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REACTION OF (*Z*)-5-(BROMOMETHYLENE)THIOPHEN-2(5*H*)-ONE WITH SOME NUCLEOPHILES IN SEARCH FOR NEW BIOFILM INHIBITORS

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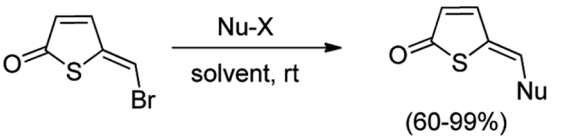
GRAPHICAL ABSTRACT



Nu-X = amines, N_3-Na, PPh_3, 4-$NO_2C_6H_4O$-H, thiols, $PhSO_2$-Na

Abstract *(Z)-5-(Bromomethylene)thiophen-2(5H)-one reacts in a Michael-type addition across the methylene side chain with nucleophiles followed by elimination of bromide. The reaction was done at room temperature and the yields were good. The aromatic sulfur substitution products showed potent activity as biofilm inhibitors.*

Keywords Biofilm; biofilm inhibitors; elimination; Michael addition; thiophenones

INTRODUCTION

Bacteria form biofilms on all kinds of surfaces. Such biofilms may have substantial implications in fields ranging from various industrial processes to health and disease.[1] In many bacteria, the mechanisms of biofilm formation include a cell-density-regulated gene expression called quorum sensing (QS).[2] QS communication leads to regulation of a variety of physiologic functions, including biofilm formation. QS thus represents an interesting novel target for control of biofilm-related problems and has lately created great interest in the scientific community.[3]

(*Z*)-5-(Bromomethylene)thiophen-2(5*H*)-one (**1**) is a QS inhibitor.[4] It is also an analog of the well-known brominated furanones **2** that have biofilm-modulating poperties.[5] In a comparison between thiophenones and furanones, we found that compound **1** was four times more active than the furanone **2** (R=H) in inhibiting

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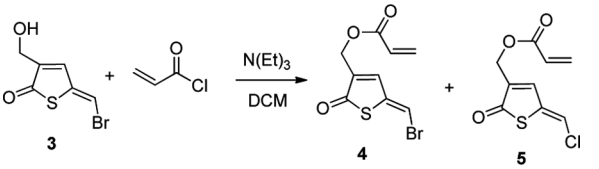
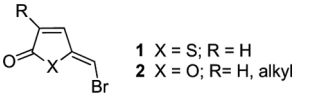


Scheme 1. Reaction of 3-hydroxymethyl-5-(bromomethylene)thiophen-2(5H)-one with acroyl chloride.

biofilm formation in the marine bacteria *V. harveyi*.[6] In a survey of structure–activity relationship for the thiophenones, we were interested to see if replacement of the bromo substituent with other heteroatoms would give biologically active molecules.

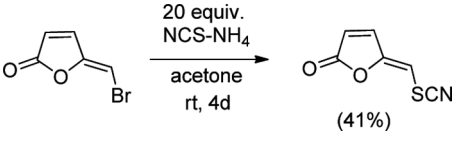


In an acylation reaction between the thiophenone **3** and acroyl chloride we noted that the product was a mixture of the 5-(bromomethylene) compound **4** and the 5-(chloromethylene) compound **5** (Scheme 1). A mixture of these compounds was obtained even if the acylation was carried out at 0 °C and with a relatively short reaction time (15 min).

This observation led us to believe that it might be possible to exchange the bromo substituent in **1** with different nucleophiles. Hence we have shown that the bromo substituent in **1** can be exchanged with other halides and pseudohalides.[6] In this article, we are concerned with other nucleophiles and their reaction with compound **1**.

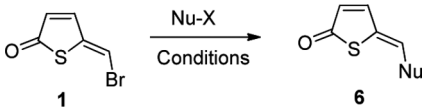
The mechanism for the exchange reaction between compound **1** and halides or pseudohalides is thought to be a Michael-type addition of the nucleophile to the methylene group followed by elimination of the bromide ion. However, the reactivity in this reaction is dependent on the ring system. For instance, compound **1** reacted with 1 equivalent of ammonium thiocyanate in acetone at 0 °C to room temperature to the substitution product **6k** in 62% yield (Table 1, entry 13) while the corresponding furanone needed a large excess (20 equivalents) of ammonium thiocyanate and a prolonged reaction time (4 d) for the exchange reaction to take place (Scheme 2).

