Supporting Information

Novel Hybrid-Type Antimicrobial Agents Targeting the Switch Region of Bacterial RNA Polymerase

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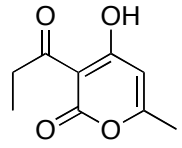
Experimental Section

General Procedure

^{1}H NMR were measured in DMSO-d_6 and $CDCl_3$ solution, and referenced to TMS (0.00 ppm) using Varian Mercury–300 NMR (300 MHz), Bruker DPX–400 NMR (400 MHz), Bruker AV–600 NMR (600 MHz) spectrophotometers, unless otherwise noted. ^{13}C NMR were measured in DMSO-d_6 and $CDCl_3$ solution, and referenced to DMSO-d_6 (39.7 ppm) and $CDCl_3$ (77.0 ppm) using Bruker DPX–400 NMR (400 MHz) and Bruker AV–600 NMR (600 MHz) spectrophotometers. Chemical shifts are reported in ppm (from TMS). When peak multiplicities are reported, the following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broadened. IR spectra were recorded on JASCO FT/IR 4100 spectrometer. Mass spectra were obtained on Waters MICRO MASS LCT–premier. For the preparative purpose, HITACHI L–6250 Intelligent Pump were used. Column chromatography was performed on silicagel 60N (spherical, neutral) (40–50 μM or 63–210 μM), thin layer chromatography (TLC) was performed on precoated plates (0.25 mm, silica gel Merk Kieselgel 60F245), and compounds were visualized with UV light and phosphomolybdic acid stain. Melting points were measured with Yanaco MP–500D melting point apparatuses. All reactions were performed in oven–dried glassware under positive pressure of argon, unless otherwise noted. Reaction mixtures were stirred magnetically. Solvents were freshly distilled prior to use or purchased from KANTO Kagaku: tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl or purchased from KANTO Kagaku (tetrahydrofuran, Dehydrated Stabilizer free): methylene chloride (CH_2Cl_2) was distilled from calcium hydride or purchased from KANTO Kagaku (methylene chloride, Dehydrated): *N, N*-dimethylformamide (DMF) were purchased from KANTO Kagaku (DMF, Dehydrated): methanol (MeOH), and *tert*–butanol (*t*BuOH) were purchased from KANTO Kagaku (MeOH, or *t*BuOH, Dehydrated): toluene was purchased from KANTO Kagaku (toluene, Dehydrated): acetone was purchased from KANTO Kagaku (acetone, Dehydrated): triethylamine (Et_3N) was distilled from KOH and kept over KOH tablets: dimethyl sulfoxide (DMSO) for a biological evaluation was purchased from Nacalai tesque (Dimethyl Sulfoxide).

1: Synthetic Study

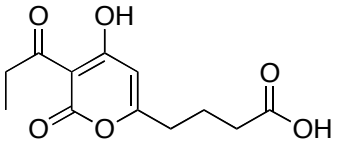
4-Hydroxy-6-methyl-3-propionyl-2*H*-pyran-2-one (9)



To a solution of 4-hydroxy-6-methyl-2-pyrone (**8**) (3.00 g, 23.7 mmol) in toluene (60 mL) were added propionic acid (1.77 mL, 23.7 mmol), DMAP (579 mg, 4.74 mmol), and DIC (3.49 mL, 23.7 mmol). The mixture was stirred at rt for 3 h then the solution was heated at 100 °C for 5 h. The mixture was filtered and concentrated. The residue was purified through silica gel column chromatography to give the title compound **9** (3. 13 g, 72 %) as a white powder.

mp 104.7–105.5 °C; IR (neat) cm^{-1}: 1724, 1642, 1552, 1448; ^1H NMR (400 MHz, $CDCl_3$): δ 5.94 (1H, s), 3.11 (2H, q, J = 7.2 Hz), 2.27 (3H, s), 1.16 (3H, t, J = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 208.30, 181.01, 168.75, 160.99, 101.43, 99.35, 35.24, 20.61, 7.71; HRMS (ESI) *m/z* calcd for $C_9H_{11}O_4$ $[M+H]^+$ 183.0657, found 183.0653.

