Synthesis of 5-(bromomethylene)furan-2(5*H*)-ones and 3-(bromomethylene)isobenzofuran-1(3*H*)-ones as inhibitors of microbial quorum sensing

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(*E*)- and (*Z*)-5-(Bromomethylene)furan-2(5*H*)-ones and (*E*)- and (*Z*)-3-(bromomethylene)isobenzofuran-1(3*H*)-ones have been prepared starting from commercially available maleic anhydrides and phthalic anhydrides, respectively. A debrominative decarboxylation or a bromodecarboxylation reaction is a key step in the synthesis. The furanones were investigated for their ability to interfere with microbial communication and biofilm formation by *Staphylococcus epidermidis*.

Introduction

Many microorganisms communicate *via* chemical signal molecules such as autoinducer-1 (AI-1) and autoinducer-2 (AI-2) to control gene expression in response to population density.[1] This phenomenon, called quorum sensing (QS), regulates various virulence factors, for instance proteolytic activity, carbohydrate metabolism and biofilm formation.[2,3] Thus interference with QS, in principle, would reduce the pathogenic potential of a bacterium. Since this occurs without exerting a selective pressure on microbial viability, resistance development is not likely.

Most microorganisms in nature prefer a biofilm mode of growth[4] and biofilm formation is regulated by QS in a number of microorganisms.[5,6] *Staphylococcus epidermidis* is a Gram-positive biofilm forming commensal bacterium on human skin and mucous membranes, as well as a major nosocomial pathogen associated with medical implant infections.[7] The virulence is related to *S. epidermidis*'s ability to form biofilm on implanted devices. There are presently few effective means to prevent medical implant infections,[8] mainly due to increased resistance of biofilm microorganisms to both antimicrobials and the human immune system, being up to 10–1000 times more tolerant to antimicrobials than their planktonic counterparts.[9] Thus antimicrobial treatment of implant infections often fails, necessitating removal of the implanted device. Control of biofilm formation by for instance *S. epidermidis* represents a novel, non-antimicrobial approach of implant infection prevention. Thus identification of compounds that inhibit QS has become an area of intense research.[2] The macro algae *Delisea pulchra* is known to prevent microbial colonisation of its surface by producing brominated furanones.[10] Such furanones are thought to interfere with microbial communication, mainly in Gram-negative microorganisms.

The aim of this study was to synthesize 5-(bromomethylene)furan-2(5*H*)-ones (**2** and **3**, Scheme 1) and 3-(bromomethylene)isobenzofuran-1(3*H*)-ones (**4** and **5**, Scheme 2) and to assess their ability to interfere with microbial communication and biofilm formation by *S. epidermidis*.

5-(Bromomethylene)furan-2(5*H*)-ones have previously been prepared from levulinic acid[11] or its derivatives,[12] propenoates[13] and allenic esters.[14] 3-(Bromomethylene) isobenzofuran-1(3*H*)-ones have been prepared from 3-alkylidene isobenzofuran-1(3*H*)-ones[15] and by dehydration of bromo keto acids.[16]

5-(Alkylidene)furan-2(5*H*)-ones, without the bromo substituent in the alkylidene group, have been synthesized from maleic anhydrides in a Wittig reaction using stabilized phosphorus ylides.[17] We wanted to use this method in our synthesis since a number of maleic and phthalic anhydrides are commercially available. The necessity to use stabilized phosphorus ylides in the Wittig reaction indicates a bromodecarboxylation step of an α,β-unsaturated acid in order to prepare the 5-bromomethylene substituent. A number of methods have been used in the bromodecarboxylation of α,β-unsaturated acids having a β-aryl substituent.[18] Without such a substituent the methods are much more limited.[19]

Results and discussion

Synthesis of furanones

The unsaturated esters **1** (Scheme 1) were prepared in good yields from commercially available maleic anhydrides in the Wittig reaction and the esters were transformed into the 5-(bromomethylene)furan-2(5*H*)-ones (**2** and **3**) in three ways (Method A, B and C, Table 1). Methods A and B have three steps, while Method C has two. All methods are one-pot procedures—with only solvent evaporation between the steps.

In Method A the *tert*-butyl ester of **1** is first cleaved by TFA in dichloromethane and then brominated before the resulting

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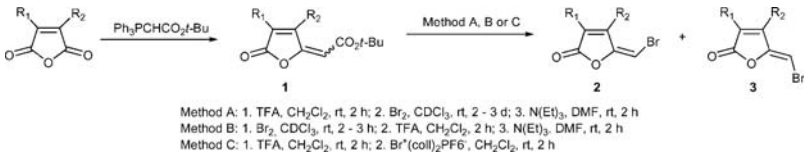
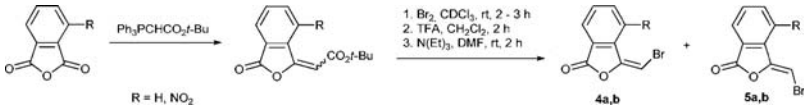