Of course, there are possibilities for nucleophiles to react with compound **1** at other places than the exocyclic double bond. For instance, the ring system itself, which is a thioester, should be inclined to react at the ester function with nucleophiles. Indeed, when compound **1** was treated with an aqueous base, then an immediate decomposition of the ring system was observed, indicating a thioester hydrolysis.



Scheme 2. Reaction of 5-(bromomethylene)furan-2(5H)-one with ammonium thiocyanate.

Table 1. Exchange reactions in thiophenone **1** with different nucleophiles (Nu-X)



1 **6**

Entry	6	Nu-X	Conditions	Yield (%)[a]
1	**a**	BuNH-H	DCM, 0 °C, 2 h	85
2	**b**	Et_2N-H	$CDCl_3$, 0 °C–rt, 2.5 h	99
3	**c**	N_3-Na	DMF, 0 °C, 20 min	89[b]
4	**d**	PPh_3	$CDCl_3$, rt, 24 h	87
5	**e**	4-$NO_2C_6H_4O$-H	$CDCl_3$, $N(Et)_3$, rt, 18 h	60
6	—	AcO-NH_4	Acetone, rt, 2 d	0
7	—	NCO-K	MeOH, rt, 3 d	0[b]
8	**f**	EtS-H	$CDCl_3$, $N(Et)_3$, 0 °C–rt, 2 h	76
9	**g**	4-ClC_6H_4S-H	$CDCl_3$, $N(Et)_3$, 0 °C–rt, 2 h	96
10	**h**	Pyridin-2-ylthio-H	DCM, $N(Et)_3$, 0 °C, 30 min	80
11	**i**	Pyrimidin-2-ylthio-H	$CDCl_3$, $N(Et)_3$, 0 °C–rt, 3 h	75
12	**j**	$C_6H_5SO_2$-Na	DMF, 0 °C, 15 min	71
13	**k**	NCS-NH_4	Acetone, 0 °C–rt, 4 h	62[c]

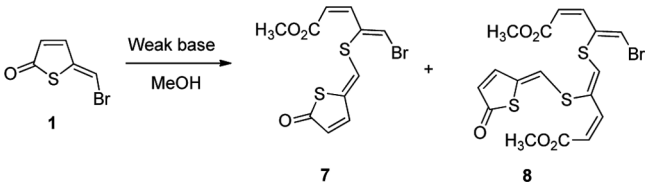
[a]*Z*-isomer. From the ^{1}H NMR of the crude product, in some cases signals seem to come from minor amounts of the *E*-isomer.
[b]Ring-opening reaction occurs; see text.
[c]Ref. 6.

In Table 1 are the results from the reaction of compound **1** with different nucleophiles.

The nitrogen nucleophiles *n*-butylamine, diethylamine, and sodium azide in nonhydroxylic solvents reacted rapidly with compound **1** in an exchange reaction with the bromo substituent (entries 1–3, Table 1). The exchange reaction of *n*-butylamine is somewhat surprising because the furan analog 4-bromo-3-butyl-5-(bromomethylene)furan-2(5*H*)-one reacts with *n*-butylamine under the same conditions at the ester functionality to give only the corresponding 1,5-dihydropyrrol-2-one.[7] Pyridine and the anion of 5-chloropyrimidin-2(1*H*)-one in dichloromethane (DCM) did not react with **1** at room temperature.

Triphenyl phosphine reacted with **1** at room temperature to give a vinyl phosphonium bromide in good yield (entry 4). Triphenyl arsine, on the other hand, did not react even after heating in dichloroethane for 24 h.

A mixture of *p*-nitrophenol and triethylamine in $CDCl_3$ reacted with **1** to give the corresponding enol ether (entry 5). Ammonium acetate in acetone gave mainly starting material after stirring for 2 d at room temperature (entry 6) while ammonium acetate in dimethylformamide (DMF) led to complete decomposition of the starting material without observation of any substitution products. Other types of oxygen nucleophiles as KOCN in DMF and NaOMe in methanol gave complex reaction mixtures, indicating degradation of the ring system. In fact, if compound **1** was reacted with KOCN in methanol/water at room temperature, a ring-opening reaction with methanol occurred and the dimer **7** and the trimer **8** were



Scheme 3. Reaction of 5-(bromomethylene)thiophen-2(5H)-one with a weak base in methanol.

isolated in 47% and 8% yields, respectively, together with 29% of recovered starting material (entry 7 and Scheme 3).

