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Bicyclic brominated furanones: A new class of quorum sensing modulators that inhibit bacterial biofilm formation

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ABSTRACT

Both natural and synthetic brominated furanones are known to inhibit biofilm formation by bacteria, but their toxicity to mammalian cells is often not reported. Here, we designed and synthesized a new class of brominated furanones (BBFs) that contained a bicyclic structure having one bromide group with well-defined regiochemistry. This class of molecules exhibited reduction in the toxicity to mammalian cells (human neuroblastoma SK-N-SH) and did not inhibit bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) growth, but retained the inhibitory activity towards biofilm formation of bacteria. In addition, all the BBFs inhibited the production of virulence factor elastase B in *P. aeruginosa*. To explore the effect of BBFs on quorum sensing, we used a reporter gene assay and found that **6-BBF** and **7-BBF** exhibited antagonistic activities for LasR protein in the *lasI* quorum sensing circuit, while **5-BBF** showed agonistic activity for the *rhlI* quorum sensing circuit. This study suggests that structural variation of brominated furanones can be designed for targeted functions to control biofilm formation.

1. Introduction

Biofilms on different surfaces cause enormous detrimental effects in medical and industrial settings[1] and are the source of many diseases, including endocarditis, otitis media, chronic prostatitis, periodontal disease, chronic urinary tract infections, and osteomyelitis,[2,3] and In particular, biofilms formed by *Pseudomonas aeruginosa* are often related to serious infections in immunocompromised patients,[4] particularly lung infection in cystic fibrosis patients.[3,5] The formation of biofilm is regulated by multiple genes, which results in highly complex film structures on the surface of microbes.[6] Controlling the formation of biofilm has been challenging because inhibition of biofilm formation and dispersion of already formed biofilm are difficult.[7] Also, the bacteria reside in the biofilm often appear to be more tolerant to antibiotic treatments than planktonic bacteria.[8] One rational approach to control biofilm formation is to interfere with the chemical communication that results in a quorum sensing (QS) between bacteria, which is one of the key events leading to the biofilm formation.[9] Several synthetic autoinducer analogs have been reported to induce or inhibit quorum sensing and biofilm formation of *P. aeruginosa*.[10,11] Other small molecules that are not structurally similar to natural autoinducers have also been proven to modulate quorum sensing and inhibit and disperse proteobacterial biofilms.[12,13] The agonistic/antagonistic activities of these molecules could be tuned by structural modifications. Chemical library screening has also been utilized to discover biofilm formation inhibitors.[14]

In this work, we aim to develop new structures of inhibitors of biofilm formation that are both nonmicrobicidal to bacteria and nontoxic to mammalian cells. Based on our previous study on what constitute the important structural elements of brominated furanones,[15] we propose that a bicyclic version of brominated furanones, which retain the conjugated exocyclic vinyl bromide in the furanone moiety, that could potentially reduce their toxicity while retaining the biofilm inhibitory activities. Here, we designed a new class of bicyclic brominated furanones (BBFs), **5-BBF**, **6-BBF**, and **7-BBF**, with [3,3,0], [4,3,0], and [5,3,0] fused ring structures, respectively (Fig. 1). Compared to the known brominated furanones, such as **BF8**[15] and **BF4** (some literature use the name 'C30'),[16] the fused bicyclic systems bear only one bromo-substitution, and introduce bulkier but semi-rigid cyclic hydrocarbon skeletons into the molecules that can potentially increase the binding and selectivity to the receptor proteins.

In this study, the toxicity of these molecules is evaluated for bacteria *P. aeruginosa* and *Escherichia coli* and human neuroblastoma SK-N-SH cells. We found that BBFs exhibited reduced toxicity to bacteria and mammalian cells compared to **BF8** and **BF4**. It was

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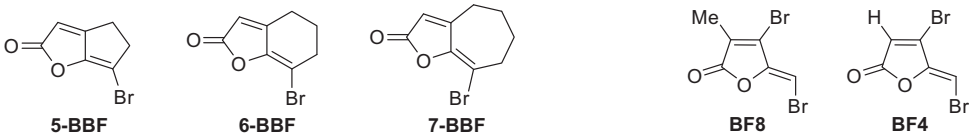


Figure 1. Structures of BBFs and known brominated furanones.

also found that BBFs inhibited the production of virulence factor elastase B by *P. aeruginosa*. To explore a mechanistic understanding, we examined their interference (agonist or antagonist) with two quorum sensing pathways (*las* and *rhl*) in *P. aeruginosa* by using reporter gene assays.

2. Results and discussion

2.1. Synthesis of BBFs

Bicyclic brominated furanones, **5-BBF**, **6-BBF** and **7-BBF**, were synthesized via a similar route starting with keto acids (Scheme 1). The synthesis of **7-BBF** is described as an example here. Under basic condition, coupling of cycloheptanone and dimethyl carbonate provided methyl 2-oxo-1-cycloheptanecarboxylate **2**, which underwent acetoacetic ester synthesis to give 2-oxocycloheptaneacetic acid **3**. This intermediate was then subjected to bromination and dehydration to build the fused ring framework, followed by elimination to yield the conjugated final product **7-BBF** without isolating the intermediates (Scheme 1). Known brominated furanones **BF8**[15] and **BF4**[17] were also synthesized to compare their toxicities and biofilm inhibition activities with BBFs.