Scheme 1



Scheme 2

α,β-dibromo acid is debromodecarboxylated with triethylamine in DMF. Method B is similar to Method A except that the two first steps are switched. The yields in Method A are somewhat better than the yields in Method B, but the reaction times in Method A are much longer than in Method B. The bromination of the unsaturated acids takes 3–4 days with a large excess of bromine, while the bromination of the unsaturated esters is over in a couple of hours with a slight excess of bromine. The product distribution of the *E*- and *Z*-isomer of the 5-(bromomethylene)furan-2(5*H*)-one (**2** and **3**) is almost the same in these two methods (Table 1). The *Z*-isomer is always the major isomer except in one case (R_1 and R_2 = Me). The identity of the *E*- and *Z*-isomers were determined by NOE experiments and/or by ^{1}H NMR chemical shift of the methine proton and any ring protons.[20]

In Method C the unsaturated esters are cleaved to the acids and then bromodecarboxylated using bis(2,4,6-trimethylpyridine)bromine(I) hexafluorophosphate in dichloromethane. The yield in this reaction is low to moderate, 10–58% over two steps (Table 1). The reaction shows some degree of stereospecificity since treatment of the pure *E*-isomer of the corresponding acid of **1** (R_1 = R_2 = Me) gives mainly the *E*-isomer (*E* : *Z* ratio 71 : 29) of the bromodecarboxylated product. The pure *Z*-isomer gives only the *Z*-isomer.

The product ratios in Table 1 are probably not equal to the thermodynamic ratios of the products, since treatment of either pure **2c** or pure **3c** with iodine in $CDCl_3$ gives the same mixture of **2c** and **3c** (29 : 71) which is significantly different from the ratio in Table 1 (12 : 88)

Phthalic anhydrides can also be used as starting material in the synthesis discussed above. Thus the 3-(bromomethylene)-isobenzofuran-1(3*H*)-ones **4a** and **5a** (R = H, Scheme 2) were formed as a 1 : 2 mixture in 53% yield while the nitro compounds **4b** and **5b** (R = NO_2, Scheme 2) were formed as a 1 : 1 mixture in 27% yield. We could separate the *E*- and *Z*-isomers **4a** and **5a** by flash chromatography but not the nitro compounds **4b** and **5b**.

Bromination of the unsaturated ester **1** (Method B, step 1) or the corresponding unsaturated acid (Method A, step 2) is completely regioselective: only the exocyclic carbon–carbon double bond is brominated at room temperature even if an excess of bromine is used.

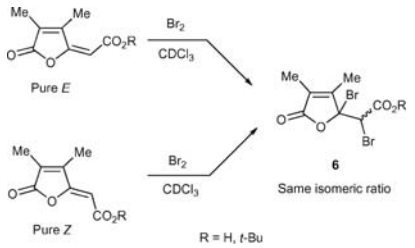
Bromination of ordinary alkenes is stereospecific.[22] The bromination of the unsaturated ester **1** (R_1 = R_2 = Me) or the corresponding unsaturated acid is, however, not stereospecific because the pure *E*-isomer and the pure *Z*-isomer of **1** give the same mixture of dibrominated ester or acid (Scheme 3).

The isomerization occurs only in the bromination step, since no isomerization was observed when a pure dibromide

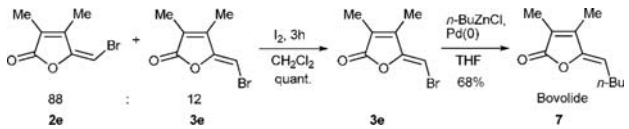
Table 1 Yields and product distribution in the synthesis of **2** and **3**

R_1	R_2	*E* : *Z* Starting material **1**	Method[a]	Product	Yield[b] **2**	Yield[b] **3**	Total yield[b]	*E* : *Z* Product **2** : **3**
H	H	100 : 0	A	**2a**/**3a**[c]	—	—	61	5 : 85
H	H	100 : 0	B	**2a**/**3a**[c]	2	44	46	4 : 96
H	H	100 : 0	C	**2a**/**3a**[c]	22	0	22	100 : 0
Me	H	100 : 0	A	**2b**/**3b**	10	77	87	11 : 89
Me	H	100 : 0	B	**2b**/**3b**	7	46	53	13 : 87
Me	H	100 : 0	C	**2b**/**3b**	32	6	38	84 : 16
Ph	H	100 : 0	A	**2c**/**3c**	7	53	60	12 : 88
Ph	H	100 : 0	B	**2c**/**3c**	6	45	51	12 : 88
Ph	H	100 : 0	C	**2c**/**3c**	54	4	58	93 : 7
Br	H	100 : 0	A	**2d**/**3d**	6	50	56	11 : 89
Br	H	100 : 0	B	**2d**/**3d**	1	48	49	2 : 98
Br	H	100 : 0	C	**2d**/**3d**	6	4	10	60 : 40
Me	Me	0 : 100	A	**2e**/**3e**	66	9	75	88 : 12
Me	Me	0 : 100	B	**2e**/**3e**	52	14	66	79 : 21
Me	Me	0 : 100	C	**2e**/**3e**	—	—	—	0 : 100[d]
Me	Me	100 : 0	C	**2e**/**3e**	34	14	48	71 : 29