The same reactivity pattern was observed with ammonium acetate in methanol. The synthesis of compounds **7** and **8** actually represents a stereospecific synthesis of penta-2,4-dienoates.

Thiols under basic conditions reacted readily with compound **1** to give substitution products (entries 8–11). Even sodium phenylsulfinate in DMF gave a clean exchange reaction (entry 12).

The solvent seems to play an important role in the exchange reaction, but the softness of the nucleophile may be more important, as can be seen from entries 6 and 13. The soft thiocyanate anion reacted nicely at room temperature in acetone, whereas the relatively hard acetate anion did not react at all under the same conditions.

The compounds **6** were tested for biofilm inhibition activity on *V. harveyi* in a Calgary biofilm model[6] (compound **6a** was not tested). Only the aromatic sulfur compounds **6g–j** showed any substantial activity. Details will be published elsewhere.

In summary the bromo substituent in (*Z*)-5-(bromomethylene)thiophen-2(5-*H*)-one (**1**) can be exchanged with fairly soft nucleophiles in nonhydroxylic solvents such as DCM, $CDCl_3$, acetone, and DMF. The reaction takes place at room temperature and the yields are good. Only the aromatic sulfur compounds gave substitution products (**6g–j**) with any essential activity against bacterial biofilm formation.

EXPERIMENTAL

Typical Procedure for the Synthesis of 6 (see also Table 1)

A mixture of 4-chlorobenzenthiol (55 mg, 0.38 mmol) and triethylamine (43 mg, 0.38 mmol) in DCM (1 mL) was added dropwise to a solution of (*Z*)-5-(bromomethylene)thiophen-2(5*H*)-one (71 mg, 0.38 mmol) in DCM (2 mL) at 0 °C. The mixture was stirred for 1 h at room temperature before ether was added, and the organic phase was washed with brine. The dried solution ($MgSO_4$) was evaporated, and the crude product was purified by flash chromatography using hexane/EtOAc 8:1 for elution to give (*Z*)-5-(((4-chlorophenyl)thio)methylene)thiophen-2(5*H*)-one **6g**. Yield 93 mg (96%); mp 107–108 °C; δ_H (200 MHz, $CDCl_3$) 7.47 (1 H, d, *J* 5.8), 7.42–7.28 (4 H, m), 7.06 (1 H, s), 6.30 (1 H, d, *J* 5.8); δ_C (75 MHz, $CDCl_3$) 193.75, 147.91, 136.03, 135.49, 133.28, 132.63, 131.62, 130.23, 128.72; *m/z* (EI): 256 ($M^+ + 2$, 50%), 254 (M + 2, 100), 228 (17), 226 (40), 191 (19), 98 (56). HRMS (EI) calc. for $C_{11}H_7ClOS_2$ 253.9627. Found 253.9629.

(*Z*)-5-((Butylamino)methylene)thiophen-2(5*H*)-one 6a

Eluent: hexane/EtOAc 1:1; δ_H (300 MHz, $CDCl_3$) 7.46 (1 H, d, *J* 5.3), 6.95 (1 H, d, *J* 13.1), 5.90 (1 H, d, *J* 5.3), 3.32 (2 H, dd, *J* 13.2, 6.9), 1.69–1.52 (2 H, m), 1.39 (2 H, m), 0.95 (3 H, t, *J* 7.3); δ_C (75 MHz, $CDCl_3$) 194.14, 149.34, 142.62, 117.38, 108.49, 48.68, 33.27, 20.05, 14.05; *m/z* (EI) 183 (M+, 100), 141 (12), 140 (52), 127 (22), 112 (24), 99 (10); HRMS (EI) $C_9H_{13}NOS$ 183.0718. Found 183.0718.

