Bicyclic Brominated Furanones: A New Class of Quorum Sensing Modulators that Inhibit Bacterial Biofilm Formation

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Supporting information

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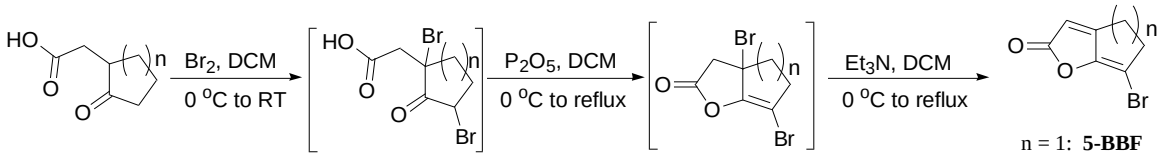
Part 1: Chemical Synthesis

Chemicals. Minimum essential medium with Eagle's salts and L-glutamine (EMEM) was obtained from Mediatech (Herndon, VA). Trypan blue stain was purchased from Sigma-Aldrich (Milwaukee, WI). Cell counting kit 8 (CCK-8) was purchased from Dojindo Molecular Technologies, Inc. (Gaithersburg, MD). Water used for preparing all buffers and solutions had resistivity of 18 MΩ cm (Millipore, Billerica, MA).



n = 1: **5-BBF**
n = 2: **6-BBF**
n = 3: **7-BBF**

General procedure for synthesis: All air sensitive reactions were performed in oven dried glassware under an atmosphere of argon unless otherwise notified. All reagent grade starting materials were obtained from commercial supplies and used as received. Anhydrous solvents were purchased from Sigma-Aldrich. Analytical thin layer chromatography was performed on EM silica gel 60 F254 glass plates (0.25 mm). Visualization of analytical thin layer chromatography was achieved using UV absorbance (254 mm), $KMnO_4$, and ceric ammonium molybdate stains. Flash column chromatography was performed using SiliaFlash P60 silica gel (40-60 Å) from SiliCycle, Inc. 1D ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Advance DPX-300 spectrometer. Chemical shifts are reported in ppm, using tetramethylsilane as the internal standard. Mass spectra were measured using a MAT 95 XP mass spectrometer, carried out by the Mass Spectroscopy Facility at Indiana University.

5-BBF: Bromine (0.79 mL, 15 mmol) was added dropwise to a solution of 2-oxocyclopentaneacetic acid (1.13 g, 7.69 mmol) in anhydrous methylene chloride (7.7 mL) at 0 ^{o}C. The reaction mixture was stirred at 0 ^{o}C for 40 min and then ambient temperature for 100 min.

The resulting solution was washed with water (10 mL) followed by aqueous 1M $Na_2S_2O_3$ solution (10 mL) and then extracted with methylene chloride (10 mL x 3). The combined organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude oil was taken up in anhydrous methylene chloride (30 mL) and cooled to 0 ^oC, followed by addition of P_2O_5 (2.65 g, 18.6 mmol) in portions. The reaction mixture was stirred at 0 ^oC for 30 min, allowed to warm to ambient temperature and then stirred under reflux for 2 h. The solid thus formed was filtered off and the filtrate concentrated under reduced pressure. The crude oil was dissolved in anhydrous methylene chloride (15 mL), cooled to 0 ^oC and treated with anhydrous Et_3N (1.12 mL, 8.0 mmol). The reaction mixture was stirred at ambient temperature for 30 min and then under reflux for 2 h. After cooled to ambient temperature, the reaction mixture was washed with aqueous saturated NH_4Cl and extracted with methylene chloride (10 mL x 3). The combined organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash chromatography (SiO_2, hexane:ethyl acetate, gradient) provided **5-BBF** (54.3 mg, 3% over 3 steps) as off white solid. TLC R_f = 0.29 (hexane:ethyl acetate, 4:1). ^1H NMR (300 MHz, $CDCl_3$): δ_H 5.67 (t, 1H, *J* = 0.9 Hz), 3.12 (t, 2H, *J* = 4.2 Hz), 2.95 (td, 2H, *J* = 3.9, 1.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 173.0, 167.0, 153.3, 105.5, 105.0, 39.8, 24.7. HRMS (ESI+): Cacld. for M^+: 199.9467, found: 199.9466 (96.3%), 201.9442 (100.0%).

6-BBF: To a mixture of ethyl 2-cyclohexaneacetate (2.00 g, 10.6 mmol) in THF (72 mL) and water (32 mL) was added lithium hydroxide (688.0 mg, 34.4 mmol). The reaction mixture was stirred vigorously at ambient temperature overnight. The reaction mixture was concentrated to remove THF and acidified with hydrochloric acid until pH was one. The mixture was extracted with ethyl acetate (30 mL x 3), washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. 2-Oxocyclohexaneacetic acid **1** was obtained as a light yellow crude oil that was carried