[a] Method A: 1. TFA, CH_2Cl_2, rt, 2 h; 2. Br_2, $CDCl_3$, rt, 2–3 d; 3. $N(Et)_3$, DMF, rt, 2 h; Method B: 1. Br_2, $CDCl_3$, rt, 2–3 h; 2. TFA, CH_2Cl_2, 2 h; 3. $N(Et)_3$, DMF, rt, 2 h; Method C: 1. TFA, CH_2Cl_2, rt, 2 h; 2. $Br^+(coll)_2PF_6^-$, CH_2Cl_2, rt, 2 h. [b] Isolated yield. [c] Ref. 21. [d] From the ^{1}H NMR of the crude product.



Scheme 3



Scheme 4

(**6**, R = *t*-Bu) was carried through to the end product. The 5-bromomethylenefuran-2(5*H*)-ones will, however, isomerize if the compounds are kept in a polar solvent like $CDCl_3$ for a couple of weeks. This tendency to isomerize can be utilized in a synthesis of the natural product bovolide[23] (**7**, Scheme 4). A mixture of **2e** and **3e** was isomerized to pure **3e** with iodine in CH_2Cl_2 and coupled with butylzinc chloride in THF at room temperature in a Pd(0)-catalyzed reaction.

The brominated furanone **3d** (Table 1) has proven to be a valuable intermediate in the synthesis of another γ-alkylidene butenolide: lissoclinolide.[24]

Interference with microbial communication and biofilm formation

All synthesized furanones (**2e**, **3b–e**, **4a**, **5a**) and the reference **F202**[21] (Fig. 1) at 6.0 μM reduced bioluminescence in *V. harveyi* BB170 significantly ($P < 0.01$ compared to control without furanone), with **5a** being slightly more effective than the **F202** reference (Fig. 2).

V. harveyi BB170 lacks the receptor for AI-1 and thus responds only to intermicrobial communication *via* the AI-2 QS molecule. We assume therefore that the tested furanones interfered with AI-2 QS communication. This is in line with previous data in both Gram-negative[25] and Gram-positive microorganisms.[26] More recently, furanones were found to structurally alter LuxR in *V. harveyi* thus preventing binding to promoter sequences.[27] Further studies are needed to verify the mechanism of action against *S. epidermidis*.

The two most effective bioluminescence reducers (**5a** and **F202**) were subsequently tested for effect on biofilm formation by *S. epidermidis*. *S. epidermidis* carries the AI-2 synthase gene, although its role in biofilm formation is not clearly defined. The biofilm assay clearly showed the biofilm inhibitory potential of both furanone **5a** and **F202** ($P < 0.01$ compared to control without furanone). Furanone **5a** reduced *S. epidermidis* biofilm by 57%, while the reference **F202** reduced biofilm formation by

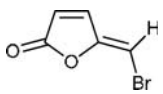


Fig. 1 Structure of compound **F202**, (*Z*)-5-(bromomethylene)furan-2(5*H*)-one.[21]

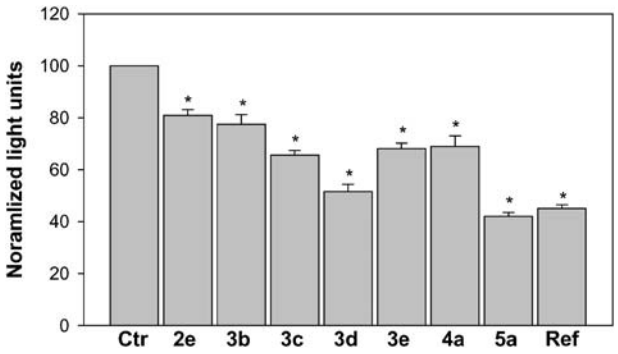


Fig. 2 Bioluminescence response in the reporter strain *V. harveyi* BB 170 induced by *V. harveyi* BB152 supernatant and repressed by 6.0 μM of furanones **2e**, **3b–e**, **4a**, **5a** or the reference **F202**. The results are mean values and standard errors from three independent experiments with three parallels. * Significantly different from control (ctr) without furanone ($P < 0.01$).