4-(4-Hydroxy-2-oxo-3-propionyl-2*H*-pyran-6-yl)butanoic acid (11)

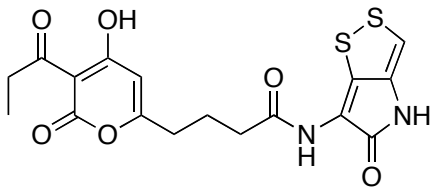


To a solution of pyrone **9** (300 mg, 1.65 mmol) in THF (5.0 mL) was added 2 M solution of LDA in heptane/THF/ethylbenzene (2.90 mL, 5.76 mmol) at –78 °C. After being stirred for 1 h at –78 °C, iodide **10** (595 mg, 1.98 mmol) and HMPA (1.00 mL, 5.78 mmol) were added to the solution and the mixture was stirred for additional 1 h at –78 °C. The reaction was quenched by sat. NH_4Cl aq. and the aqueous layer was extracted with AcOEt. The extract was dried over $MgSO_4$, filtered and concentrated. The residue was purified through silica gel column chromatography to give the silyl ether (439 mg, 75 %).

To a flask with the silyl ether (130 mg, 0.37 mmol) was added a solution of acetic acid/H_2O/THF (3/1/1) (5.0 mL) with additional stirring for 26 h at rt. The mixture was diluted with H_2O and the aqueous layer was extracted with AcOEt. The extract was dried over $MgSO_4$, filtered and concentrated. The residue was purified through silica gel column chromatography to give the alcohol (78.4 mg, 89 %).

To a solution of the alcohol (50.4 mg, 0.21 mmol) in CH_2Cl_2 (3.0 mL) was added PCC (136 mg, 0.63 mmol) at 0 °C and the mixture was stirred at rt for 30 min. The residue was filtered through a pad of silica gel and concentrated. The synthesized aldehyde was used in the following step without further purification.
To a solution of aldehyde (0.21 mmol) in ^{t}BuOH/H_2O/THF (1/1/1) (3.0 mL) were added 2-methyl-2-butene (0.45 mL, 4.20 mmol), NaH_2PO_4 (75.6 mg, 0.63 mmol) and $NaClO_2$ (38.0 mg, 0.42 mmol) at 0 °C. After being stirred for 4 h at rt, the mixture was diluted with $CHCl_3$ and extracted with $CHCl_3$. The extract was dried over $MgSO_4$, filtered and concentrated. The residue was purified through silica gel column chromatography to give the title compound **11** (33.6 mg, 63 % (2 steps)) as a white powder.
mp 111.8–112.4 °C; IR (neat) cm^{-1}: 3079, 2982, 2944, 1724, 1643, 1558, 1233; ^{1}H NMR (400 MHz, $CDCl_3$ (O*H* was exchanged with D_2O.)): δ 5.97 (1H, s), 3.11 (2H, q, *J* = 7.2 Hz), 2.59 (2H, t, *J* = 7.5 Hz), 2.46 (2H, t, *J* = 7.2 Hz), 2.03 (2H, quint, *J* = 7.5 Hz), 1.17 (3H, t, *J* = 7.2 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): δ 208.37, 180.91, 177.66, 170.69, 160.92, 101.26, 99.68, 35.33, 33.23, 32.51, 21.23, 7.72; HRMS (ESI) *m/z* calcd for $C_{12}H_{15}O_6$ $[M+H]^+$ 255.0869, found 255.0859.

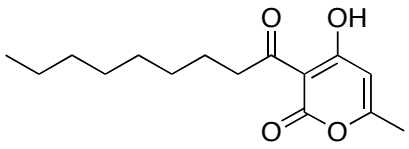
4-(4-Hydroxy-2-oxo-3-propionyl-2*H*-pyran-6-yl)-*N*-(5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)butanamide (13)



To a solution of carboxylic acid **11** (20.5 mg, 0.080 mmol) in toluene (2.0 mL) was added oxalyl chloride (10.2 μL, 0.12 mmol) at rt and the solution was stirred for 1 h. The mixture was concentrated and used in the following step without further purification.
To a solution of acid chloride in toluene (1.0 mL) were added a solution of holothin hydrochloride **12** (25.0 mg, 0.12 mmol) in THF (2.0 mL) and Et_3N (0.022 mL, 0.16 mmol) at rt. After being stirred for 30 min at rt, the mixture was concentrated and the residue was purified through silica gel column chromatography to give the title compound **13** (19.9 mg, 61%) as a yellow amorphous.
IR (neat) cm^{-1}: 3418, 1645, 1457; ^{1}H NMR (600 MHz, $CDCl_3$ (N*H* and O*H* were exchanged with D_2O.)): δ 6.76 (1H, s), 5.98 (1H, s), 3.10 (2H, q, *J* = 7.1 Hz), 2.61 (2H, t, *J* = 7.4 Hz), 2.45 (2H, t, *J* = 7.2 Hz), 2.10 (2H, quint, *J* = 7.2 Hz), 1.16 (3H, t, *J* = 7.2 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): δ 208.34, 180.92, 170.80, 169.87, 167.75, 160.96, 132.66, 130.97, 128.98, 111.19, 101.27, 99.69, 35.06, 33.35, 31.92, 22.69, 7.73; HRMS

