HHS Public Access

Author manuscript

Bioorg Med Chem. Author manuscript; available in PMC 2019 April 09.

Published in final edited form as:

Bioorg Med Chem. 2014 February 15; 22(4): 1313–1317. doi:10.1016/j.bmc.2014.01.004.

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

Sijie Yang[a], **Osama A. Abdel-Razek**[b], **Fei Cheng**[a], **Debjyoti Bandyopadhyay**[a], **Gauri S. Shetye**[a], **Guirong Wang**[b,*], and **Yan-Yeung Luk**[a,c,*]

[a]Department of Chemistry, Syracuse University, Syracuse, NY 13244, United States

[b]Department of Surgery, Upstate Medical University, State University of New York, Syracuse, NY 13210, United States

[c]Department of Biomedical and Chemical Engineering, Syracuse University, Syracuse, NY 13244, United States

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.

Keywords

Furanones; Biofilm inhibition; Virulence factor; Cell signaling; Toxicity

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

*Corresponding authors. Tel.: +1 315 443 7440; fax: +1 315 443 4070 (Y.-Y. Luk), tel.: +1 315 464 6283 (G. Wang). yluk@syr.edu (Y.-Y. Luk).

Supplementary data

Supplementary data (the synthesis procedures, NMR characterization, protocols for biological assays, and supplemental graph of data for biological activities) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.01.004.

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 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 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 400 μM **BF4** showed the least fluorescence signals (see Fig. S1). To characterize the biofilm inhibition quantitatively, we accessed the biomass, mean thickness and surface area of biofilms formed with and without agents using COMSTAT software (Fig. 3).[19] The results were normalized by the controls without agents. Compound **6-BBF** exhibited the strongest inhibition as it reduced the biofilm formation by 71% ($P < 0.01$), followed by **5-BBF** with 53% inhibition ($P < 0.01$) and **7-BBF** with 50% inhibition ($P < 0.05$). The mean thickness of biofilm was reduced by **6-BBF** to 60% ($P < 0.01$) and by **7-BBF** to 74% ($P < 0.05$). We note that the mean thickness of biofilm treated by **5-BBF** did not seem to reduce. One possibility is that the biofilm becomes more 'fluffy' in the presence of 400 μM **5-BBF**. The surface areas of biofilm in the presence of all three BBFs were about 60% of that formed in the absence of BFs ($P < 0.05$). Similar results were obtained when *E. coli* RP437 (pRSH103) biofilms were grown in the absence and presence of 200 μM BBFs and **BF8** (see Fig. S2).

These results suggest that **6-BBF** is a stronger biofilm inhibitor than **5-BBF** and **7-BBF** for both *P. aeruginosa* and *E. coli*. We followed up with a dose-dependence study on inhibitory activity of BBFs on *P. aeruginosa* biofilm using a colorimetric assay in 96-well plates employing a dye molecule, crystal violet (CV) (see Fig. S3).[20] The half maximal inhibitory concentration (IC_{50}) values obtained from the dose–response curves were more than 400 μM for **5-BBF**, and 145.8 and 139.7 μM for **6-BBF** and **7-BBF**, respectively. These results suggest that **6-BBF** and **7-BBF** are stronger biofilm inhibitors than **5-BBF** for *P. aeruginosa*. We note that the percentage of relative biofilm formation in the presence of 400 μM BBFs obtained from confocal laser scan microscopy did not match exactly that obtained from the CV-dye staining assay. However, the general trend that **6-BBF** and **7-BBF** is more active than **5-BBF** against *Pseudomonas* biofilm formation is consistent for both assays.

2.3. BBFs do not influence the growth of *P. aeruginosa* or *E. coli*

The toxicity of brominated furanones to the growth of bacteria is unpredictable based on structures. For example, **BF8** does not inhibit the growth of *E. coli*, whereas similar structures do.[15] Here, we compared the toxicity of BBFs and **BF4** to the growth of *P. aeruginosa* and *E. coli* to study if the biofilm inhibition was due to bactericidal effect. At 400 μM, none of the three BBFs inhibited the growth of *P. aeruginosa* PA01 (Fig. 4), and bacteria grown in the presence of BBFs reached the same optical density (OD_{600}) as those in the absence of BBFs after 24 h. Under the same conditions, **BF4** completely inhibited bacterial growth ($P < 0.01$), which suggested that the significant biofilm inhibition observed for **BF4**

at this concentration was due to bactericidal effect. The effect of the BBFs on the growth of *E. coli* strain RP437 was also studied, with **BF8** (a known biofilm inhibitor to this strain at 200 μM) as a positive control. At 200 μM, none of the BBFs exhibited obvious impact on the growth of *E. coli* RP437 (see Fig. S4). The growth curve of bacteria in the presence of **BF8** deviated from that of the control for up to 8 h and then the OD_{600} values were essentially the same to the control after then.