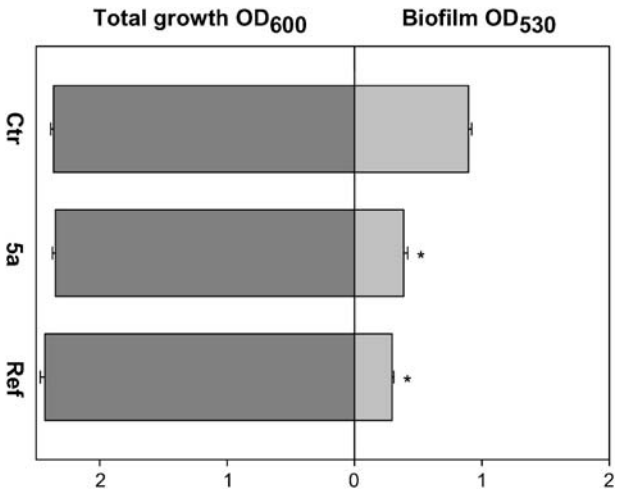


Fig. 3 24 h Biofilm formation by *S. epidermidis* on discs coated with furanone **5a** and the reference **F202**. The results are mean values and standard errors from three independent experiments done in triplicate.* Significantly different from control (ctr) without furanone ($P < 0.01$).

68%. Notably, this reduction could not be ascribed to an antimicrobial effect since for both compounds, total growth was unaffected (Fig. 3). These findings are interesting in view of the increased use of implanted medical devices and the concomitant implant infections. We suggest that synthetic compounds like **5a** and **F202**, immobilised on implant surfaces, may prevent implant infection. **F202** was more effective than **5a** in reducing biofilm formation by *S. epidermidis*. **F202** has previously been reported to interfere with colonization by the Gram-negative *Pseudomonas aeruginosa* in lungs of experimentally infected mice.[28] Thus **F202** appear to be able to interfere with both AI-1 and AI-2 communication. The present and other studies confirm the possibility of interfering with microbial virulence without inhibiting microbial growth.

Conclusion

We have shown that 5-(bromomethylene)furan-2(5*H*)-ones and 3-(bromomethylene)isobenzofuran-1(3*H*)-ones can be

easily synthesized in 3 or 4 steps from commercially available maleic anhydrides and phthalic anhydrides, respectively. This study also shows that furanones may be potential inhibitors of microbial communication. The most effective furanones, **5a** and **F202**, also reduced biofilm formation by *S. epidermidis* without affecting the growth. The bioluminescence assay indicates that the efficacy may be structurally dependent. We see a potential for furanones and isobenzofuranones in preventing implant infections.

Experimental

The ^{1}H NMR and the ^{13}C NMR spectra were recorded on Bruker Avance DPX instruments. Mass spectra, under electron impact conditions, were recorded at 70 eV ionizing energy on a Fision ProSpec instrument.

Preparation of 5-(bromomethylene)furan-2(5*H*)-ones and 3-(bromomethylene)isobenzofuran-2(3*H*)-ones

Method A. The α,β-unsaturated ester (1.0 mmol) was dissolved in a mixture of CH_2Cl_2 (1 mL) and TFA (1 mL). The reaction mixture was stirred at room temperature for 2 h, evaporated and redissolved in a mixture of $CDCl_3$ (2 mL) and TFA (0.1 mL). Bromine (2 mL, 2 M in CCl_4) was added and the mixture was stirred at room temperature until ^{1}H NMR showed that all starting material had been consumed (2–3 d). The solvents were evaporated off and the residue was dissolved in DMF (2 mL). Triethylamine (0.15 mL, 1.08 mmol) was added at 0 °C and the mixture stirred at room temperature for 45 min before water was added. The product was extracted into Et_2O, washed with brine (3 × 10 mL), dried ($MgSO_4$) and evaporated. The *E*- and *Z*-isomers were separated by flash chromatography on silica gel.

Method B. Bromine (0.55 mL, 1.10 mmol, 2 M in CCl_4) was added to a solution of the α,β-unsaturated ester (1.0 mmol) in CH_2Cl_2 (2 mL). The mixture was stirred at room temperature for 2 h before the solvent was evaporated and the residue dissolved in a mixture of CH_2Cl_2 (1 mL) and TFA (1 mL). The mixture was stirred at room temperature for 2 h before the solvent was evaporated off. The residue was dissolved in DMF (2 mL) and triethylamine (0.15 mL, 1.08 mmol) was added at 0 °C. The mixture was stirred at room temperature for 45 min before water was added and the product was extracted into Et_2O, washed with brine (3 × 10 mL), dried ($MgSO_4$) and evaporated. The *E*- and *Z*-isomers were separated by flash chromatography on silica gel.

Method C. The α,β-unsaturated ester (1.0 mmol) was dissolved in a mixture of CH_2Cl_2 (1 mL) and TFA (1 mL). The reaction mixture was stirred at room temperature for 2 h, evaporated and redissolved in CH_2Cl_2 (4 mL). Bis(2,4,6-trimethylpyridine)bromine(I) hexafluorophosphate[29] (700 mg, 1.50 mmol) was added at 0 °C and the mixture was stirred at 0 °C for 15 min and at room temperature for 2 h before Et_2O was added. The Et_2O was washed with 1M HCl, saturated $NaHCO_3$ and brine before it was dried ($MgSO_4$) and evaporated. The *E*- and *Z*-isomers were separated by flash chromatography on silica gel.