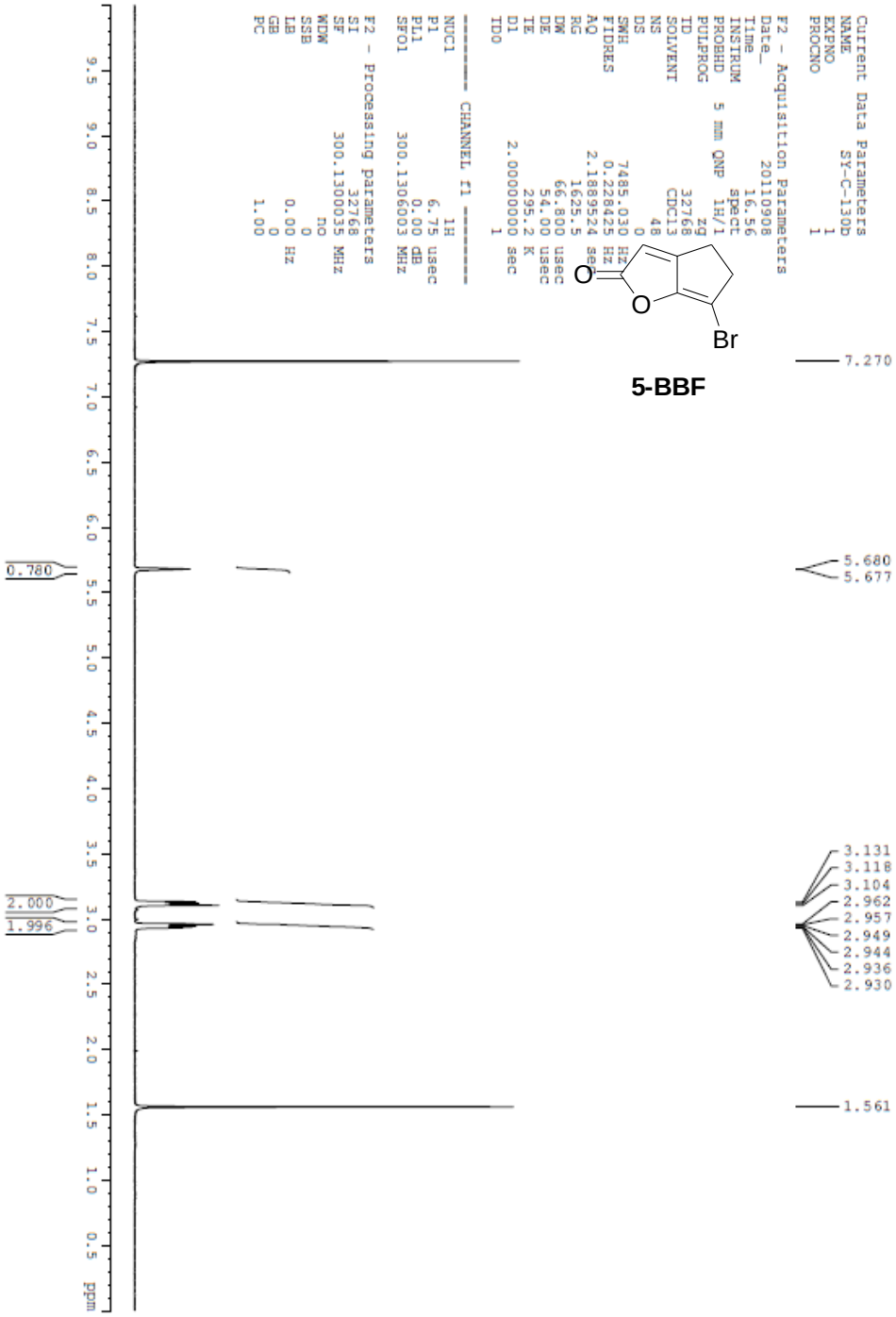
on to the next step without further purification. Bromine (1.14 mL, 22.3 mmol) was added dropwise to a solution of crude 2-oxocyclohexaneacetic acid **1** (1.65 g, 10.6 mmol) in anhydrous methylene chloride (10.6 mL) at 0 ^{o}C. The reaction mixture was stirred at 0 ^{o}C for 30 min and then ambient temperature for 2 h. The resulting solution was washed with water (6 mL) followed by aqueous 1M $Na_2S_2O_3$ solution (6 mL) and then extracted with methylene chloride (6 mL x 3). The combined organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude oil was taken up in anhydrous methylene chloride (42 mL) and cooled to 0 ^{o}C, followed by addition of P_2O_5 (3.67 g, 25.9 mmol) in portions. The reaction mixture was stirred at 0 ^{o}C for 30 min, allowed to warm to ambient temperature and then stirred under reflux for 2 h. The solid thus formed was filtered off and the filtrate concentrated under reduced pressure. The crude oil was dissolved in anhydrous methylene chloride (26 mL), cooled to 0 ^{o}C and treated with anhydrous Et_3N (1.50 mL, 10.8 mmol). The reaction mixture was stirred at ambient temperature for 30 min and then under reflux for 1 h. After cooled to ambient temperature, the reaction mixture was washed with aqueous saturated NH_4Cl and extracted with methylene chloride (18 mL x 3). The combined organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash chromatography (SiO_2, hexane:ethyl acetate, gradient) provided **6-BBF** (350.5 mg, 16% over 4 steps) as off white solid. TLC R_f = 0.66 (hexane:ethyl acetate, 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 5.83 (s, 1H), 2.79 (t, 2H, *J* = 6.0 Hz), 2.73 (td, 2H, *J* = 6.0, 1.2 Hz), 2.00 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 168.5, 154.9, 148.3, 111.0, 107.4, 34.0, 24.0, 23.6. HRMS (ESI+): Cacld. for M^+: 213.9624, found: 213.9626 (100.0%), 215.9596 (99.6%).

7-BBF: To a stirred suspension of NaH (5.10 g, 60% suspension in mineral oil, 128 mmol, 15 mL x 2 hexane washed) in anhydrous benzene (80 mL) was added dimethyl carbonate (7.6 mL, 90 mmol) via syringe. The reaction was stirred under reflux for 1 h. A solution of cycloheptanone

(4.3 mL, 36 mmol) in anhydrous benzene (6.0 mL) was then added via cannula and the reaction mixture was stirred under reflux for 3 h. The heterogeneous mixture was coloed to ambient temperature and then quenched with acetic acid (8 mL). The mixture was diluted with water (200 mL), extracted with ethyl acetate (50 mL x 4), washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Methyl 2-oxo-1-ccloheptanecarboxylate **2** was obtained as yellow oil that was carried on to the next step without further purification. To a solution of crude methyl 2-oxo-1-ccloheptanecarboxylate **2** in acetone (100 mL) was added potassium carbonate (23.2 g, 168 mmol) followed by ethyl bromoacetate (3.8 mL, 34 mmol). The reaction mixture was stirred under reflux overnight (~17 h). The suspension was cooled to ambient temperature and concentrated under reduced pressure to remove ~ half of the acetone. The residue was diluted with water (50 mL), extracted with diethyl ether (50 mL x 3), washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting yellow oil was treated with a solution of hydrochloric acid (27 mL, 6M) and acetic acid (27 mL). The reaction mixture was stirred under reflux for 2 days and cooled to ambient temperature. The mixture was diluted with water (50 mL), extracted with methylene chloride (30 mL x 5), washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash chromatography (SiO_2, methylene chloride:ethyl acetate, gradient) provided 2-oxocycloheptaneacetic acid **3** (5.35 g, 87% over 3 steps) as a light yellow oil. TLC R_f = 0.31 (methylene chloride:ethyl acetate, 1:1). The identity of this compound was confirmed by comparing ^{1}H NMR data with that previously reported.[1] Bromine (0.93 mL, 18 mmol) was added dropwise to a solution of 2-oxocycloheptaneacetic acid **3** (1.47 g, 8.66 mmol) in anhydrous methylene chloride (8.7 mL) at 0 ^{o}C. The reaction mixture was stirred at 0 ^{o}C for 30 min and then ambient temperature for 2 h. The resulting solution was washed with water (5 mL) followed by aqueous 1M $Na_2S_2O_3$ solution (5 mL) and then extracted with methylene

chloride (5 mL x 3). The combined organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude oil was taken up in anhydrous methylene chloride (35 mL) and cooled to 0 ^oC, followed by addition of P_2O_5 (3.00 g, 21.1 mmol) in portions. The reaction mixture was stirred at 0 ^oC for 40 min, allowed to warm to ambient temperature and then stirred under reflux for 2 h. The solid thus formed was filtered off and the filtrate concentrated under reduced pressure. The crude oil was dissolved in anhydrous methylene chloride (22 mL), cooled to 0 ^oC and treated with anhydrous Et_3N (1.50 mL, 8.93 mmol). The reaction mixture was stirred at ambient temperature for 30 min and then under reflux for 1 h. After cooled to ambient temperature, the reaction mixture was washed with aqueous saturated NH_4Cl and extracted with methylene chloride (15 mL x 3). The combined organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash chromatography (SiO_2, hexane:ethyl acetate, gradient) provided **7-BBF** (194.8 mg, 10% over 3 steps) as off white solid. TLC R_f = 0.62 (hexane:ethyl acetate, 1:1). ^1H NMR (300 MHz, $CDCl_3$): δ_H 6.01 (s, 1H), 2.98 (t, 2H, J = 6.0 Hz), 2.82 (td, 2H, J = 6.0, 1.5 Hz), 1.91 (m, 2H), 1.81 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 168.0, 157.0, 148.2, 117.2, 115.4, 39.5, 29.3, 27.8, 23.9. HRMS (ESI+): Cacld. for M^+: 227.9780, found: 227.9785 (97.3%), 229.9766 (100.0%).