2.4. BBFs interfere with the quorum sensing in *P. aeruginosa*

To investigate whether BBFs inhibit biofilm formation via interference with QS, we studied the effects of BBFs on the QS systems in *P. aeruginosa*. There are two identified *N*-acyl homoserine lactone (AHL)-mediated quorum sensing circuits in *P. aeruginosa*. One is the *las* circuit that includes an AHL synthase gene *lasI* responsible for the synthesis of *N*-(3-oxo-dodecanoyl)-L-homoserine lactone (3-oxo-C12-HSL or PAI1);[21] the PAI1 binds to LasR to activate a range of quorum sensing genes. The other is the *rhl* circuit that includes *rhlI* gene responsible for synthesis of *N*-butanoyl-L-homoserine lactone (C4-HSL, or PAI2);[22] the PAI2 binds to RhlR to further activate other genes.

Reporter strain PA01 (p*lasI*-LVAgfp)[12] was used to evaluate the *las* quorum sensing system. The GFP production is quantified by correcting the measured fluorescence signal for cell density. Both **6-BBF** and **7-BBF** decreased fluorescent signals as the concentration was increased from 50 to 150 to 300 μM (Fig. 5), indicating an inhibition of GFP production. At 300 μM, the presence of **6-BBF** or **7-BBF** caused a reduction in GFP production to 78 ± 1.1% ($P < 0.05$) and 69 ± 4.0% ($P < 0.001$), respectively, as compared to that of the BF-free controls. These results suggest that **6-BBF** and **7-BBF** are weak antagonists of LasR protein. The known biofilm inhibitor **BF8**[15] resulted in less GFP production, 46 ± 4.8%, compared to that of the BF-free control ($P < 0.001$). Compound **5-BBF** inhibited the GFP production slightly at 50 and 150 μM, and reduced the GFP expression by approximately half at 300 μM. These results suggest that the BBFs are weak to moderate antagonists of the *las* QS circuit, possibly by binding to LasR receptor protein. The BBFs were also examined for antagonism of QS using a double-knockout reporter strain *P. aeruginosa* PAO-JP2[23] (ΔlasIΔrhlI) harboring the plasmid p*lasI*-LVAgfp.[24] When bacteria are growing in the presence of exogenously introduced autoinducer 3-oxo-C12-HSL or analogues, LasR receptor binds to the AI and activates transcription of the *lasI* promoter which controls green fluorescence protein (GFP) expression. Similar inhibition of GFP production was obtained when PAO-JP2 (p*lasI*-LVAgfp) was used (see Fig. S5). We note that the inhibition of GFP production within the concentration range tested is not due to bactericidal effect.

We also tested the effects of BBFs on the *rhl* quorum sensing system using PA01 (p*rhlI*-LVAgfp). Interestingly, **5-BBF** promoted, instead of suppressed, the GFP expression significantly as its concentration was increased (See Fig. S5). At 150 and 300 μM, **5-BBF** increased the GFP expression by 82.6 ± 4.4% and 165 ± 17.6%, respectively ($P < 0.01$). On the contrary, **6-BBF**, **7-BBF** and **BF8** had no effect on the GFP expression at all concentrations tested. These results suggest that **5-BBF** is a strong agonist of RhlR, while **6-BBF** and **7-BBF** are not. Similar results were also obtained when reporter strain PAO-JP2 (p*rhlI*-LVAgfp) was used (see Fig. S6).

The expression of the virulence factor metalloprotease elastase B (LasB) in *P. aeruginosa* is also controlled by the QS.[25] This highly toxic virulence factor facilitates the invasion and destruction of host tissues,[26] induces inflammatory responses from the host[27] and also signals the biofilm formation.[28] Thus, molecules that inhibit virulence factor production without bactericidal effect are of potential use without invoking the bacterial resistance. Because BBFs appeared to be potent antagonists of LasR, we also tested their ability to inhibit elastase B production. At 300 μM, all BBFs significantly reduced the production of elastase B by ~80% (see Fig. S7). In a dose-dependent study, the expression of elastase B decreased as the concentration of **6-BBF** increased. These results are consistent with BBFs being antagonists of LasR in *P. aeruginosa*.

These results suggest that the ring size of BBFs have an impact on their ability to modulate QS in *P. aeruginosa*. For the *las* system, BBFs with larger rings (**6-BBF** and **7-BBF**) seemed to have slightly stronger antagonistic activity than that with smaller rings (**5-BBF**). For the *rhl* system, the agonistic activity seemed to be more sensitive to the ring size, as **5-BBF** was able to enhance the activity of RhlR protein while **6-BBF** or **7-BBF** had no effect. We believe that the interference of only one quorum sensing signaling pathway will likely elicit an effect on biofilm formation, and response in an in vivo environment, but does not ensure a therapeutic development.[29]

2.5. BBFs are less cytotoxic than other BFs to human cells

To avoid invoking bacterial resistance,[30–33] nonmicrobicidal agents that do not kill bacteria or influence bacterial growth are being sought over. However, for any potential use of the agents, the toxicity of the molecules towards mammalian or human cells should be minimized. Although many natural and non-natural brominated furanones inhibit biofilm formation by a wide range of bacteria, few studies have addressed their toxicity to mammalian or human cells.[11,34] Here, we compare the toxicity of BBFs to a non-microbicidal brominated furanone, **BF8**, against human neuroblastoma SK-N-SH. This human cell line were allowed to grow in 96-well tissue culture-treated microtiter plates for 24 h, after which they were treated with at 400 μM (the highest concentration tested for biofilm inhibition) of each agent (**5-BBF**, **6-BBF**, **7-BBF** and **BF8**) for 1 h. The agent-containing medium was replaced with fresh medium and the cells were then allowed to recover for 0, 24, and 48 h (recovery time), at which time the number of live cells was determined by the CCK 8 assay.[35] Survival (%) was calculated based on BF-free control.