(ESI) *m/z* calcd for $C_{17}H_{17}N_2O_6S_2$ $[M+H]^+$ 409.0528, found 409.0535.

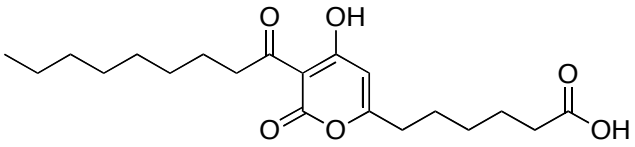
4-Hydroxy-6-methyl-3-nonanoyl-2*H*-pyran-2-one (14)



To a solution of **8** (300 mg, 2.38 mmol) in toluene (10 mL) were added nonanoic acid (0.42 mL, 2.38 mmol), DMAP (58.2 mg, 0.48 mmol) and DIC (0.37 mL, 2.38 mmol). After being stirred for 1.5 h at rt, the solution was stirred at 100 °C for additional 14 h. The mixture was filtered and concentrated. The residue was purified through silica gel column chromatography to give the title compound **14** (461 mg, 74 %) as a white powder.

mp 80.2–81.6 °C; IR (neat) cm^{-1}: 3430, 2957, 2922, 2851, 1716, 1652, 1637, 1614, 1561, 1454, 995; ^1H NMR (400 MHz, $CDCl_3$): δ 5.93 (1H, s), 3.07 (2H, t, J = 7.5 Hz), 2.27 (3H, s), 1.65 (2H, quint, J = 7.3 Hz), 1.38–1.28 (10H, m), 0.88 (3H, t, J = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 208.02, 181.26, 168.74, 160.92, 101.50, 99.45, 41.60, 31.79, 29.37, 29.20, 29.11, 23.95, 22.60, 20.60, 14.04; HRMS (ESI) *m/z* calcd for $C_{15}H_{23}O_4$ $[M+H]^+$ 267.1596, found 267.1597.

6-(4-Hydroxy-3-nonanoyl-2-oxo-2*H*-pyran-6-yl)hexanoic acid (16)

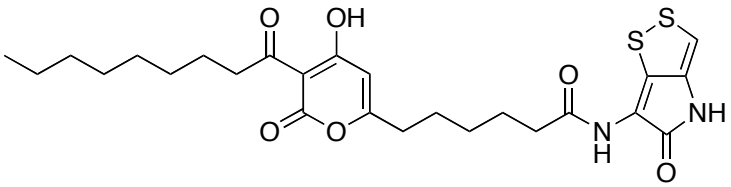


To a solution of pyrone **14** (43.0 mg, 0.16 mmol) in THF (3.0 ml) was added 2 M solution of LDA in heptane/THF/ethylbenzene (0.28 mL, 0.57 mmol) at –78 °C. After being stirred for 1 h at –78 °C, iodide **15** (78.8 mg, 0.24 mmol) and HMPA (0.10 mL, 0.54 mmol) were added to the solution and the mixture was stirred for additional 1 h at –78 °C. The mixture was quenched by sat. NH_4Cl aq. and the aqueous layer was extracted with AcOEt. The extract was dried over $MgSO_4$, filtered and concentrated. The residue was purified through silica gel column chromatography to give the silyl ether.

To a flask with the silyl ether (0.16 mmol) was added a solution of acetic acid/H_2O/THF (3/1/1) (5.0 mL) and the mixture was stirred at rt for 26 h. The reaction was diluted with H_2O and the aqueous layer was extracted with AcOEt. The extract was dried over