^1H NMR and ^{13}C NMR spectra of BBFs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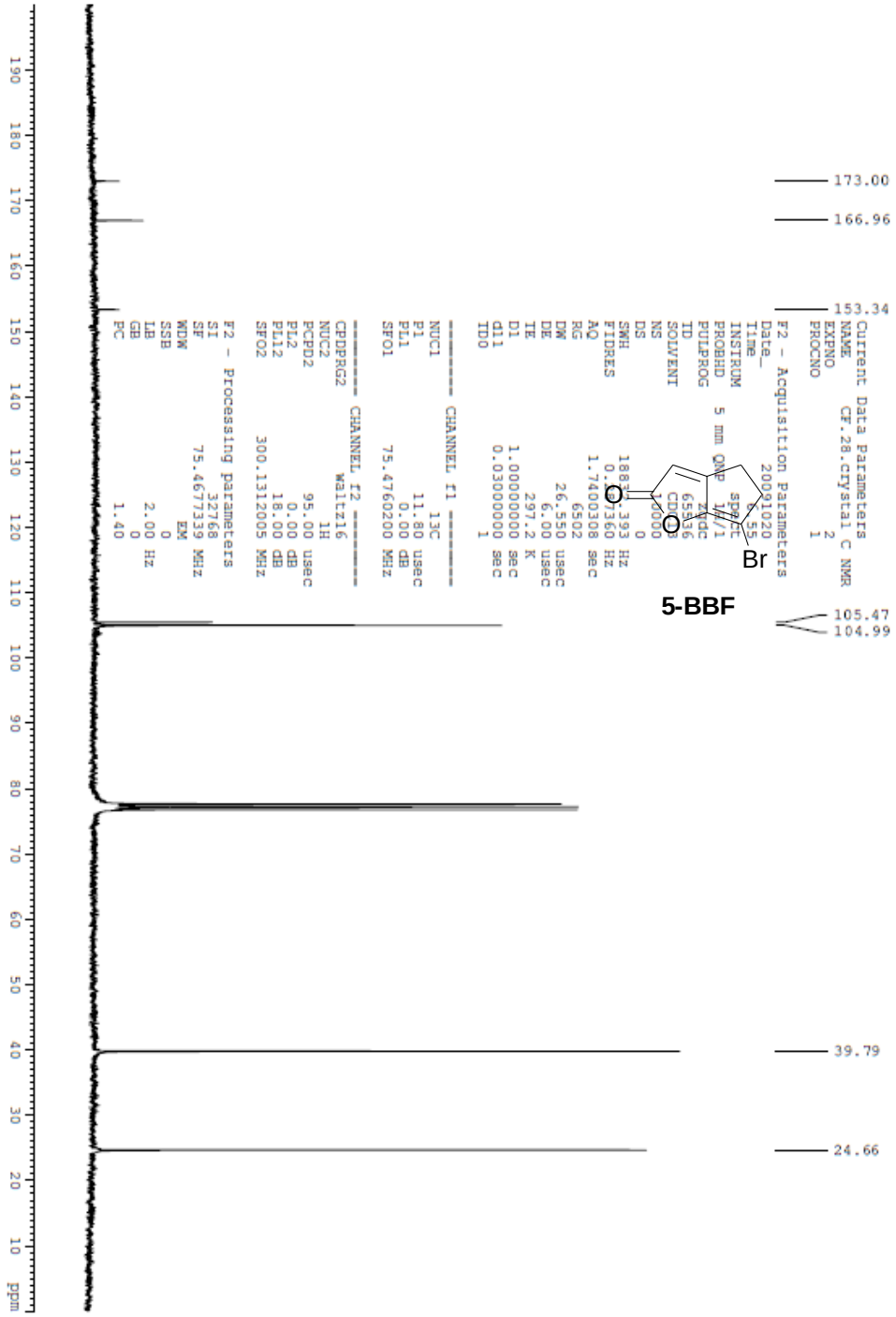
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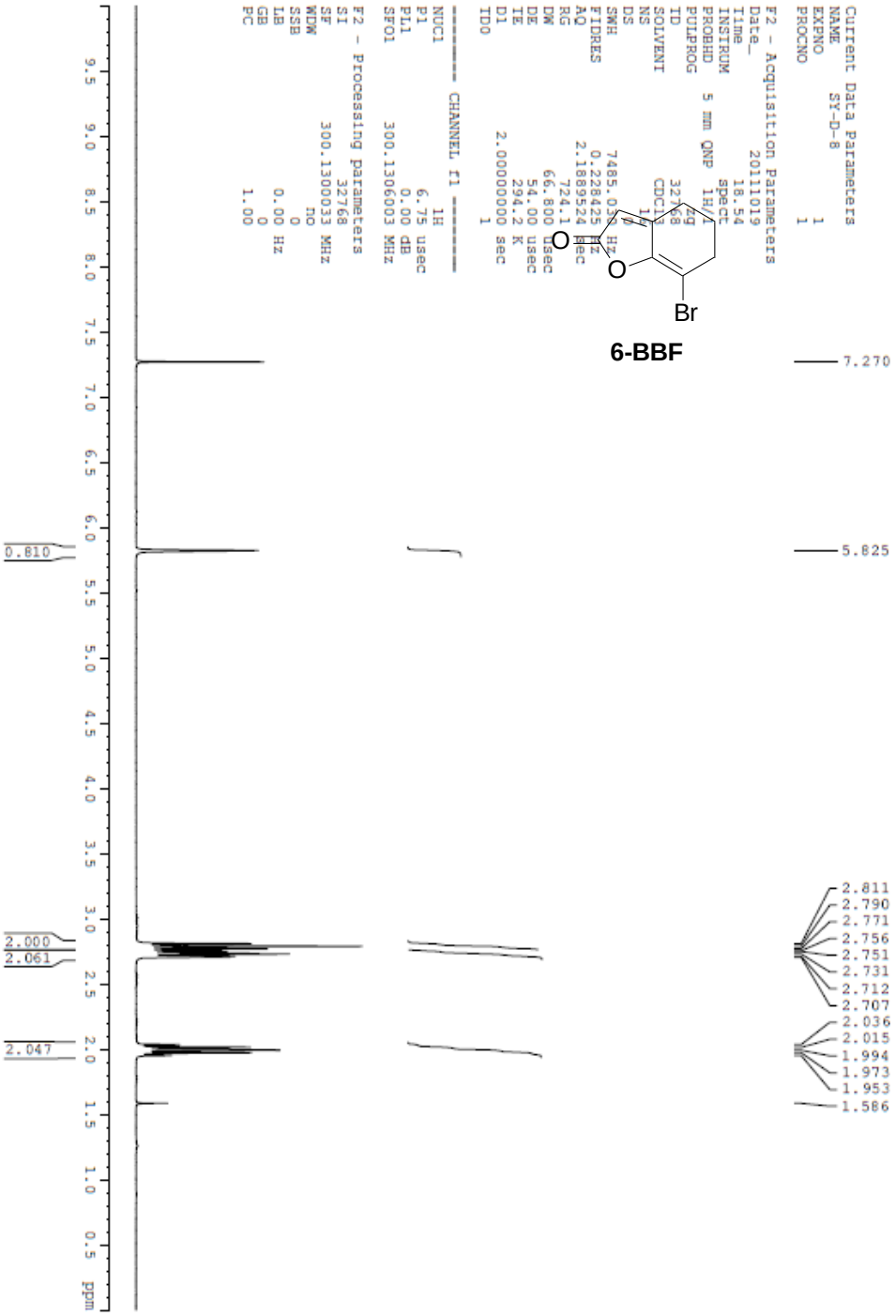
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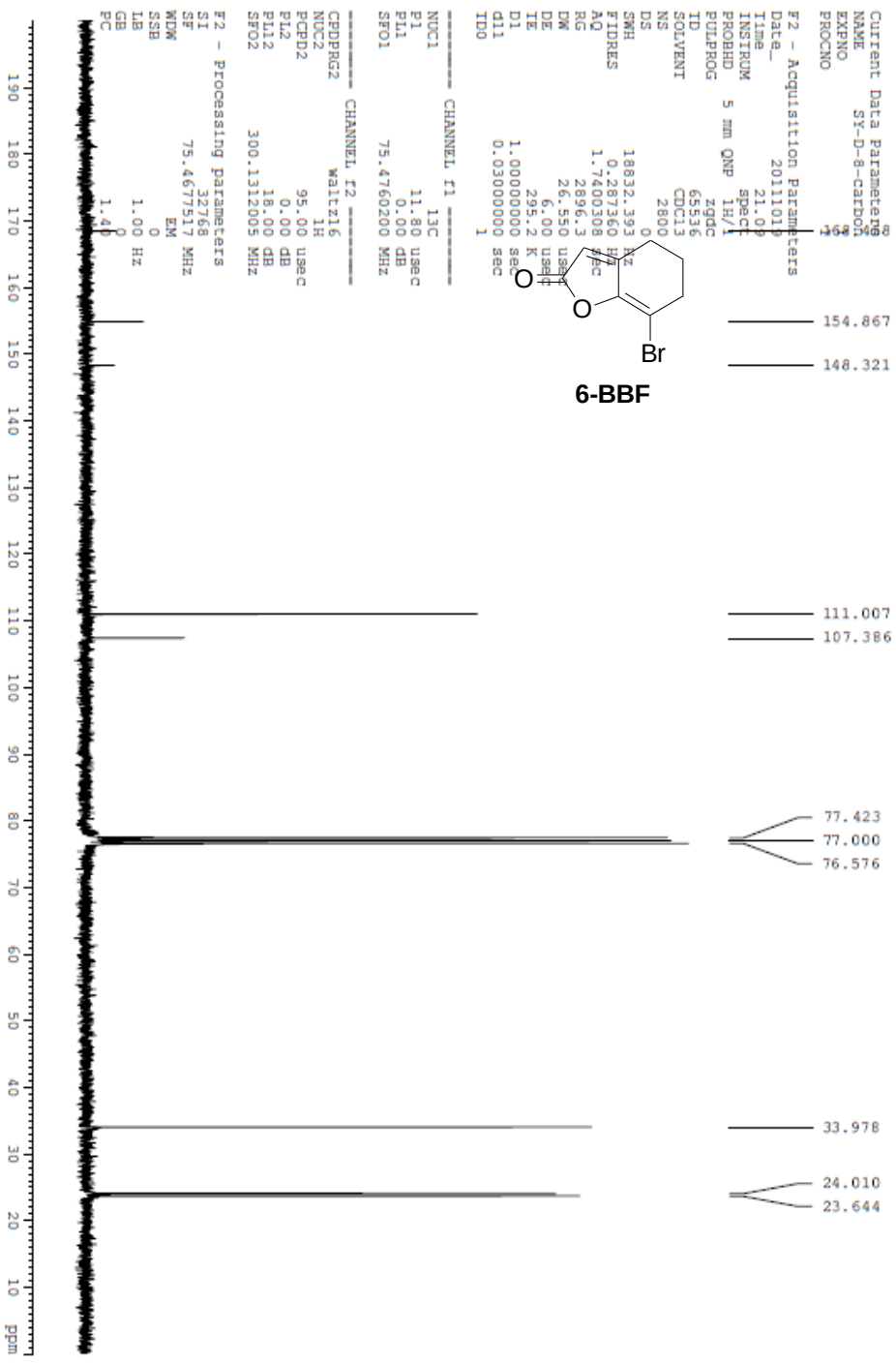