After 1 h treatment of agents and 0 h recovery time, **BF8** exhibited the strongest cytotoxic effect toward human neuroblastoma SK-N-SH cells; 29.0 ± 14.1% of cells were alive compare to the BF-free control ($P < 0.001$). Bicyclic brominated furanones **5-BBF** and **6-BBF** showed mild toxicity (~76% cell survival for both), and **7-BBF** resulted in ~44% of cell survival (Fig. 6). After 24 and 48 h of recovery time, survival (%) for cells treated with **5-BBF** dropped to ~21% and ~6%, respectively. For cells treated with **BF8**, almost no live cell remained (<2%) after 24 and 48 h. In the presence of **7-BBF**, cell survival dropped to ~7% after 24 h of recovery time, but appeared to increase to 13% after 48 h. In contrast, **6-BBF** exhibited the least cytotoxic effect to this human cell line. After 24 and 48 h of recovery time, about 50% survival was observed for cells treated with **6-BBF**. We note this

cytotoxicity assay was also performed at lower concentration (100 μM). At this lower concentration, all three BBFs showed little to no toxicity, with **6-BBF** being the least toxic one. Brominated furanone, **BF8**, was still toxic at this low concentration (see Fig. S8). The cytotoxicity exhibited by the BBFs to the human neuroblastoma limited their use in further internal clinical study.

3. Conclusion

The bicyclic brominated furanones are a new series of synthetic brominated furanones that interfere with quorum sensing and QS-controlled activities in Gram-negative bacteria. They inhibit biofilm formation by *E. coli* and *P. aeruginosa* and inhibit elastase B production by *P. aeruginosa* at nonmicrobicidal concentrations. The BBFs are also less cytotoxic to human neuroblastoma cells compared to other known brominated furanones. This work indicates some structure–bioactivity relationship for brominated furanones. The relative high IC_{50} of biofilm inhibition and moderate cytotoxicity to human cells of these BBFs need further improvement for pharmaceutical area. However, they may be useful in industrial and other ex vivo settings. The exploration of structures that give high potency for biofilm inhibition while maintaining a low toxicity is an ongoing subject of our research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was partially supported by NSF-CAREER (#0845686, PI Y.Y.L), NSF-EFRI (#1137186, coPI, Y.Y.L), and NIH HL096007 (G.W.). We thank Bonnie B. Toms (Upstate Medical University, SUNY) for the generous donation of human neuroblastoma cell line SK-N-SH. We thank Dr. Helen E. Blackwell (Univ. of Wisconsin-Madison) for the generous donation of strains PAO-JP2 (p*lasI*-LVAgfp) and PAO-JP2 (p*rhlI*-LVAgfp), Dr. Dacheng Ren (SU) for the generous donation of strains *E. coli* RP437, *E. coli* RP437(pRSH103), and Dr. Hiroaki Suga (The University of Tokyo) for the generous donation of plasmids p*lasI*-LVAgfp and p*rhlI*-LVAgfp. We also thank Dr. James L. Hougland (SU) for the use of fluorescence plate reader and the review of the manuscript.

References and notes

1. Costerton JW; Lewandowski Z; Caldwell DE; Korber DR; Lappin-Scott HM Annu. Rev. Microbiol 1995, 49, 711. [PubMed: 8561477]
2. Costerton JW; Stewart PS; Greenberg EP Science 1999, 284, 1318. [PubMed: 10334980]
3. Parsek MR; Singh PK Annu. Rev. Microbiol 2003, 57, 677. [PubMed: 14527295]
4. Van Delden C; Iglewski BH Emerg. Infect. Dis 1998, 4, 551. [PubMed: 9866731]
5. Campana S; Taccetti G; Ravenni N; Masi I; Audino S; Sisi B; Repetto T; Doring G; de Martino MJ Cyst. Fibrosis 2004, 3, 159.
6. Stoodley P; Sauer K; Davies DG; Costerton JW Annu. Rev. Microbiol 2002, 56, 187. [PubMed: 12142477]
7. Kaplan JB J. Dent. Res 2010, 89, 205. [PubMed: 20139339]
8. Davies D Nat. Rev. Drug Disc 2003, 2, 114.
9. Camilli A; Bassler BL Science 2006, 311, 1113. [PubMed: 16497924]
10. Geske GD; O'Neill JC; Blackwell HE Chem. Soc. Rev 2008, 37, 1432. [PubMed: 18568169]
11. Lowery CA; Abe T; Park J; Eubanks LM; Sawada D; Kaufmann GF; Janda KD J. Am. Chem. Soc 2009, 131, 15584. [PubMed: 19824634]
12. Frei R; Breitbach AS; Blackwell HE Angew. Chem., Int. Ed. 2012, 51, 5226.