$MgSO_4$, filtered and concentrated. The residue was purified through silica gel column chromatography to give the alcohol (37.2 mg, 66 % (2 steps)).
To a solution of the alcohol (23.5 mg, 0.067 mmol) in CH_2Cl_2 (5.0 mL) was added PCC (43.1 mg, 0.20 mmol) at 0 °C and the mixture was stirred at rt for 30 min. The residue was filtered through a pad of silica gel and the solvent was removed in vacuo. The synthesized aldehyde was used in the following step without further purification.
To a solution of aldehyde (0.067 mmol) in ^{t}BuOH/H_2O/THF (1/1/1) (3.0 mL) were added 2-methyl-2-butene (0.14 mL, 1.34 mmol), NaH_2PO_4 (24.1 mg, 0.20 mmol) and $NaClO_2$ (12.1 mg, 0.13 mmol) at 0 °C. After being stirred for 4 h at rt, the mixture was diluted with $CHCl_3$ and extracted with $CHCl_3$. The extract was dried over $MgSO_4$, filtered and concentrated. The residual oil was purified through silica gel column chromatography to give the title compound **16** (20.6 mg, 84 % (2 steps)) as a white powder.
mp 108.2–108.9 °C; IR (neat) cm^{-1}: 3423, 2923, 2853, 1713, 1696, 1643, 1557, 1454; ^{1}H NMR (600 MHz, $CDCl_3$ (O*H* was exchanged with D_2O.)): δ 5.92 (1H, s), 3.07 (2H, t, *J* = 7.3 Hz), 2.50 (2H, t, *J* = 7.6 Hz), 2.38 (2H, t, *J* = 7.3 Hz), 1.74–1.63 (6H, m), 1.45–1.25 (12H, m), 0.88 (3H, t, *J* = 7.1 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): δ 208.07, 181.24, 177.72, 171.91, 161.05, 100.92, 99.68, 41.68, 34.02, 33.37, 31.82, 29.40, 29.24, 29.16, 28.25, 26.00, 24.14, 23.95, 22.64, 14.10; HRMS (ESI) *m/z* calcd for $C_{20}H_{31}O_6$ $[M+H]^+$ 367.2121, found 367.2114.

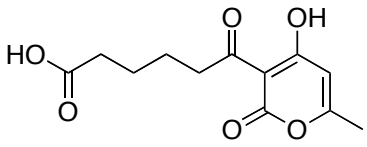
6-(4-Hydroxy-3-nonanoyl-2-oxo-2*H*-pyran-6-yl)-*N*-(5-oxo-4,5-dihydro-[1,2]dithiolo [4,3-*b*]pyrrol-6-yl)hexanamide (17)



To a solution of carboxylic acid **16** (20.6 mg, 0.060 mmol) in toluene (2.0 mL) was added oxalyl chloride (7.10 μL, 0.080 mmol) at rt and the solution was stirred for 1 h. The mixture was concentrated and used in the following step without further purification.
To a solution of acid chloride in toluene (0.50 mL) were added a solution of holothin hydrochloride **12** (16.7 mg, 0.080 mmol) in THF (2.0 mL) and Et_3N (0.020 mL, 0.12 mmol) at rt. After being stirred for 30 min at rt, the mixture was concentrated and the residue was purified through silica gel column chromatography to give the title compound **17** (20.3 mg, 65 %) as a yellow amorphous.
IR (neat) cm^{-1}: 3417, 2923, 1645, 1541, 1457, 1287; ^{1}H NMR (600 MHz, $CDCl_3$ (N*H*

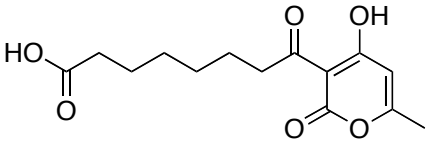
and O*H* were exchanged with D_2O.)): δ 6.75 (1H, s), 5.92 (1H, s), 3.07 (2H, t, *J* = 7.2 Hz), 2.50 (2H, t, *J* = 7.6 Hz), 2.38 (2H, t, *J* = 7.4 Hz), 1.74–1.65 (6H, m), 1.47–1.23 (12H, m), 0.88 (3H, t, *J* = 7.3 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): δ 208.10, 181.24, 171.94, 170.85, 167.72, 161.07, 132.15, 130.98, 128.90, 109.99, 100.92, 99.67, 41.69, 34.03, 33.15, 31.88, 29.35, 29.24, 29.16, 28.24, 25.88, 24.19, 23.97, 22.68, 14.11; HRMS (ESI) *m/z* calcd for $C_{25}H_{33}N_2O_6S_2$ $[M+H]^+$ 521.1780, found 521.1780.