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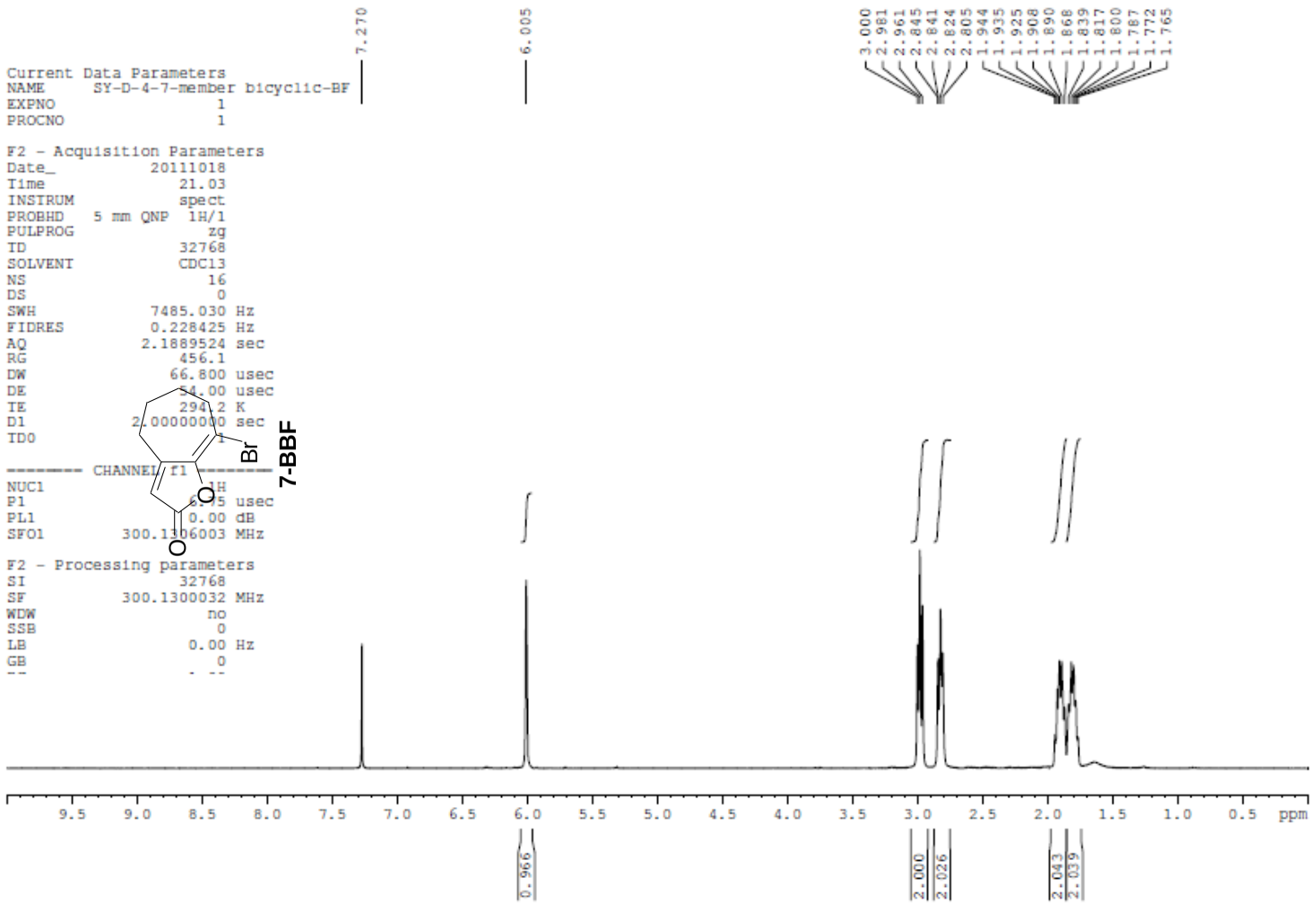
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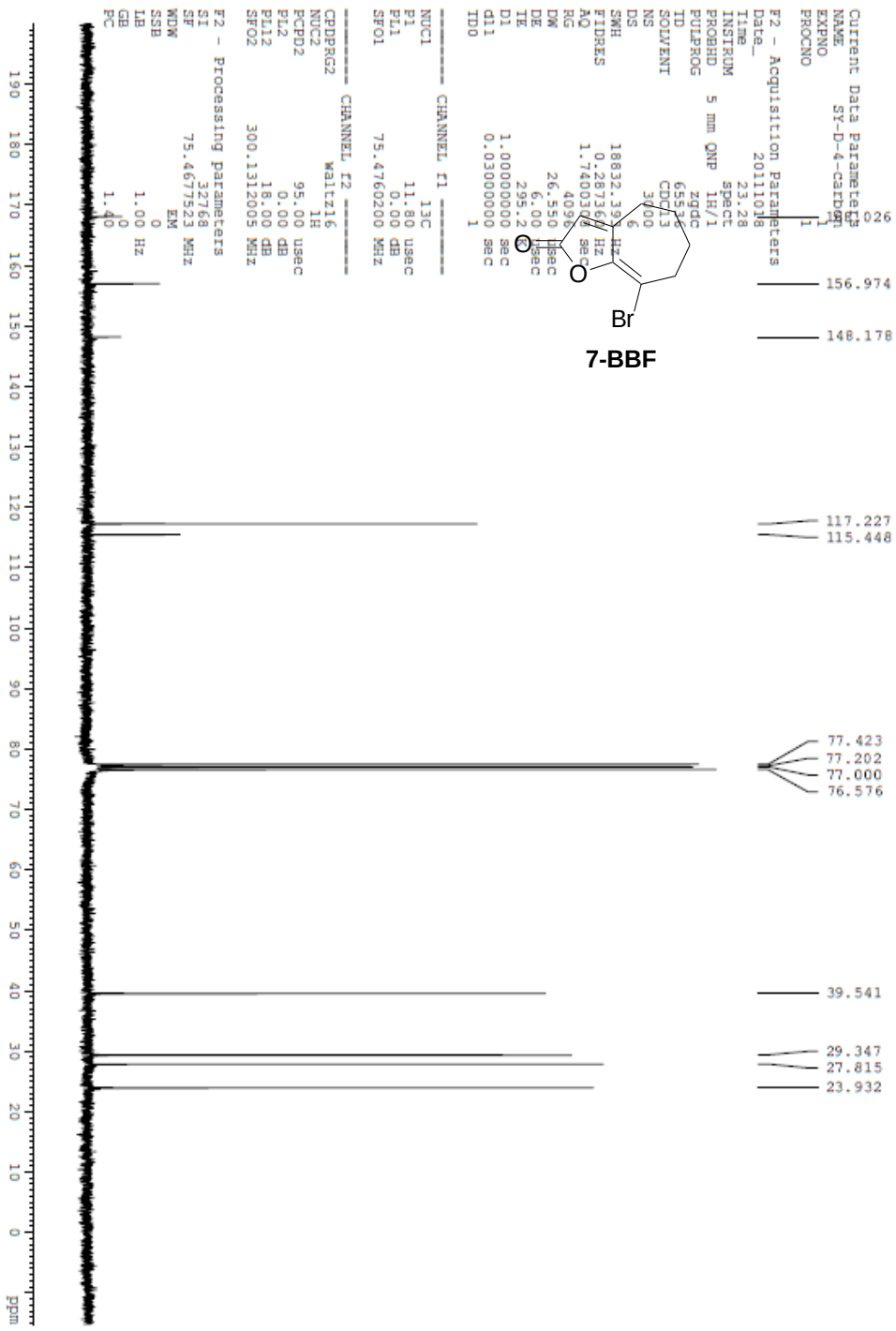
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190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