(*E*)-5-(Bromomethylene)-3-methylfuran-2(5*H*)-one (2b). Eluent EtOAc–hexane 1 : 6 R_f 0.30; mp 52–56 °C; δ_H (300 MHz; $CDCl_3$) 2.01 (3H, Me), 6.33 (1H, s, CHBr), 7.37 (1H, m, H4); δ_C (75 MHz; $CDCl_3$) 11.03, 91.96, 133.42, 134.52, 150.93, 170.02; *m*/*z* (EI) 190 (M^+ + 2, 63%), 188 (M^+, 64), 125 (65), 122 (40), 120 (42), 97 (42), 68 (49), 39 (100); HRMS (EI) calcd. for $C_6H_5O_2Br$: 187.9472, found 187.9467.

(*Z*)-5-(Bromomethylene)-3-methylfuran-2(5*H*)-one (3b). Eluent EtOAc–hexane 1 : 6 R_f 0.13; mp 91–94 °C; δ_H (200 MHz; $CDCl_3$) 1.95 (3H, Me), 5.91 (1H, s, CHBr), 7.03 (1H, m, H4); δ_C (75 MHz; $CDCl_3$) 10.75, 89.57, 131.24, 135.81, 151.18, 169.52; *m*/*z* (EI): 190 (M^+ + 2, 99%), 188 (M^+, 100), 162 (16), 160 (16), 122 (55), 120 (57), 53 (76); HRMS (EI) calcd. for $C_6H_5O_2Br$: 187.9472, found 187.9469.

(*E*)-5-(Bromomethylene)-3-phenylfuran-2(*H*)-one (2c). Eluent EtOAc–hexane 1 : 3 R_f 0.48; δ_H (200 MHz; $CDCl_3$) 6.48 (1H, s, =CHBr), 7.41–7.47 (3H, m, Ph), 7.82 (1H, s, H4), 7.91–7.96 (2H, m, Ph); δ_C (75 MHz; $CDCl_3$) 93.89, 124.50, 128.78, 128.95, 130.43, 131.12 , 133.15, 150.75, 167.74; *m*/*z* (M^+) 252 (M^+ + 2, 59%), 250 (M^+, 60), 171 (100), 115 (74), 102 (88), 57 (20); HRMS (EI) calcd. for $C_{11}H_7O_2Br$: 249.9629, found 249.9626.

(*Z*)-5-(Bromomethylene)-3-phenylfuran-2(*H*)-one (3c). Eluent EtOAc–hexane 1 : 3 R_f 0.32; mp 123–125 °C; δ_H (200 MHz; $CDCl_3$) 6.10 (1H, s, =CHBr), 7.40–7.44 (3H, m, Ph), 7.47 (1H, s, H4), 7.85–7.90 (2H, m, Ph); δ_C (75 MHz; $CDCl_3$) 91.58, 127.15, 128.69, 128.91, 130.13, 131.30, 132.50, 151.03, 167.1; *m*/*z* (EI): 252 (M^+ + 2, 68%), 250 (M^+, 69), 172 (14), 171 (100), 116 (10), 115 (93), 102 (100), 76 (17); HRMS (EI) calcd. for $C_{11}H_7O_2Br$: 249.9629, found 249.9623.

(*E*)-3-Bromo-5-(bromomethylene)furan-2(5*H*)-one (2d). Eluent EtOAc–hexane 1 : 6 R_f 0.23; mp 52–55 °C; δ_H (200 MHz; $CDCl_3$) 6.53 (1H, s, =CHBr), 7.83 (1H, s, H4); *m*/*z* (EI) 256 (M^+ + 4, 49%), 254 (M^+ + 2, 100), 252 (M^+, 52), 228 (9), 226 (19), 224 (10), 175 (11), 173 (11), 149 (15), 147 15), 145 (15), 122 (18), 120 (19) 119 (17), 117 (17).

(*Z*)-3-Bromo-5-(bromomethylene)furan-2(5*H*)-one (3d).[24] Eluent EtOAc–hexane 1 : 6 R_f 0.15; mp 73–76 °C; δ_H (300 MHz; $CDCl_3$) 6.19 (1H, s, =CHBr), 7.49 (1H, s, H4); δ_C (75 MHz; $CDCl_3$) 93.27, 114.42, 139.42, 150.80, 164.01; *m*/*z* (EI) 256 (M^+ + 4, 49%), 254 (M^+ + 2, 100), 252 (M^+, 51), 228 (8), 226 (16), 224 (8), 145 (16), 147 (15), 53 (37). HRMS (EI) calcd. for $C_5H_2O_2Br_2$: 251.8421, found 251.8422.