13. Richards JJ; Melander C Anti-Infect. Agents Med. Chem 2009, 8, 295.
14. Junker LM; Clardy J Antimicrob. Agents Chemother 2007, 51, 3582. [PubMed: 17664319]
15. Han Y; Hou S; Simon KA; Ren D; Luk Y-Y Bioorg. Med. Chem. Lett 2008, 18, 1006. [PubMed: 18164977]
16. Hentzer M; Wu H; Andersen JB; Riedel K; Rasmussen TB; Bagge N; Kumar N; Schembri MA; Song Z; Kristoffersen P; Manefield M; Costerton JW; Molin S; Eberl L; Steinberg P; Kjelleberg S; Hoiby N; Givskov M EMBO J. 2003, 22, 3803. [PubMed: 12881415]
17. Sorg A; Siegel K; Brueckner R Synlett 2004, 321.
18. Bloemberg GV; O'Toole GA; Lugtenberg BJJ; Kolter R Appl. Environ. Microbiol 1997, 63, 4543. [PubMed: 9361441]
19. Heydorn A; Nielsen AT; Hentzer M; Sternberg C; Givskov M; Ersboll BK; Molin S Microbiology 2000, 146, 2395. [PubMed: 11021916]
20. Huigens RW III; Richards JJ; Parise G; Ballard TE; Zeng W; Deora R; Melander CJ Am. Chem. Soc 2007, 129, 6966.
21. Pearson JP; Gray KM; Passador L; Tucker KD; Eberhard A; Iglewski BH; Greenberg EP Proc. Natl. Acad. Sci. U.S.A 1994, 91, 197. [PubMed: 8278364]
22. Pearson JP; Passador L; Iglewski BH; Greenberg EP Proc. Natl. Acad. Sci. U.S.A 1995, 92, 1490. [PubMed: 7878006]
23. Pesci E; Pearson JP; Seed PC; Iglewski BH J. Bacteriol 1997, 179, 3127. [PubMed: 9150205]
24. De Kievit TR; Gillis R; Marx S; Brown C; Iglewski BH Appl. Environ. Microbiol 2001, 67, 1865. [PubMed: 11282644]
25. Gambello MJ; Iglewski BH J. Bacteriol 1991, 173, 3000. [PubMed: 1902216]
26. Tamura Y; Kijima M; Suzuki S; Takahashi T; Nakamura MJ Vet. Med. Sci 1992, 54, 1049.
27. Kon Y; Tsukada H; Hasegawa T; Igarashi K; Wada K; Suzuki E; Arakawa M; Gejyo F FEMS Immunol. Med. Microbiol 1999, 25, 313. [PubMed: 10459586]
28. Kamath S; Kapatral V; Chakrabarty AM Mol. Microbiol 1998, 30, 933. [PubMed: 9988471]
29. Jensen Peter O; Bjarnsholt T; Phipps R; Rasmussen Thomas B; Calum H; Christoffersen L; Moser C; Williams P; Pressler T; Givskov M; Hoiby N Microbiology (Reading, England) 2007, 153, 1329.
30. Smith AW Hugo and Russell's Pharmaceutical Microbiology, 8th ed.; Wiley-Blackwell, 2011, 217.
31. Neu HC Science 1992, 257, 1064. [PubMed: 1509257]
32. Wu H; Song Z; Hentzer M; Andersen JB; Molin S; Givskov M; Hoiby NJ Antimicrob. Chemother 2004, 53, 1054.
33. Rogers SA; Huigens RW III; Cavanagh J; Melander C Antimicrob. Agents Chemother 2010, 54, 2112. [PubMed: 20211901]
34. Kuehl R; Al-Bataineh S; Gordon O; Luginbuehl R; Otto M; Textor M; Landmann R Antimicrob. Agents Chemother 2009, 53, 4159. [PubMed: 19620329]
35. Ishiyama M; Tominaga H; Shiga M; Sasamoto K; Ohkura Y; Ueno K Biol. Pharm. Bull 1996, 19, 1518. [PubMed: 8951178]

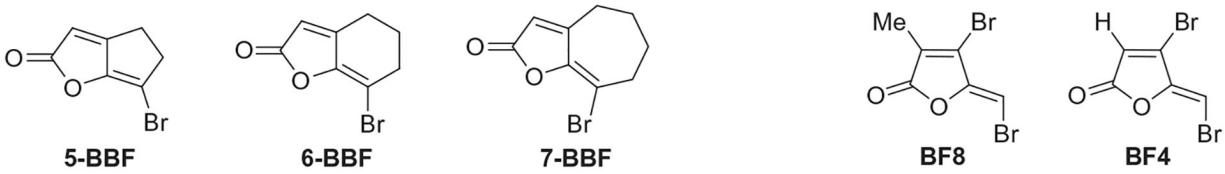


Figure 1.
Structures of BBFs and known brominated furanones.