6-(4-Hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-6-oxohexanoic acid (24)



To a solution of **8** (200 mg, 1.59 mmol) in toluene (5.0 mL) were added monomethyl adipate **18** (253 mg, 1.59 mmol), DMAP (38.8 mg, 0.32 mmol) and DIC (0.24 mL, 1.59 mmol). After being stirred for 3 h at rt, the solution was stirred at 100 °C for 6 h. The mixture was filtered and concentrated. The residue was purified through silica gel column chromatography to give the methyl ester (270 mg, 64%) as an orange powder.
The methyl ester (268 mg, 1.00 mmol) was hydrolyzed with 1M LiOH aq. in THF (1 M LiOH aq./THF = 1/4) (3.0 mL). The mixture was stirred at rt for 14 h and quenched by sat. NH_4Cl aq.. The aqueous layer was acidified with 0.1 M HCl aq. and extracted with $CHCl_3$. The extract was washed with brine, dried over $MgSO_4$, filtered and concentrated. Carboxylic acid **24** (197 mg) was obtained as a yellow crystal.
mp 121.6–122.3 °C; IR (neat) cm^{-1}: 2940, 1716, 1692, 1644, 1614, 1567, 1451, 1413, 1240; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.93 (1H, s), 3.11 (2H, t, *J* = 6.5 Hz), 2.41 (2H, t, *J* = 6.5 Hz), 2.26 (3H, s), 1.75–1.72 (4H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 207. 50, 181.53, 179.52, 169.31, 161.38, 101.85, 99.84, 41.53, 34.08, 24.47, 23.53, 21.00; HRMS (ESI) *m/z* calcd for $C_{12}H_{14}O_6Na$ $[M+Na]^+$ 277.0688, found 277.0679.

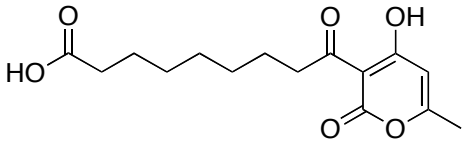
8-(4-Hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-8-oxooctanoic acid (25)



To a solution of **8** (300 mg, 2.38 mmol) in toluene (10.0 mL) were added monomethyl suberate **19** (0.42 mL, 2.38 mmol), DMAP (58.6 mg, 0.48 mmol) and DIC (0.37 mL, 2.38 mmol). After being stirred for 3 h at rt, the solution was stirred at 100 °C for 6 h.

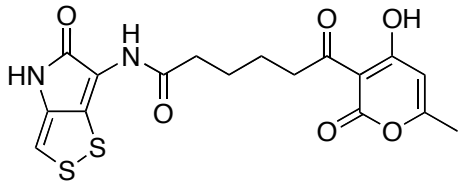
The mixture was filtered and concentrated. The residue was purified through silica gel column chromatography to give the methyl ester (656 mg, 93%) as a yellow powder.
The methyl ester (100 mg, 0.33 mmol) was hydrolyzed with 1 M LiOH aq. in THF (1 M LiOH aq./THF = 1/4) (3.0 mL). After being stirred for 14 h at rt, the mixture was quenched by sat. NH_4Cl aq.. The aqueous layer was acidified with 0.1 M HCl aq. and extracted with $CHCl_3$. The organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated. Carboxylic acid **25** (54.0 mg) was obtained as a yellow crystal.
mp 99.1–101.1 °C; IR (neat) cm^{-1}: 2938, 1713, 1664, 1555, 1461, 1245, 994; ^1H NMR (400 MHz, $CDCl_3$): δ 5.95 (1H, s), 3.08 (2H, t, J = 3.7 Hz), 2.37 (2H, t, J = 7.5 Hz), 2.29 (3H, s), 1.72–1.63 (4H, m), 1.47–1.37 (4H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 207.88, 181.37, 179.63, 168.94, 161.12, 101.63, 99.59, 41.59, 33.99, 28.91, 28.87, 24.57, 23.80, 20.74; HRMS (ESI) *m/z* calcd for $C_{14}H_{19}O_6$ $[M+H]^+$ 283.1182, found 283.1186.