Part 2: Biological activities study.

Bacteria strains an Bacteria strains and growth media.

Double-knockout mutants of *Pseudomonas aeruginosa*, PAO-JP2 (p*lasI*-LVAgfp) and PAO-JP2 (p*rhlI*-LVAgfp) were kindly provided by Dr. Helen E. Blackwell (University of Wisconsin-Madison) with permission by Dr. Babara H. Iglewski (University of Rochester Medical Center).[2] Plasmids p*lasI*-LVAgfp and p*rhlI*-LVAgfp were kindly provided by Dr. Hiroaki Suga (The University of Tokyo).[3] *Escherichia coli* RP437 and *E. coli* RP437 with pRSH103 plasmid (constitutively expresses red fluorescence proteins) were kindly provided by Dr. Dacheng Ren (Syracuse University)[4]. *E. coli* RP437 and *E.coli* RP437 (pRSH103) were grown in Luria-Bertani (LB) medium (containing 10 µg/mL of tetracycline for *E.coli* RP437 (pRSH103)) at 37 ^{o}C. *P. aeruginosa* PA01-BAA-47 (wild type) from ATCC and PA01-GFP (constitutively expresses green fluorescence proteins on plasmid pSMC2)[5] were grown in LB medium (containing 300 µg/mL of carbenicillin for PA01-GFP) at 37 ^{o}C. All the other *P. aeruginosa* strains were grown in M9 minimal medium containing 300 µg/mL of carbenicillin at 37 ^{o}C.

Stock solutions of brominated furanones

Stock solutions of all brominated furanones (BFs) were prepared in DMSO, sterilized by filtering through a 0.2 µm syringe filter, and stored at -20 °C in sealed vials. Appropriate amount of DMSO was added to controls in all assays to eliminate solvent effect. The amount of DMSO in all cases was no more than 0.8%.

Confocal laser scanning microscopy (CLSM) analysis of biofilms

Biofilms were grown on 316 stainless steel coupons (ca. 3/8 in. × 3/8 in., McMaster-Carr, Elmhurst, IL) with or without BBFs in a 24-well microplate. The plate was wrapped in a Saran wrap and incubated at 37 ºC for 24 h without shaking. Each steel coupon was then washed gently by dipping into 0.9% NaCl saline buffer 3 times (fresh saline buffer was used for each dipping) and then placed upside down on a microscope cover glass (50 x 24mm, No. 2, Fisher Scientific, Pittsburgh, PA). The biofilms were visualized using a Zeiss LSM 710 Confocal Laser Scanning Microscope (Carl Zeiss, Jena, Germany). The biofilms formed by strain *E.coli* RP437 (pRSH103) were visualized by excitation with a HeNe laser at 543 nm and fluorescent emission was detected with a LP 560 nm emission filter. A 488 nm laser line was used to visualize biofilms formed by PA01-GFP. Z-stacks from four randomly picked spots were taken for each steel coupon. Quantification analysis of biomass, mean thickness, and surface area of the biofilms formed in the absence and presence of brominated furanones were obtained from fluorescence image using COMSTAT software.[6] Values are normalized by that of the BF-free control.