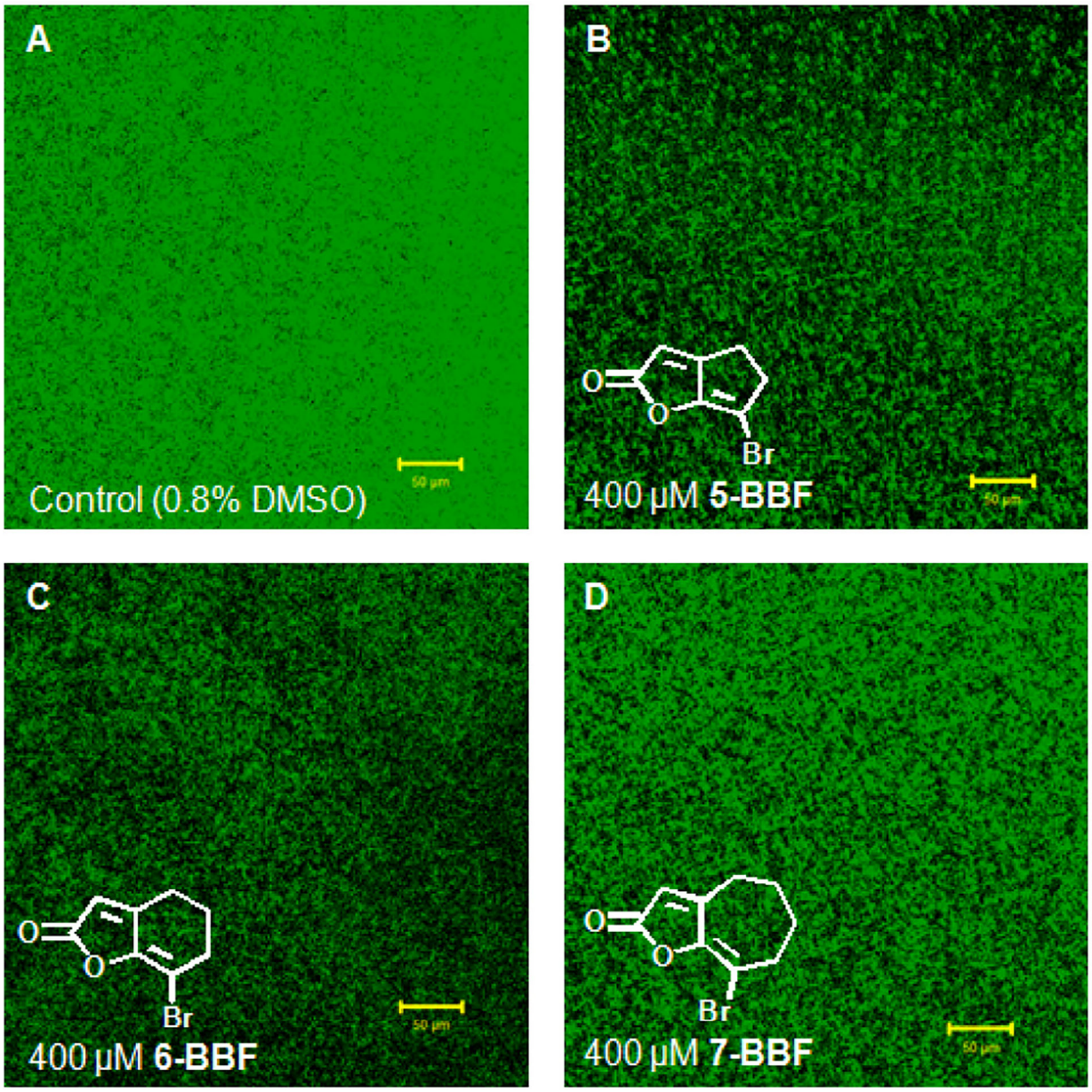


Figure 2.

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.