9-(4-Hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-9-oxononanoic acid (26)



To a solution of **8** (200 mg, 1.59 mmol) in toluene (5.0 mL) were added azelaic acid monomethyl ester **20** (318 mg, 1.59 mmol), DMAP (38.8 mg, 0.32 mmol) and DIC (0.24 mL, 1.59 mmol). After being stirred for 3 h at rt, the solution was stirred at 100 °C for 6 h. The mixture was filtered and concentrated. The residue was purified through silica gel column chromatography to give the methyl ester (345 mg, 70%) as a yellow powder.
The methyl ester (100 mg, 0.32 mmol) was hydrolyzed with 1 M LiOH aq. in THF (1 M LiOH aq./THF = 1/4) (3.0 mL). After being stirred for 14 h at rt, the mixture was quenched by sat. NH_4Cl aq.. The aqueous layer was acidified to pH 2 with 0.1 M HCl aq. and extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated. Carboxylic acid **26** (84.0 mg) was obtained as a yellow crystal.
mp 101.6–102.5 °C; IR (neat) cm^{-1}: 2942, 1717, 1645, 1607, 1557, 1459, 1427, 1241, 995; ^1H NMR (400 MHz, $CDCl_3$): δ 5.96 (1H, s), 3.09 (2H, t, J = 7.3 Hz), 2.38 (2H, t, J = 7.5 Hz), 2.30 (3H, s), 1.70–1.65 (4H, m), 1.39 (6H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 207.94, 181.32, 179.55, 168.85, 161.05, 101.58, 99.53, 41.59, 33.97, 29.03, 28.99, 28.89, 24.63, 23.88, 20.67; HRMS (ESI) *m/z* calcd for $C_{15}H_{20}O_6Na$ $[M+Na]^+$ 319.1158, found 319.1146.

***N*-(4,5-Dihydro-5-oxo-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)-6-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-6-oxohexanamide (27)**

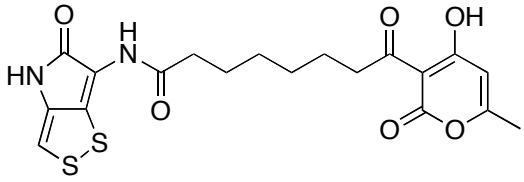


To a solution of carboxylic acid **24** (23.1 mg, 0.091 mmol) in toluene (2.0 mL) was added oxalyl chloride (0.023 mL, 0.27 mmol) at rt and the mixture was stirred for 1 h. The mixture was concentrated and used in the following step without further purification.

To a solution of acid chloride in toluene (0.50 mL) were added a solution of holothin hydrochloride **12** (19.0 mg, 0.091 mmol) in THF (2.0 mL) and Et_3N (0.038 mL, 0.27 mmol) at rt and the mixture was stirred for 30 min at rt. The solution was concentrated and the residual oil was purified through silica gel column chromatography to give the title compound **27** (24.2 mg, 65 % from **24**) as a yellow amorphous.

IR (neat) cm^{-1}: 3393, 2921, 2850, 1715, 1644, 1557, 1455, 1246; ^1H NMR (400 MHz, DMSO-d_6): δ 10.58 (1H, s), 9.79 (1H, s), 7.05 (1H, s), 6.27 (1H, s), 3.01–2.96 (2H, m), 2.38 (2H, t, J = 6.4 Hz), 2.26 (3H, s), 1.58 (2H, br s), 1.21 (2H, br s); ^{13}C NMR (100 MHz, DMSO-d_6): δ 207.27, 180.91, 174.75, 172.10, 168.40, 160.78, 134.44, 134.17, 115.82, 111.01, 101.51, 99.60, 41.09, 34.95, 24.99, 23.37, 20.54; HRMS (ESI) *m/z* calcd for $C_{17}H_{16}N_2O_6NaS_2$ $[M+Na]^+$ 431.0347, found 431.0330.

***N*-(4,5-Dihydro-5-oxo-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)-8-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-8-oxooctanamide (28)**

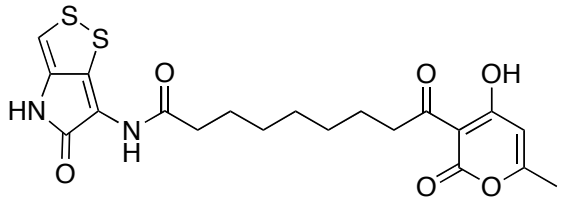


To a solution of carboxylic acid **25** (26.6 mg, 0.094 mmol) in toluene (2.0 mL) was added oxalyl chloride (0.024 mL, 0.28 mmol) at rt and the solution was stirred for 1 h. The mixture was concentrated and used in the following step without further purification.