(*E*)-5-(Bromomethylene)-3,4-dimethylfuran-2(5*H*)-one (2e). Eluent EtOAc–hexane 1 : 4 R_f 0.40; mp 48–50 °C; δ_H (200 MHz, $CDCl_3$) 1.87 (3H, m, 4-Me), 2.35 (3H, m, 3-Me), 6.38 (1H, s, =CHBr); δ_C (75 MHz, $CDCl_3$) 8.8, 13.7, 91.3, 129.0, 146.2, 150.8, 169.0; *m*/*z* (EI) 204 (M^+ + 2, 100%), 202 (M^+, 100), 191(9), 189(9), 139(10), 127 (17), 122 (26), 120 (26); HRMS (M^+) calcd. for $C_7H_7O_2Br$: 201.9629, found 201.9631.

(*Z*)-5-(Bromomethylene)-3,4-dimethylfuran-2(5*H*)-one (3e). Eluent EtOAc–hexane 1 : 4 R_f 0.26; mp 113–116 °C; δ_H (200 MHz; $CDCl_3$) 1.92 (3H, m, 4-Me), 2.10 (3H, m, 3-Me), 5.98 (1H, s, =CHBr); δ_C (75 MHz; $CDCl_3$) 8.5, 9.6, 86.5, 125.7, 145.7, 152.7, 169.0; *m*/*z* (EI) 204 (M^+ + 2, 100%), 202 (M^+,

100), 191(10), 189 (10), 176 (6), 174 (6), 122 (33), 120 (33), 111 (17), 67 (68); HRMS (M^+) calcd. for $C_7H_7O_2Br$ 201.9629, found 201.9634.

(*E*)-3-(Bromomethylene)isobenzofuran-1(3*H*)-one (4a). Eluent EtOAc–hexane 1 : 6 R_f 0.23; mp 80–85 °C; δ_H (200 MHz; $CDCl_3$) 6.56 (1H, s, =CHBr), 7.62–8.46 (Ar); δ_C (75 MHz; $CDCl_3$) 91.05, 124.56,125.73, 125.97, 131.05, 134.70, 137.23, 147.49, 165.65; *m*/*z* (EI) 226 (M^+ + 2, 98%), 224 (M^+, 100), 170 (11), 168 (11), 104 (48), 89 (82), 76 (52); HRMS (M^+) calcd. for $C_9H_5O_2Br$: 223.9472 found 223.9475.

(*Z*)-3-(Bromomethylene)isobenzofuran-1(3*H*)-one (5a).[15] Eluent EtOAc–hexane 1 : 6 R_f 0.15; mp 128–131 °C; δ_H (200 MHz; $CDCl_3$) 6.33 (1H, s, =CHBr), 7.57–7.91 (Ar); δ_C (75 MHz; $CDCl_3$) 85.72, 119.96, 124.47, 125.84, 130.56, 134.87, 137.90, 148.72, 165.54; *m*/*z* (EI) 226 (M^+ + 2, 98%), 224 (M^+, 100), 170 (10), 168 (10), 104 (36), 89 (83), 76 (41); HRMS (EI) calcd. for $C_9H_5O_2Br$: 223.9472, found 223.9476.

(*E*)- and (*Z*)-3-(Bromomethylene)-4-nitroisobenzofuran-1(3*H*)-one (4b and 5b). Eluent EtOAc–hexane 2 : 3 R_f 0.35 gave a 1 : 1 mixture of **4b** and **5b**; δ_H (200 MHz; $CDCl_3$) 6.95 (1H, s, =CHBr), 7.59 (1H, s, =CHBr), 7.80–8.50 (2 × 3H, m, Ph); *m*/*z* (EI): 271 (M^+ + 2, 23%), 269 (M^+, 23), 218 (34), 190 (49), 161 (55), 104 (60), 75 (100), 57 (59).

(*Z*)-3,4-Dimethyl-5-pentylidenefuran-2(5*H*)-one (7).[23] Zinc chloride (3.0 mL, 1.5 mmol, 0.50 M in THF) was added dropwise to a solution of *n*-butyllithium (0.15 mL, 1.5 mmol, 10 M) at −78 °C under N_2. After 1 h a solution of (*Z*)-5-(bromomethylene)-3,4-dimetylfuran-2(5*H*)-one (**3e**) (0.102 g, 0.5 mmol) in dry THF (5 mL) was added, followed by tetrakis(triphenylphosphine)palladium [generated *in situ* from tris(dibenzylideneacetone)dipalladium chloroform adduct (0.026 g, 0.025 mmol) and triphenylphosphine (0.026 g, 0.1 mmol)] in dry THF (4 mL). The mixture was stirred at room temperature for 18 h before the solvent was evaporated off. The residue was dissolved in diethyl ether, washed with a saturated solution of NH_4Cl (10 mL) and brine. The solution was dried ($MgSO_4$), evaporated and the crude product purified by flash chromatography on silica gel. Eluent 0–15% EtOAc in hexane; 0.61 g (68%) yellow oil; δ_H (200 MHz; $CDCl_3$) 0.88 (3H, t, J = 7.0 Hz, –CH_3), 1.30–1.42 (2 × 2H, m, 2 × CH_2), 1.86 (3H, s, 4-Me), 1.99 (3H, s, 3-Me), 2.32 (2H, q, J = 7.3 Hz, −CH_2), 5 .17 (1H, t, J = 7.8 Hz, CH*n*Bu); δ_C (75 MHz; $CDCl_3$) 8.48, 9.77, 13.74, 22.28, 25.60, 31.26, 110.86, 123.89, 146.83, 149.93, 170.93; *m*/*z* (EI): 180 (M^+, 38%), 137 (98), 138 (16), 125 (27), 124 (100), 110 (16), 82 (17), 55 (65). HRMS (EI) calcd. for $C_{11}H_{16}O_2$: 180.1150, found 180.1147.