(*Z*)-5-((Diethylamino)methylene)thiophen-2(5*H*)-one 6b

Eluent: $CHCl_3$/MeOH 20:1; δ_H (200 MHz, $CDCl_3$) 7.39 (2 H, d, *J* 5.3), 6.91 (2 H, s), 5.85 (2 H, d, *J* 5.3), 3.42 (4 H, q, *J* 7.2), 1.26 (6 H, t, *J* 7.2); δ_C (75 MHz, $CDCl_3$) 195.83, 151.21, 144.07, 114.98, 105.75, 14.78; *m/z* (EI) 183 (M+, 100%), 168 (46), 154 (6), 140 (37), 112 (15), 71 (17), 56 (40). HRMS (EI) calc. for $C_9H_{13}NOS$ 183.0718. Found 183.0722.

(*Z*)-5-((Bromotriphenylphosphoranyl)methylene)thiophen-2(5*H*)-one 6d

Mp > 260 °C; δ_H (200 MHz, $CDCl_3$) 9.45 (1 H, d, *J* 15.7), 9.34 (1 H, dd, *J* 5.9, 1.4), 7.93–7.54 (15 H, m), 6.45 (1 H, dd, *J* 5.8, 3.5); δ_C (75 MHz, $CDCl_3$) 192.25 (s), 162.45 (s), 153.33 (d, *J* 18.6), 136.12 (d, *J* 3.1), 134.64 (d, *J* 11.1), 131.93 (s) 131.11 (d, *J* 13.2), 117.93 (d, *J* 91.2), 111.29 (d, *J* 88.1); *m/z* (ESI) 373 (M + -Br, 100%). HRMS (ESI) calc. for $C_{23}H_{18}OPS$ 373.0816. Found 373.0810.

(*Z*)-5-((4-Nitrophenoxy)methylene)thiophen-2(5*H*)-one 6e

Eluent: CH_2Cl_2; mp 205–207 °C; δ_H (200 MHz, $CDCl_3$) 8.16 (2 H, d, *J* 9.1), 7.52 (1 H, d, *J* 5.8), 7.19–7.07 (3 H, m), 6.21 (1 H, d, *J* 5.9); δ_C (75 MHz, CDCl3) 160.41, 146.73, 141.40, 129.31, 126.58, 124.61, 117.51; *m/z* (EI) 249 (M+, 100%), 192 (6), 147 (17), 146 (8), 71 (11). HRMS (EI) calc. for $C_{11}H_7NO_4S$ 249.0096; found 249.0101.

(*Z*)-5-((Ethylthio)methylene)thiophen-2(5*H*)-one 6f

Eluent: hexane/EtOAc 4:1; δ_H (200 MHz, $CDCl_3$) 7.43 (1 H, d, *J* 5.7), 7.03 (1 H, s), 6.19 (1 H, d, *J* 5.7), 2.92 (2 H, q, *J* 7.4), 1.38 (3 H, t, *J* 7.4); δ_C (75 MHz, $CDCl_3$) 194.21, 147.87, 136.11, 134.24, 127.21, 29.74, 16.00; *m/z* (EI) 172 (M+, 100%), 143 (25), 116 (27), 115 (15), 112 (15), 71 (21). HRMS (EI) Calc. for $C_7H_8OS_2$ 172.0017. Found 172.0019.

(*Z*)-5-((Pyridin-2-ylthio)methylene)thiophen-2(5*H*)-one 6h

Eluent: hexane/EtOAc 3:1; mp 133–135 °C; δ_H (200 MHz, $CDCl_3$) 8.52 (1 H, d, *J* 4.9), 8.20 (1 H, s), 7.73–7.55 (2 H, m), 7.31 (1 H, d, *J* 8.0), 7.22–7.14 (1 H, m), 6.30 (1 H, d, *J* 5.7); δ_C (75 MHz, $CDCl_3$) 193.72, 153.51, 149.66, 148.54, 138.28, 136.17, 128.76, 128.32, 123.71, 122.27; *m/z* (EI) 221 (M+, 26%), 193 (15), 192 (20), 161 (68), 160 (100), 135 (15), 78 (27). HRMS (EI) calc. for $C_{10}H_7NOS_2$ 220.9969. Found 220.9965.