Crystal violet biofilm assay in 96-well plates

The protocol for biofilm assay employing crystal violet was adapted from literature with modification.[7] An overnight bacterial culture was subcultured at an optical density (OD_{600}) of 0.1 in LB broth. Appropriate amount of stock solutions of BBFs in DMSO were added to each well. The outermost wells of the 96-well polystyrene microtiter plates (Costar 3370) were filled with 100 µL of sterile autoclaved water to prevent uneven evaporation. The plates were wrapped in Saran wrap and incubated under stationary conditions at 37 ºC for 24 h. The media was then discarded and the plates were washed with deionized water (125 µL × 1) and dried for 30 min.

Biofilms were stained with 100 µL of 0.1% aqueous solution of crystal violet for 30 min at room temperature. The crystal violet was discarded and then the plates were washed with deionized water (125 µL × 3) and dried again for 30 min. The remaining stained cells were solubilized with 200 µL of 95% EtOH and 125 µL of the solubilized CV stain was transferred to a new polystyrene microtiter dish. The amount of biofilm formation was determined by measure the absorbance at 540 nm. The relative biofilm formation was determined using the following equation: relative biofilm formation (%) = (A_{540} sample – A_{540} medium)/(A_{540} control – A_{540} medium) × 100. The sample absorbance values were obtained from the wells containing bacteria and brominated furanones, and the control absorbance values were obtained from BF-free wells containing bacteria and DMSO.

Growth curve of planktonic bacteria

An overnight bacterial culture was diluted and grown to an OD_{600} of 0.05. The subculture was aliquoted into 96-well plates at 200 µL per well. Appropriate amount of stock solutions of brominated furanones in DMSO was added to each well. The outermost wells of the 96-well plates were filled with 200 µL of sterile autoclaved water to prevent uneven evaporation. The plates were incubated at 37 ºC with shaking (250 rpm). The OD_{600} readings were acquired aseptically at 0, 2, 4, 6, 8, 10, 12, and 24 h, using Biotek ELx800 ™ absorbance microplate reader (BioTek Instruments, Inc., Winooski, VT) and Gen5™ data analysis software. The growth curves were plotted as OD_{600} values (mean ± SD) versus time from six replicates.

Reporter gene assay for *P. aeruginosa*

The protocols used for reporter gene assay was adapted from literature.[8, 9] An overnight culture of PA01 (p*lasI*-LVAgfp) and PA01 (p*rhlI*-LVAgfp) in M9 medium (300 µg/mL carbenicillin) was grown from a single colony picked from an LB agar plate supplemented with 300 µg/mL carbenicillin. The cell culture was diluted and grew to an OD_{600} of 0.1 in M9 medium containing 300 µg/mL of carbenicillin. Bacteria culture (200 µL) was added to each well of a polystyrene 96-well microplate (Costar 3370, Corning Incorporated, Corning, NY) containing appropriate amount of natural autoinducers with and without brominated furanones. The plate was incubated at 37 ºC for 24 h in a rotary shaking incubator (250 rpm). The culture from each well was then transferred to a 96-well plate with black wall (µClear, 655096, Greiner Bio-One North America, Inc., Monroe, NC). The fluorescence (an excitation wavelength of 500 nm and an emission wavelength of 540 nm) and OD absorbance (OD_{600}) in each well was measured by Synergy 2 multi-mode microplate reader with a Gen5 data analysis software. Background signals from M9 medium were deducted from all samples.

Production of elastase B

The production of virulence factor elastase B by *P. aeruginosa* was measured as described previously.[10, 11] Bacteria were grown overnight in PTSB media (5% Peptone, 0.1% Tryptic Soy Broth) at 37 ºC, diluted and grown to midlog phase, and subcultured to an OD_{600} of 0.05. The culture was then added to test tubes containing BBFs at the desired final concentrations. The tubes were incubated for 24 h at 37 ºC with shaking (250 rpm). Culture supernatants were recovered by centrifugation at 3000 rpm (Galaxy 5D centrifuge, VWR, Radnor, PA) for 10 min at room temperature and then passed through a 0.45 µm PVDF syringe filter (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). A 100 µL aliquot of the supernatant was added to 900

μL of Elastin-Congo red (ECR) buffer (100 mM Tris-HCl, 1 mM $CaCl_2$, pH 7.2) containing 4.5 mg of Elastin-Congo red and incubated for 24 h at 37 ^oC with shaking (250 rpm). After incubation, 0.2 mL of 0.12 M EDTA was added to stop the reaction. Insoluble ECR was removed by centrifugation and the absorbance of the supernatant (A_{490}) was measured. Elastase B activity was represented by A_{490} of the samples treated with BBFs minus the A_{490} of the bacteria-free samples.

Cytotoxicity assay for human cells

All cell cultures were maintained in a humidified incubator, at 37 °C, with 5% CO_2 atmosphere. Human neuroblastoma SK-N-SH cells (a donation from Bonnie B. Toms at SUNY Upstate Medical University) were cultured in 96-well plates with 100 μL of culture medium (EMEM + 10% FBS, 100 μg/mL streptomycin, 100 IU/mL penicillin, and 2.0 mM L-glutamine). Each well contained 100 μL of cell suspension with a concentration of 5×10^4 cells/mL. The viability of SK-N-SH was determined with a hemacytometer using standard trypan blue protocol. For cytotoxicity assay, the colorimetric cell counting assay (CCK 8 assay) was used as described previously.[12] In brief, after being plated, the SK-N-SH cells were allowed to adhere to the bottom of the wells for 24 h. The medium was then replaced with fresh ones (supplemented with DMSO to eliminate solvent effect for the negative control) with or without brominated furanones. After 1 h, the BF-containing media were removed and the cells were washed twice with fresh culture medium without DMSO. 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.[12] The survival (%) was calculated using the equation: survival (%) = (A_{450} sample – A_{450} medium)/(A_{450} control – A_{450} medium) × 100. The sample

absorbance values were obtained from the wells containing cells and brominated furanones, and the control absorbance were obtained from BF-free wells containing cells and DMSO.

Statistical analysis

Experimental data were analyzed by SigmaStat 3.5 software (Systat Software, Inc., San Jose, CA) and presented as means ± standard error. Two-group comparisons were performed using Student's *t* test. A *P* value of <0.05 was considered to be statistically significant.

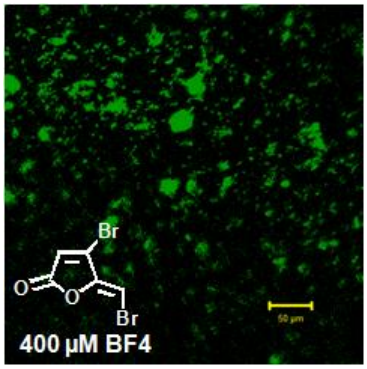

Fig S1. Effect of 400 µM **BF4** on biofilm formation by *P. aeruginosa* strain PA01-GFP. Representative confocal laser scan microscopy (CLSM) image of biofilm formed by PA01-GFP (expresses green fluorescence on plasmid pSMC2) in the presence of 400 µM **BF4**. Scale bar = 50 µm.