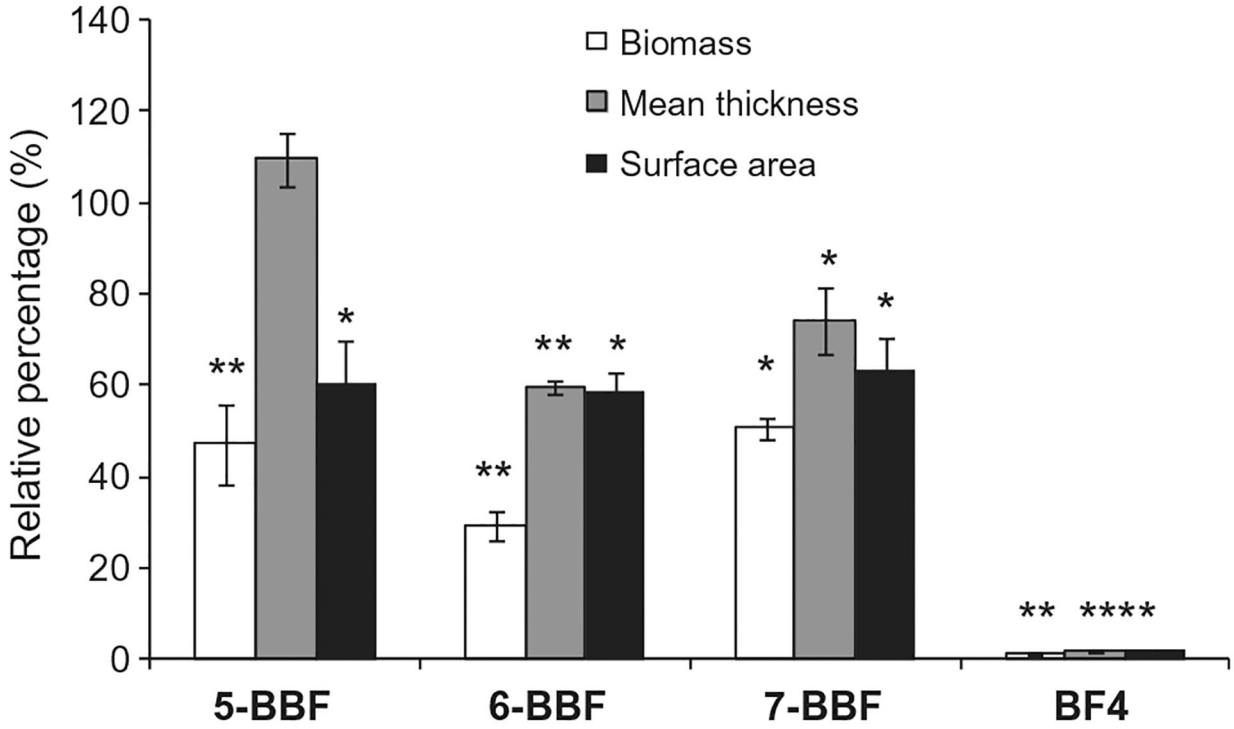


Figure 3.

Quantification of biofilm formation by PA01-GFP in the absence and presence of 400 μM brominated furanones. Biomass, mean thickness, and surface area were quantified from fluorescence image using COMSTAT software. Z-Stack images from four different locations were used. Values are normalized by that of the BF-free control and represent the means ± standard deviation from 4 replicates. Significant differences in biofilm formation with BF-free control are indicated by asterisks: $*P < 0.05$; $**P < 0.01$.

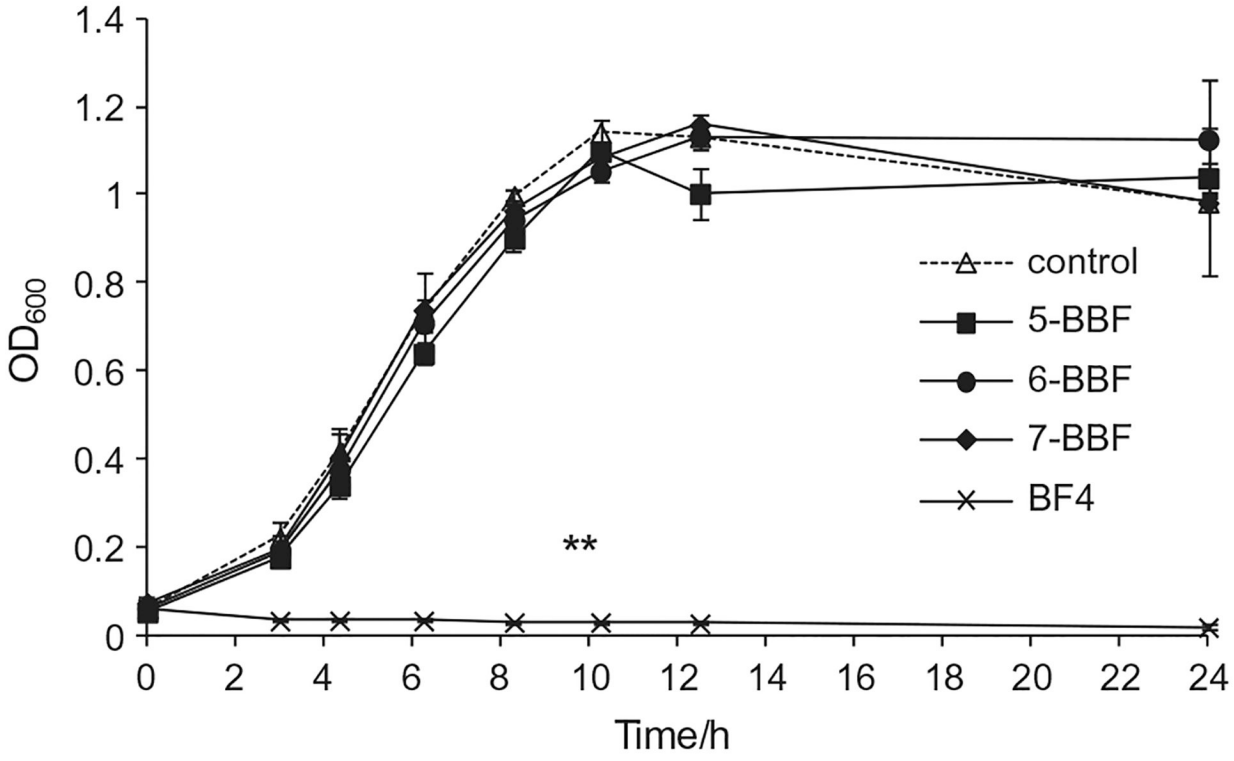


Figure 4.

Growth curves of *P. aeruginosa* PA01 in the absence (control) and presence of 400 μM brominated furanones. Values represent the means ± standard deviation from six replicates. Significant differences in the optical density with BF-free control are indicated by asterisks: $^{**}P < 0.01$.

