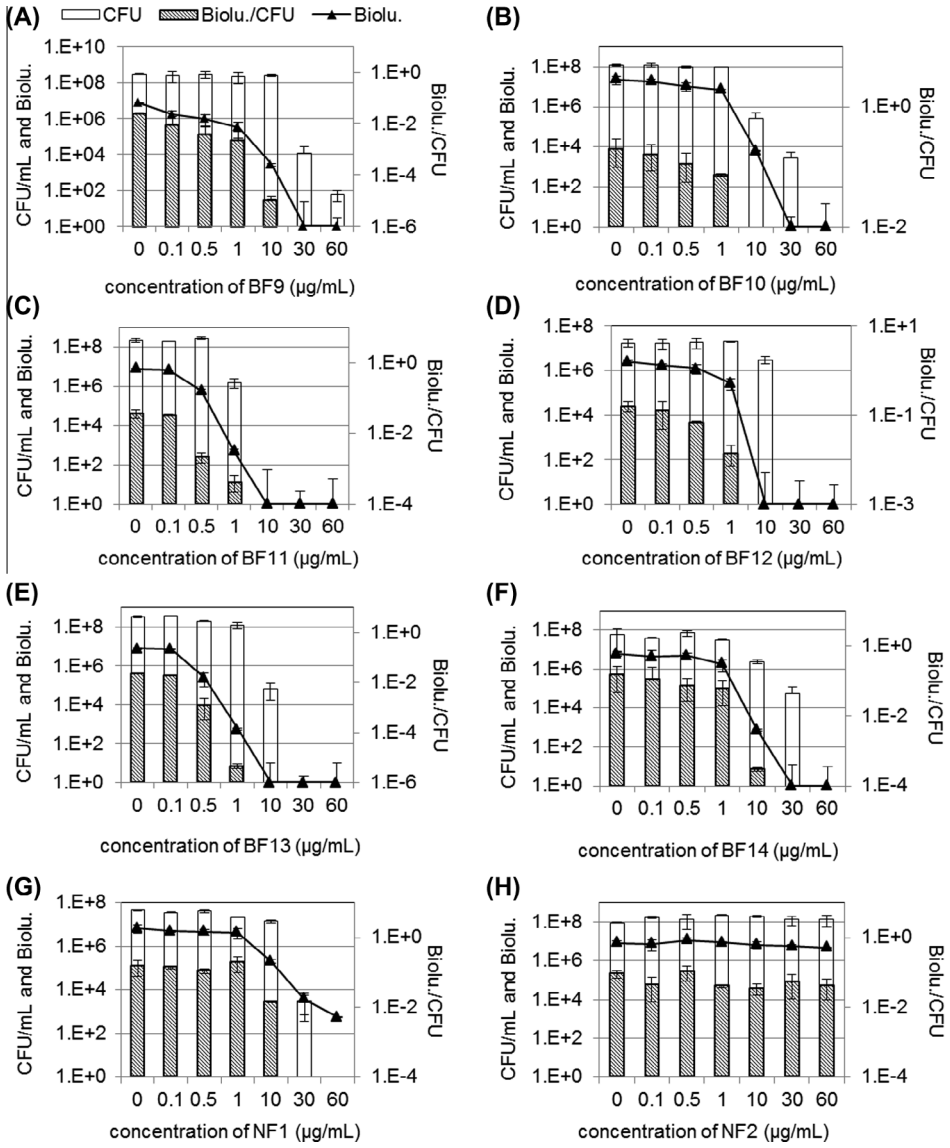

Figure 2. Effects of BFs and NFs on AHL-mediated quorum sensing. *V. harveyi* BB886 was challenged with BFs and NFs at different concentrations. The number of viable *V. harveyi* BB886 cells after BF treatment, the level of bioluminescence, and the relative bioluminescence (bioluminescence normalized by CFU) are shown.