To a solution of acid chloride in toluene (0.50 mL) were added a solution of holothin hydrochloride **12** (19.6 mg, 0.094 mmol) in THF (2.0 mL) and Et_3N (0.039 mL, 0.28

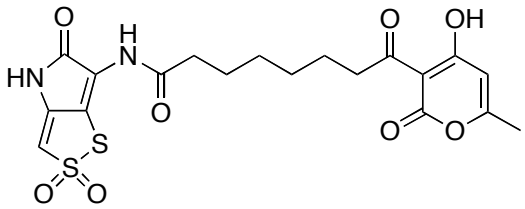
mmol) at rt. After being stirred for 30 min at rt, the mixture was concentrated and the residue was purified through silica gel column chromatography to give the title compound **28** (23.8 mg, 58% from **25**) as a yellow amorphous.
IR (neat) cm^{-1}: 3248, 2933, 2857, 1715, 1643, 1609, 1557, 1456, 1241, 996; ^1H NMR (400 MHz, $CDCl_3$): δ 9.71 (1H, br s), 8.24 (1H, br s), 6.90 (1H, s), 5.94 (1H, s), 3.09–3.04 (2H, m), 2.41–2.34 (2H, m), 2.27 (3H, s), 1.67 (4H, m), 1.41 (4H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 207.79, 181.28, 178.22, 172.11, 168.87, 161.07, 136.32, 133.35, 114.55, 113.24, 101.57, 99.51, 41.52, 35.04, 28.87, 28.81, 24.93, 23.71, 20.66; HRMS (ESI) *m/z* calcd for $C_{19}H_{20}N_2O_6NaS_2$ $[M+Na]^+$ 459.0660, found 459.0641.

_N_-(4,5-Dihydro-5-oxo-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)-9-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-9-oxononanamide (29)



To a solution of carboxylic acid **26** (35.2 mg, 0.12 mmol) in toluene (2.0 mL) was added oxalyl chloride (0.030 mL, 0.36 mmol) at rt and the solution was stirred for 1 h. The mixture was concentrated and used in the following step without further purification.
To a solution of acid chloride in toluene (0.50 mL) were added a solution of holothin hydrochloride **12** (25.0 mg, 0.12 mmol) in THF (2.0 mL) and Et_3N (0.050 mL, 0.36 mmol) at rt. After being stirred for 30 min at rt, the mixture was concentrated and the residue was purified through silica gel column chromatography to give the title compound **29** (34.6 mg, 64% from **26**) as a yellow amorphous.
IR (neat) cm^{-1}: 3248, 2927, 2852, 1716, 1644, 1601, 1556, 1455, 1288, 1245, 996; ^1H NMR (400 MHz, $CDCl_3$): δ 8.70 (1H, br s), 7.66 (1H, br s), 6.79 (1H, s), 5.93 (1H, s), 3.07 (2H, t, J = 7.3 Hz), 2.36 (2H, t, J = 7.4 Hz), 2.27 (3H, s), 1.67–1.64 (5H, m), 1.37 (5H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 207.92, 181.30, 171.74, 168.83, 168.71, 161.04, 135.86, 133.17, 114.57, 111.74, 101.58, 99.52, 41.56, 36.37, 29.01, 28.96, 25.32, 23.87, 20.67; HRMS (ESI) *m/z* calcd for $C_{20}H_{22}N_2O_6NaS_2$ $[M+Na]^+$ 473.0817, found 473.0819.

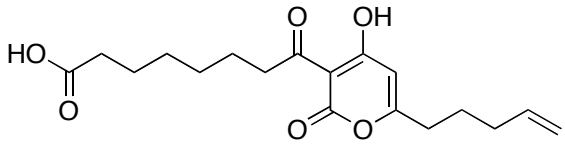
_N_-(4,5-Dihydro-2,2-dioxido-5-oxo-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)-8-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-8-oxooctanamide (30)



To a solution of **28** (20.0 mg, 0.046 mmol) in acetone/H_2O (1/1) (4.0 mL) was added oxone (70.0 mg) at 0 °C. After being stirred for 30 min at 0 °C, the mixture was quenched by sat. $NaHCO_3$ aq. and stirred for additional 30 min. The aqueous layer was extracted with AcOEt and the extract was dried over $MgSO_4$, filtered and concentrated. The residue was purified with preparative TLC to give the title compound **30** (8.60 mg, 40%) as a pale yellow amorphous.