Interference with microbial communication

Bioluminescence assay. The biofilm producing type strain *Staphylococcus epidermides* ATCC35984, the AI-2 QS signal producer *Vibrio harveyi* BB152, and the AI-2 QS signal reporter *Vibrio harveyi* BB170 (the *Vibrio*s were kind gifts from Prof. B. L. Bassler) were included in the study. Growth conditions for *V. harveyi* were as described previously.[6,26,30] The second overnight culture of the reporter strain *V. harveyi* BB170 was diluted 1 : 500 in fresh BA-medium and then stored at −70 °C until use.[31] *S. epidermidis* was grown on Brain Heart Infusion agar plates (BHI, Difco Laboratories, Detroit, MI, USA) for 24 h at 37 °C in an aerobic atmosphere before inoculation into Brain Heart Infusion medium (BHI, Difco Laboratories, USA) for biofilm formation and planktonic growth.

A slightly modified bioluminescence assay was performed as previously described[6,26,30] to assess the ability of the various furanones (**2e**, **3b–e**, **4a**, **5a**) to interfere with QS communication. Furanone **F202** was included as a reference. Briefly, cell-free supernatants prepared from *V. harveyi* BB152 were added at final concentration of 10% to BB170. The various furanones were added to a final concentration of 6.0 μmol L^{-1} where upon bioluminescence induction was followed during the next six hours in a Synergy HT Multi-Detection Microplate Reader (Biotek, VT, USA).

Biofilm assay. Biofilm by *S. epidermidis* was allowed to form during 24 h at 37 °C in an aerobic atmosphere on polystyrene discs (Nunc) coated with furanone. Coating with furanone was performed by adding 1 ml 60 μmol L^{-1} furanone dissolved in ethanol to each well. After 24 h, the solvent had evaporated. Discs similarly treated but without furanone were included as controls with no inhibitory effect on biofilm.

Biofilm mass formed was quantified as follows; the discs were rinsed twice in distilled water and stained for 10 min with a 0.1% solution of safranin in new wells. The discs were rinsed again, and bound safranin was released from stained cells using 30% glacial acetic acid. OD measurements at 530 nm were compared to the non-furanone coated control and the reference **F202**.

To exclude an antimicrobial effect of the furanones, total growth, including both scraped biofilm and planktonic cells, was measured. After vigorous shaking to evenly disperse the cells, total growth was quantified by measuring optical density at 600 nm.

Statistics. Each assay was performed in triplicate in three independent experiments. One-way ANOVA on Ranks followed by Student–Newman–Keuls method for normal distribution was used for multiple comparisons on biofilm formation and growth and for comparisons of bioluminescence induction. The level of significance was set at $P \leq 0.01$.

References

1 J. M. Henke and B. L. Bassler, *Trends Cell Biol.*, 2004, **11**, 648–656.
2 W. R. Lyon, J. C. Madden, J. C. Levin, J. L. Stein and M. G. Caparon, *Mol. Microbiol.*, 2001, **42**, 145–157; A. Vendeville, K. Winzer, K. Heurlier, C. M. Tang and K. R. Hardie, *Nat. Rev. Microbiol.*, 2005, **3**, 383–396.
3 W. C. Fuqua, S. C. Winans and E. P. Greenberg, *J. Bacteriol.*, 1994, **176**, 269–275; R. McNab, S. K. Ford, A. El-Sabaeny, B. Barbieri, G. S. Cook and R. J. Lamont, *J. Bacteriol.*, 2003, **185**, 274–284; K. B. Xavier and B. L. Bassler, *Curr. Opin. Microbiol.*, 2003, **6**, 191–197.
4 J. W. Costerton, P. S. Stewart and E. P. Green, *Science*, 1999, **284**, 1318–1322; D. Stickler, *Curr. Opin. Microbiol.*, 1999, **2**, 270–275.
5 M. R. Parsek and E. P. Greenberg, *Trends Microbiol.*, 2005, **13**, 27–33; M. B. Miller and B. L. Bassler, *Annu. Rev. Microbiol.*, 2001, **55**, 165–199.
6 M. G. Surette and B. L. Bassler, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 7046–7050.