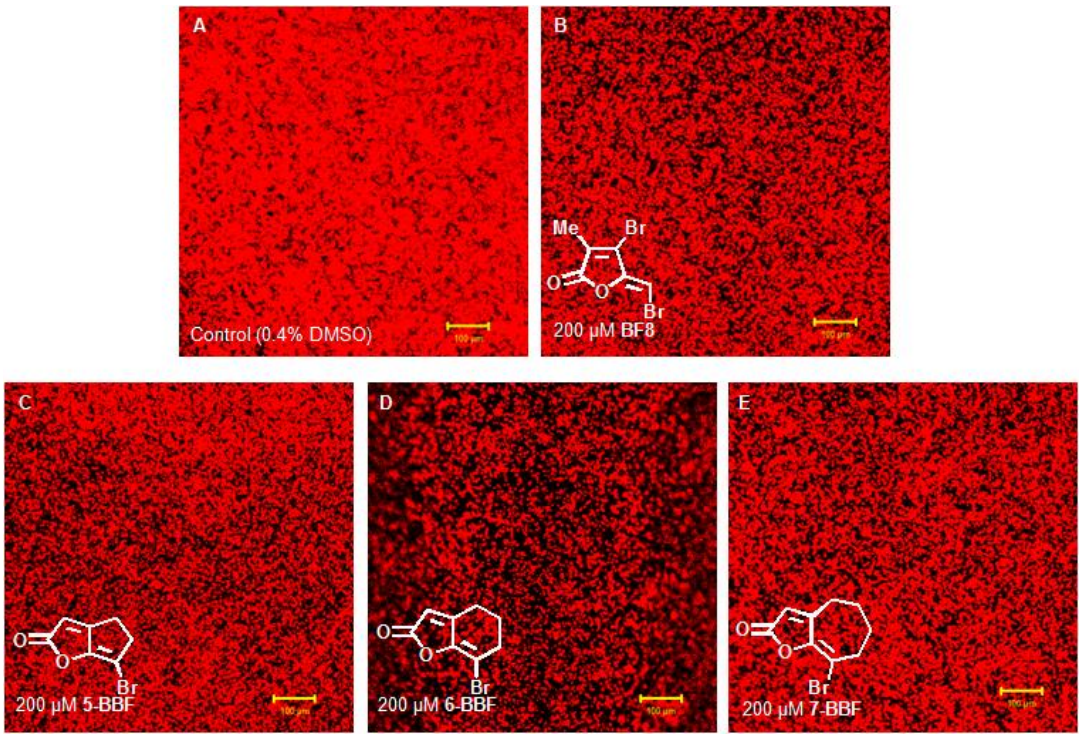

Fig S2. Effect of brominated furanones on biofilm formation by *E.coli* RP437 (pRSH103). Representative confocal laser scanning microscopy (CLSM) images of biofilm formed by *E.coli* RP437 (pRSH103) in the absence (A) and presence of 200 µM **BF8** (B), **5-BBF** (C), **6-BBF** (D) and **7-BBF** (E). The control is supplemented with the same amount (0.4%) of DMSO as present in the brominated furanone-treated conditions. Scale bar = 100 µm.

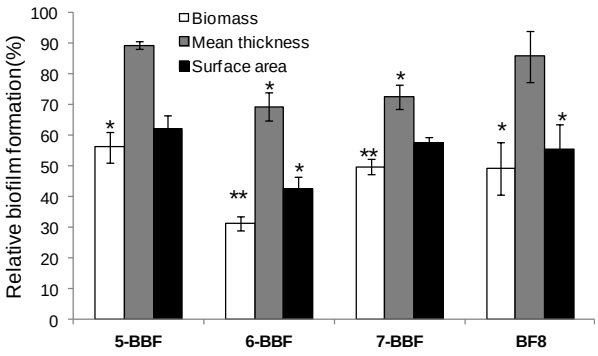

Fig S3. Quantification of biofilm formation by *E.coli* RP437 (pRSH103) in the absence and presence of 200 µM brominated furanones. Biomass, mean thickness, and surface area were quantified from fluorescence image using COMSTAT software.[6] Z-Stack images from four different locations were used. Values are normalized by that of the BF-free control and represent the means ± standard deviation from 4 replicates. Significant differences in the biofilm formation with the BF-free control are indicated by asterisks: *, $P < 0.05$; **, $P < 0.01$.

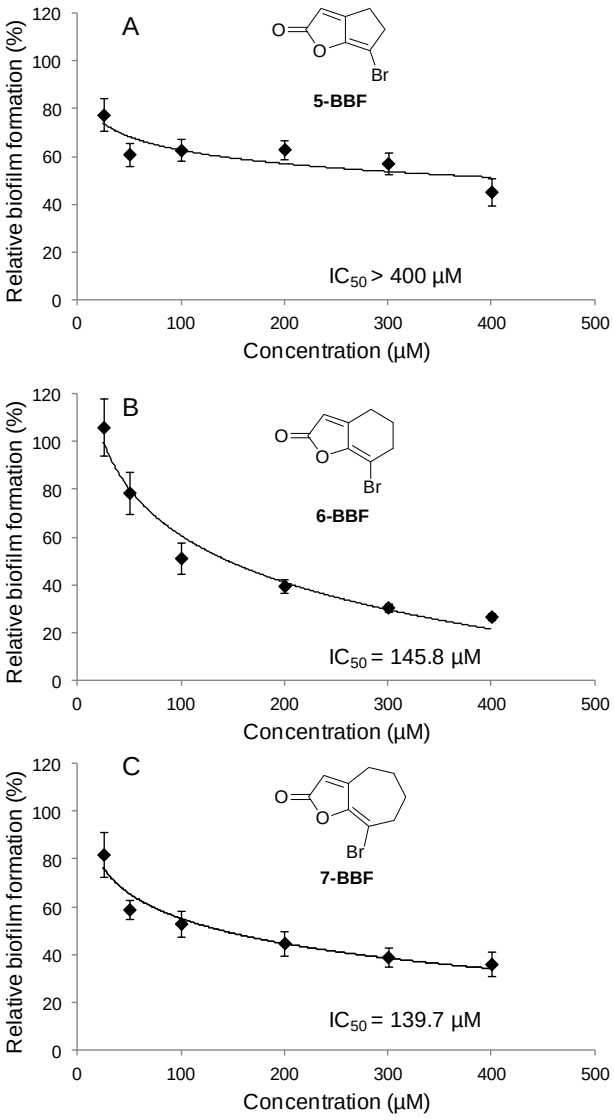

Fig S4. *P. aeruginosa* biofilm formation dose-response curves with IC_{50} values for (A) **5-BBF**, (B) **6-BBF**, and (C) **7-BBF**. Values represent means ± standard deviation from 4 replicates. Data shown is a representative of at least three separate experiments.

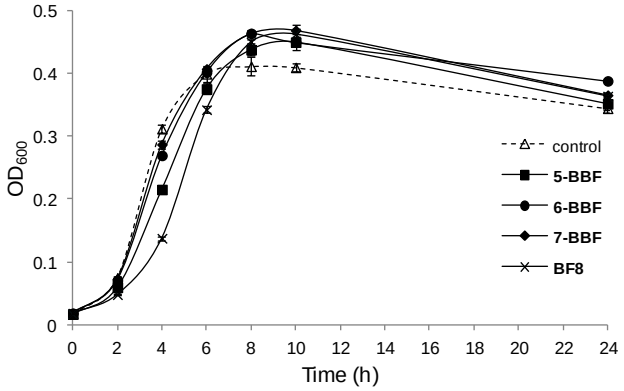

Fig S5. Effect of 200 µM BBFs or **BF8** on the growth of *E. coli* RP437. Growth curve of *E.coli* RP437 in the absence and presence of 200 µM brominated furanones. Values are normalized by that of the BF-free control and represent the means ± standard deviation from 6 replicates. Data shown is a representative of at least three separate experiments.