(*Z*)-5-((Pyrimidin-2-ylthio)methylene)thiophen-2(5*H*)-one 6i

Eluent: hexane/EtOAc 1:1; mp 163–165 °C; δ_H (200 MHz, $CDCl_3$) 8.61 (2H, d, *J* 5.4), 8.12 (1H, s), 7.62 (1 H, *J* 5.8), 7.14 (1 H, *J* 4.8), 6.33 (1 H, d 5.8); δ_C (75 MHz,

$CDCl_3$) 193.75, 167.60, 162.71, 158.32, 148.43, 135.75, 128.98, 128.79, 118.84; *m/z* (EI): 222 (M^+, 41%), 194 (22), 162 (72), 161 (100), 136 (13), 79 (10), 53 (10), 39 (10). HRMS (EI) calc. for $C_9H_6ON_2S_2$: 221.9922. Found 221.9921.

(*Z*)-5-((Phenylsulfonyl)methylene)thiophen-2(5*H*)-one 6j

Eluent: hexane/EtOAc 2:1; mp 106 °C; δ_H (600 MHz, $CDCl_3$) 7.93 (1H, d, *J* 7.5), 7.66 (1H, t, *J* 7.5), 7.63–7.50 (4H, m), 6.73 (1H, s), 6.53 (1 H, d, *J* 6.0); δ_C (151 MHz, $CDCl_3$) 194.11, 148.76, 148.71, 139.72, 134.38, 133.64, 129.62, 127.90, 127.88; *m/z* (EI) 252 (M+, 17%), 160 (5), 125 (100), 97 (8), 83 (10), 77 (35). HRMS (EI) calc. for $C_{11}H_8O_3S_2$ 251.9915. Found 251.9920.

(2*Z*,4*Z*)-Methyl 5-bromo-4-(((*Z*)-(5-oxothiophen-2(5*H*)-ylidene) methyl)thio)penta-2,4-dienoate 7

Eluent: hexane/EtOAc 3:1; δ_H (200 MHz, $CDCl_3$) 7.45 (1 H, d, *J* 5.8), 6.97 (1 H, s), 6.81 (1 H, d, *J* 1.7), 6.38 (1 H, dd, *J* 11.6, 1.7), 6.28 (1 H, d, *J* 5.8), 6.09 (1 H, d, *J* 11.6); δ_C (75 MHz, $CDCl_3$) 193.57, 165.71, 147.89, 138.35, 136.86, 133.90, 129.48, 128.85, 125.46, 112.55, 52.45; *m/z* (EI) 334 $M^+ + 2$, 1%), 332 (M+,1), 253 (100), 221 (20), 193 (19), 115 (9), 71 (12); HRMS (EI) calc. for $C_{11}H_9BrO_3S_2$ 331.9176. Found 331.9171.

(2*Z*,4*Z*)-Methyl 5-bromo-4-(((1*Z*,3*Z*)-5-methoxy-5-oxo-2-(((*Z*)-(5-oxothiophen-2(5*H*)-ylidene)methyl)thio)penta-1,3-dien-1-yl)thio)penta-2,4-dienoate 8

Eluent: hexane/EtOAc 3:1; δ_H (200 MHz, $CDCl_3$) 7.45 (1 H, d, *J* 5.6), 7.43 (1 H, d, *J* 1.0), 6.90 (1 H, s), 6.75 (1 H, t, *J* 1.8), 6.41 (1 H, dd, *J* 11.6, 1.6), 6.38 (1 H, dd, *J* 12.0, 1.0) 6.26 (1 H, d, *J* 5.3), 6.05 (1 H, d, *J* 11.6), 5.85 (1 H, d, *J* 12.1), 3.72 (3 H, s), 3.70 (3 H, s); δ_C (75 MHz, $CDCl_3$) 193.86, 166.34, 165.75, 147.91, 140.15, 139.13, 138.89, 136.56, 134.54, 131.87, 128.36, 124.97, 123.32, 121.47, 111.53, 52.30, 52.13; *m/z* (EI) 476 ($M^+ + 2$, 0.3%), 474 (M+, 0.3), 395 (37), 323 (12), 284 (10), 253 (100), 221 (22),193 (26), 243 (15), 79 (21). HRMS (EI) calc. for $C_{17}H_{15}BrO_5S_3$ 473.9265. Found 473.9255. calc. for $C_{17}H_{15}O_5S_3$ 395.0082. Found 395.0078.

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