2.2. BBFs inhibit the biofilm formation by *P. aeruginosa* and *E. coli*

We used a wild type strain PA01-GFP to study biofilm formation on steel coupons with and without BBFs. This strain constitutively expresses green fluorescent proteins (GFP)[18] and enables easy and direct visualization of biofilm by confocal laser scanning microscopy. In the initial screening, all three BBFs resulted in much less green fluorescence signals in the PA01 biofilms than the BF-free

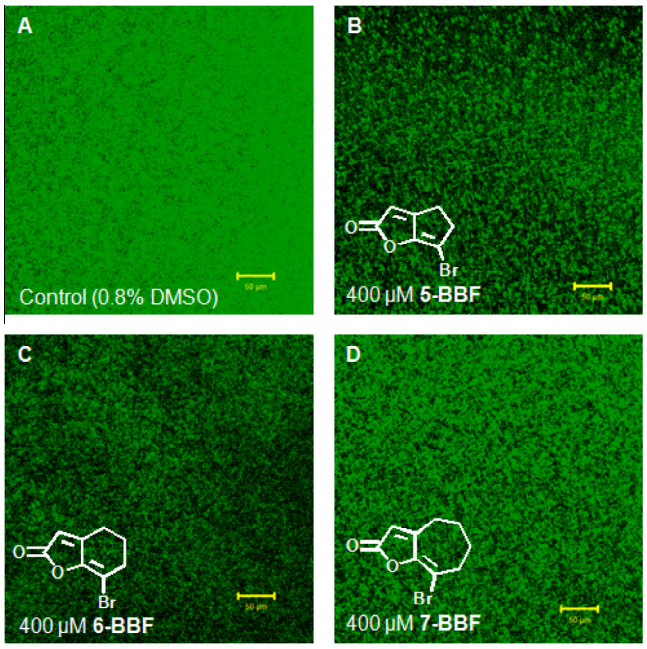
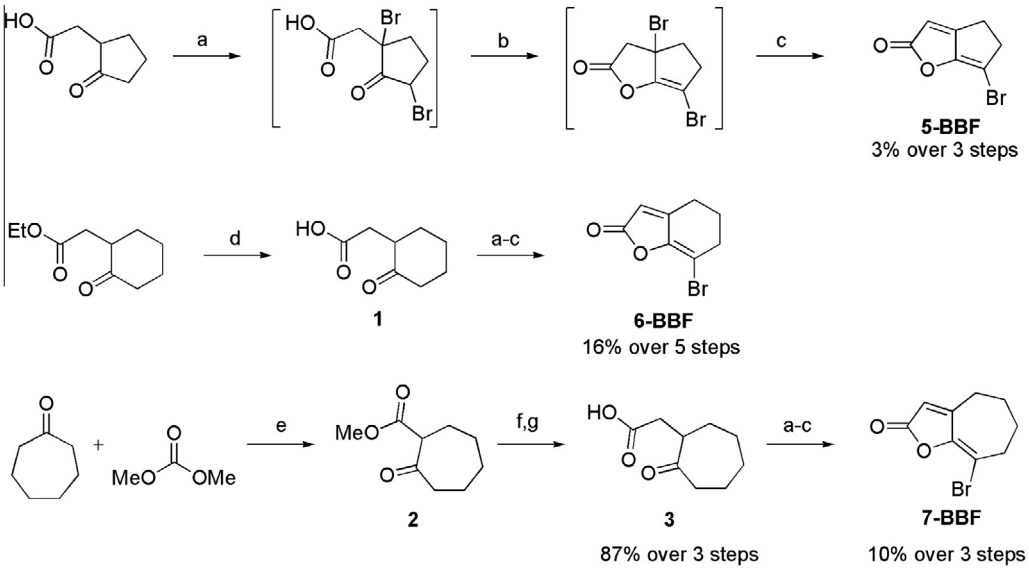


Figure 2. The effect of brominated furanones on biofilm formation by *P. aeruginosa*. Representative confocal laser scan microscopy (CLSM) images of biofilm formed by PA01-GFP (expresses green fluorescence on plasmid pSMC2) (A) in the absence of agents, and in the presence of (B) **5-BBF**, (C) **6-BBF**, (D) **7-BBF**. The control is supplemented with the same amount (0.8%) of DMSO as present in the BF-treated conditions. Scale bar = 50 μm.

control at 400 μM, with **6-BBF** provided more inhibition than that by **5-BBF** and **7-BBF** (Fig. 2). Biofilm grown in the presence of



Scheme 1. Synthesis of BBFs. Reagents and conditions: (a) Br_2, CH_2Cl_2, 0 °C–rt; (b) P_2O_5, DCM, 0 °C to reflux; (c) Et_3N, DCM, 0 °C to reflux; (d) LiOH, THF/H_2O (9:4), 23 h, 1 M HCl (aq), rt; (e) NaH, benzene, rt to 85 °C; (f) ethyl bromoacetate, K_2CO_3, acetone, rt to reflux, 16 h; (g) 6 M HCl (aq), AcOH, rt to reflux, 2 d.