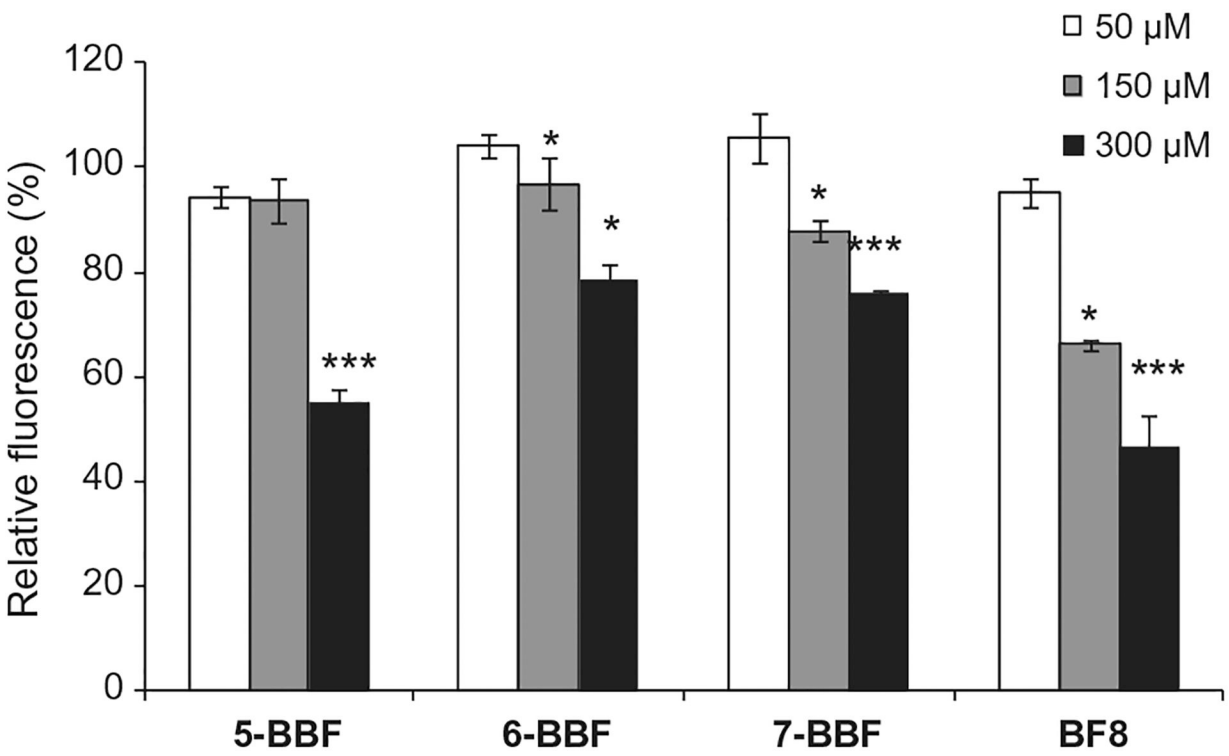


Figure 5.

GFP expression by PA01 (p*lasI*-LVAgfp) in the absence or presence of **5-BBF**, **6-BBF**, **7-BBF**, or **BF8** at 50, 150, and 300 μM. Fluorescence signals were corrected for cell density by dividing by OD_{600} of cell culture and the results were normalized to that of the BF-free control. Values represent the means ± standard deviation of the mean. Data shown is a representative of at least three separate experiments. Significant differences in the GFP expression with the control are indicated by asterisks: *$P < 0.05$;***, $P < 0.001$.