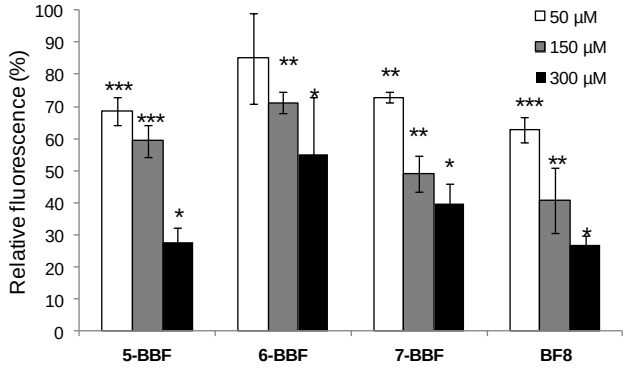

Fig S6. GFP expression by PAO-JP2 (p*lasI*-LVAgfp) in the presence of 1 µM 3-oxo-C12-HSL alone (control) or 1 µM 3-oxo-C12-HSL plus various concentration of **5-BBF**, **6-BBF**, **7-BBF**, or **BF8**. Fluorescence signals were corrected for cell density by dividing by OD_{600} of cell culture and the results were normalized to the BF-free control. Values represent the means ± standard deviation from four replicates. Data shown is a representative of duplicate experiments. Significant differences in the GFP expression with the control are indicated by asterisks: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

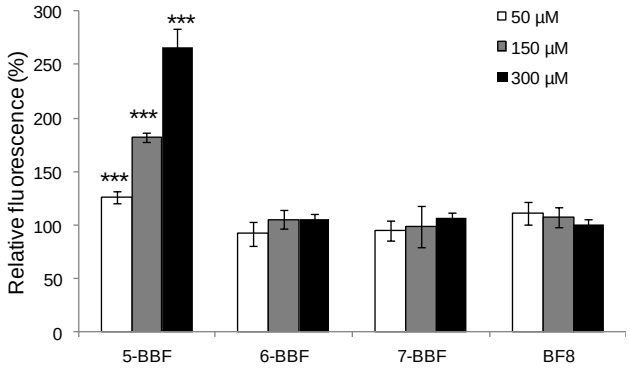

Fig S7. GFP expression by PA01 (p*rhlI*-LVAgfp) in the absence or presence of **5-BBF**, **6-BBF**, **7-BBF**, or **BF8** at 50, 150, and 300 µM. Fluorescence signals were corrected for cell density by dividing by OD_{600} of cell culture and the results were normalized to the BF-free control. Values represent the means ± standard deviation from four replicates. Data shown is a representative of at least three separate experiments. Significant differences in the GFP expression with the control are indicated by asterisks: ***, $P < 0.001$.

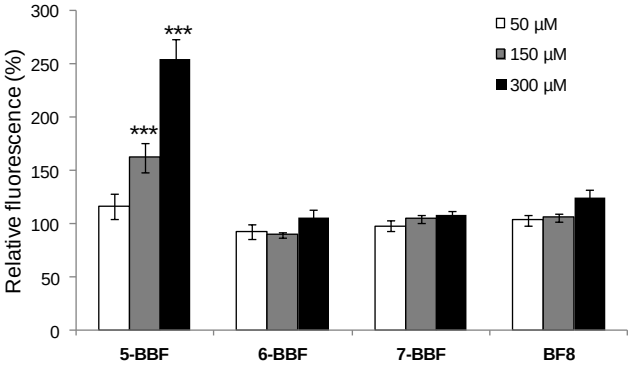

Fig S8. GFP expression by PAO-JP2 (p*rhlI*-LVAgfp) in the presence of 1 µM 3-oxo-C12-HSL and 10 µM C4-HSL alone (control) or 1 µM 3-oxo-C12-HSL and 10 µM C4-HSL plus various concentrations of **5-BBF**, **6-BBF**, **7-BBF**, or **BF8**. Fluorescence signals were corrected for cell density by dividing by OD_{600} of cell culture and the results were normalized to the BF-free control. Values represent the means ± standard deviation from four replicates. Data shown is a representative of at least three separate experiments. Significant differences in the GFP expression with the control are indicated by asterisks: ***, $P < 0.001$.

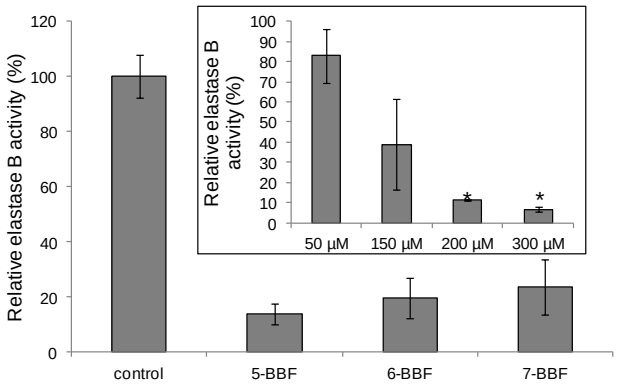

Fig S9. Effects of BBFs on the expression of elastase B by *P. aeruginosa* PAO-JP2. Elastase B activity produced by *P. aeruginosa* PAO-JP2 in the presence of 5 µM 3-oxo-C12-HSL and 10 µM C4-HSL alone (control) or 5 µM 3-oxo-C12-HSL and 10 µM C4-HSL plus 300 µM BBFs or 5 µM 3-oxo-C12-HSL and 10 µM C4-HSL plus various concentration of **6-BBF** (insert). Values were normalized to that of the BF-free control and represent the means ± standard deviation from four replicates. Data shown is a representative of at least three separate experiments. Significant differences in the elastase B activity with the control are indicated by asterisks: *, $P < 0.05$.

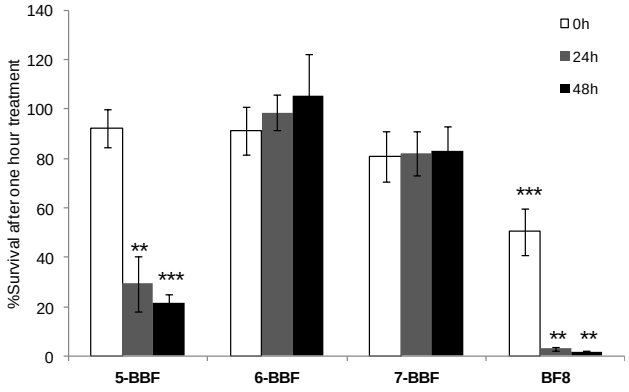

Fig S10. Survival (%) of human neuroblastoma SK-N-SH cells at 0, 24, and 48 h after 1 h treatment of 100 µM **5-BBF**, **6-BBF**, **7-BBF** and **BF8**, respectively. Values represent the means ± standard deviation. Data shown is a representative of duplicate experiments. Significant differences in the survival% with BF-free control are indicated by asterisks: **, $P < 0.01$; ***, $P < 0.001$.

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