7 J. P. O'Gara and H. Humphreys, *J. Med. Microbiol.*, 2001, **50**, 582–586; F. Götz, *Mol. Microbiol.*, 2002, **43**, 1367–1378.
8 I. Raad, A. Alarahwan and K. Rolston, *Clin. Infect. Dis.*, 1998, **26**, 1182–1187.
9 D. G. Davies, M. R. Parsek, J. P. Pearson, B. H. Iglewski, J. W. Costerton and E. P. Greenberg, *Science*, 1998, **280**, 295–298; T. F. Mah and G. A. O'Toole, *Trends Microbiol.*, 2001, **9**, 34–39.
10 S. Kjelleberg and P. Steinberg, *Microbiol. Today*, 2001, **28**, 134–135.
11 A. J. Manny, S. Kjelleberg, N. Kumar, R. de Nys, R. W. Read and P. Steinberg, *Tetrahedron*, 1997, **53**, 15813–15826; A. Sorg, K. Siegel and R. Brückner, *Synlett*, 2004, 321–325; A. Sorg, K. Siegel and R. Brückner, *Chem.–Eur. J.*, 2005, **11**, 1610–1624.
12 C. M. Beechan and J. J. Sims, *Tetrahedron Lett.*, 1979, 1649–1652.
13 D. Caine and V. C. Ukachukwu, *J. Org. Chem.*, 1985, **50**, 2195–2198.
14 P. de March, J. Font, A. Garcia and Z. Qingying, *J. Org. Chem.*, 1995, **60**, 1814–1822.
15 K. Hemmi, J. W. Harper and J. C. Powers, *Biochemistry*, 1985, **24**, 1841–1848.
16 G. A. Krafft and J. A. Katzenellenbogen, *J. Am. Chem. Soc.*, 1981, **103**, 5459–5466.
17 E. Negishi and M. Kotora, *Tetrahedron*, 1997, **53**, 6707–6738.
18 A. Graven, K. A. Jørgensen, S. Dahl and A. Stanczak, *J. Org. Chem.*, 1994, **59**, 3543–3546; H.-W. You and K.-J. Lee, *Synlett*, 2001, 105–107; C. Kuang, H. Senboku and M. Tokuda, *Synlett*, 2000, 1439–1442; D. Naskar and S. Roy, *Tetrahedron*, 2000, **56**, 1369–1377; S. C. Roy, C. Guin and G. Maiti, *Tetrahedron Lett.*, 2001, **42**, 9253–9255.
19 F. Homsi and G. Rousseau, *Tetrahedron Lett.*, 1999, **40**, 1495–1498; C. Kuang, H. Senboku and M. Tokuda, *Tetrahedron Lett.*, 2001, **42**, 3893–3896.
20 C. F. Ingham, R. A. Massy-Westropp, G. D. Reynolds and W. D. Thorpe, *Aust. J. Chem.*, 1975, **28**, 2499–2501.
21 T. Benneche, J. Lönn and A. Aamdal Scheie, *Synth. Commun.*, 2006, **36**, 1401–1404.
22 J. March, in *Advanced Organic Chemistry*, Wiley-Interscience, New York, 4th edn, 1992, pp. 737.
23 W. D. Wulff, S. R. Gilbertson and J. P. Springer, *J. Am. Chem. Soc.*, 1986, **108**, 520–522.
24 A. Sorg, F. Blank and R. Brückner, *Synlett*, 2005, 1286–1290.
25 D. Ren, L. A. Bedzyk, R. W. Ye, S. M. Thomas and T. K. Wood, *Biotechnol. Bioeng.*, 2004, **88**, 630–642.
26 J. Lönn-Stensrud, F. C. Petersen, T. Benneche and A. Aamdal Scheie, *Oral Microbiol. Immunol.*, 2007, **22**, 340–346.
27 T. Defoirdt, C. M. Miyamoto, T. K. Wood, E. A. Meighen, P. Sorgeloos, W. Verstraete and P. Bossier, *Environ. Microbiol.*, 2007, **9**, 2486–2495.
28 H. Wu, Z. Song, M. Hentzer, J. B. Andersen, S. Molin, M. Givskov and N. Høiby, *J. Antimicrob. Chemother.*, 2004, **53**, 1054–1061.
29 F. Homsi, S. Robin and G. Rousseau, *Org. Synth.*, 2000, **77**, 206–211.
30 S. Schauder, K. Shokat, M. G. Surette and B. L. Bassler, *Mol. Microbiol.*, 2001, **41**, 463–476.
31 A. H. Rickhard, R. J. Palmer, Jr, D. S. Blehert, S. R. Campagna, M. F. Semmelhack and P. G. Egland, *Mol. Microbiol.*, 2006, **60**, 1446–1456.