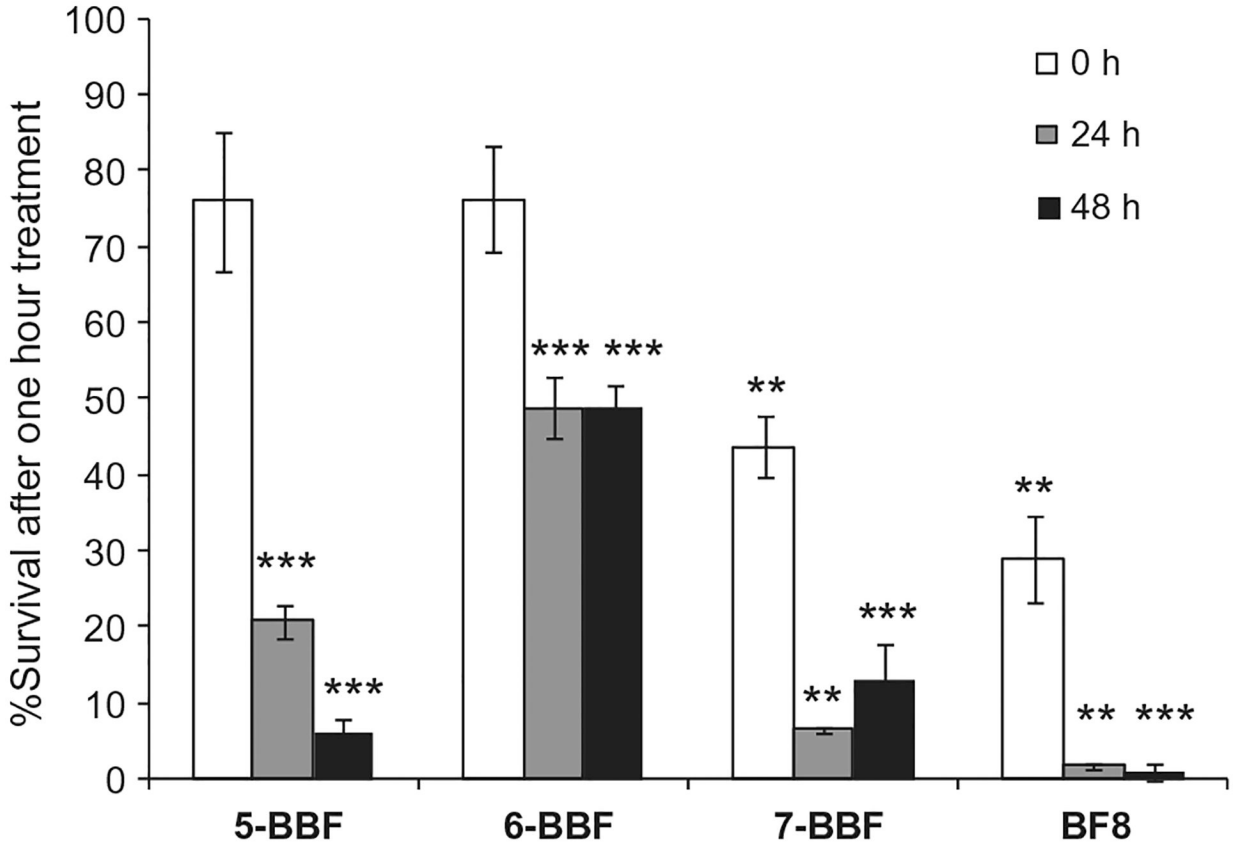
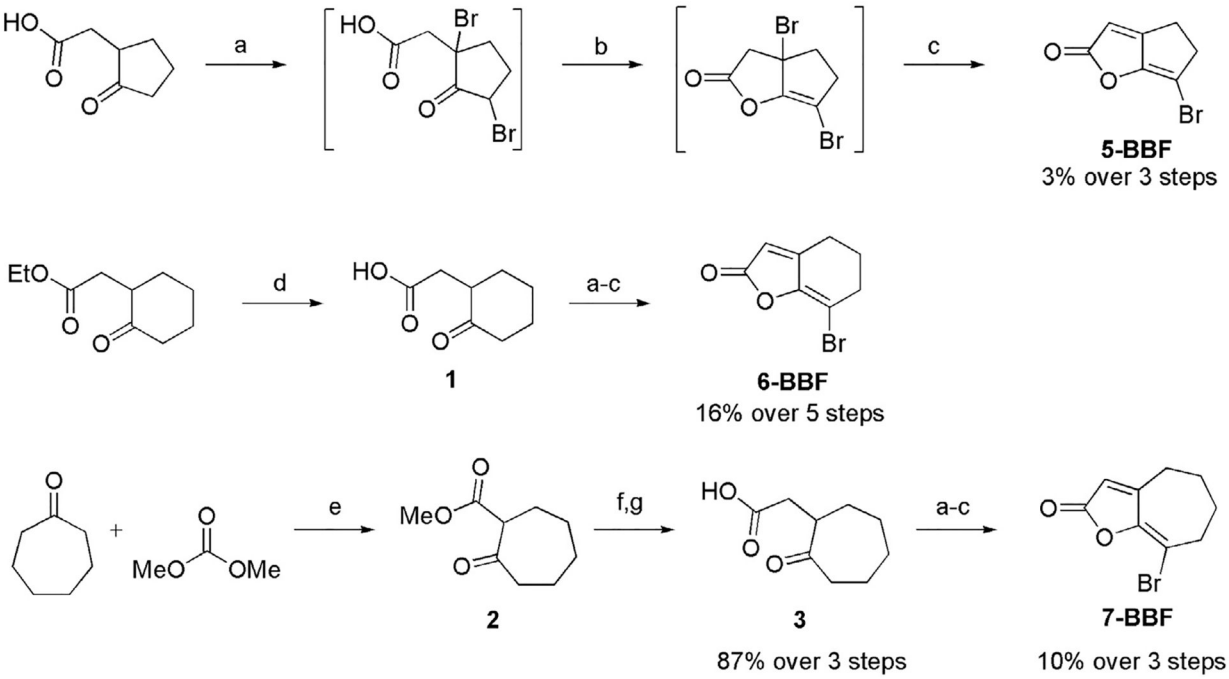


Figure 6.

Survival (%) of human neuroblastoma SK-N-SH cells at 0, 24, and 48 h after 1 h treatment of 400 μM **5-BBF**, **6-BBF**, **7-BBF** and **BF8**, respectively. Values represent the means ± standard deviation from six replicates. Significant differences between BF-treated conditions in the survival (%) and BF-free control at each time point are indicated by asterisks: *$P <$ 0.05; **$P <$ 0.01; ***$P <$ 0.001.



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.