IR (neat) cm^{-1}: 2918, 2849, 1725, 1682, 1661, 1644, 1607, 1547, 1450, 1283, 1219, 1029, 993; ^1H NMR (400 MHz, DMSO-d_6): δ 11.48 (1H, br s), 10.52 (1H, br s), 7.25 (1H, s), 6.28 (1H, s), 2.98 (2H, t, J = 7.3 Hz), 2.45 (2H, t, J = 7.3 Hz), 2.27 (3H, s), 1.61–1.48 (4H, m), 1.36–1.26 (4H, m); ^{13}C NMR (150 MHz, DMSO-d_6): δ 206.97, 180.69, 173.75, 165.95, 160.64, 143.44, 123.51, 115.37, 109.64, 101.47, 99.35, 41.14, 34.83, 28.43, 28.41, 24.64, 23.44, 20.23; HRMS (ESI) *m/z* calcd for $C_{19}H_{20}N_2O_8NaS_2$ $[M+Na]^+$ 491.0559, found 491.0548.

8-(4-Hydroxy-2-oxo-6-(pent-4-en-1-yl)-2*H*-pyran-3-yl)-8-oxooctanoic acid (31)

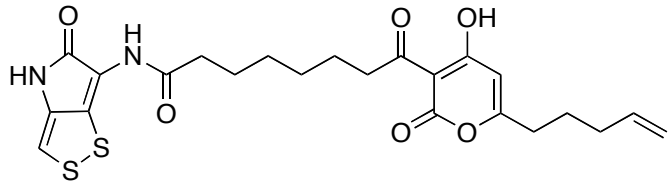


To a solution of **8** (200 mg, 1.59 mmol) in THF (4.0 mL) was added 2 M solution of LDA in heptane/THF/ethylbenzene (1.70 mL, 3.50 mmol) at –78 °C. After being stirred for 1 h at –78 °C, 4-bromo-1-butene (0.32 mL, 3.18 mmol) and HMPA (0.61 mL, 3.50 mmol) were added to the solution at –78 °C and the mixture was stirred for additional 14 h at rt. The reaction was quenched by sat. NH_4Cl aq. and the aqueous layer was extracted with AcOEt. The extract was dried over $MgSO_4$, filtered and concentrated. The residue was purified through silica gel column chromatography to give the alkylated compound (244 mg, 85 %).

To a solution of the alkylated pyrone (200 mg, 1.11 mmol) in toluene (5.0 mL) were added monomethyl suberate **19** (209 mg, 1.11 mmol), DMAP (13.4 mg, 0.11 mmol) and DIC (0.17 mL, 1.11 mmol). After being stirred for 2 h at rt, the solution was heated at 100 °C for 14 h. The mixture was filtered and concentrated. The residue was purified through silica gel column chromatography to give the methyl ester (300 mg, 77%) as a

yellow powder.
The methyl ester (100 mg, 0.33 mmol) was hydrolyzed with 1 M LiOH aq. in THF (1 M LiOH aq./THF = 1/4) (3.0 mL). After being stirred for 24 h at rt, the mixture was quenched by sat. NH_4Cl aq.. The aqueous layer was acidified with 1 M HCl aq. and extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified through silica gel column chromatography to give the title compound **31** (92.7 mg, 95%) as a white powder.
mp 78.3–78.9 °C; IR (neat) cm^{-1}: 3429, 3948, 1714, 1644, 1608, 1556, 1456, 1283, 1246; ^1H NMR (400 MHz, $CDCl_3$): δ 5.92 (1H, s), 5.82–5.72 (1H, m), 5.07–5.02 (2H, m), 3.07 (2H, t, *J* = 7.2), 2.50 (2H, t, *J* = 7.6), 2.36 (2H, t, *J* = 7.4), 2.13 (2H, dd, *J* = 14, 7.2), 1.78 (2H, quint, *J* = 7.5), 1.69–1.64 (4H, m), 1.41–1.39 (4H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 207.78, 181.23, 172.15, 164.15, 161.06, 137.02, 115.98, 100.94, 99.69, 41.51, 33.54, 32.75, 28.82, 28.79, 25.43, 24.50, 23.71; HRMS (ESI) *m/z* calcd for $C_{18}H_{25}O_6$ $[M+H]^+$ 337.1651, found 337.1653.

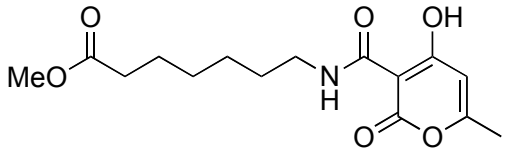
4-(4-Hydroxy-2-oxo-6-(pent-4-en-1-yl)-2*H*-pyran-3-yl)-4-oxo-*N*-(5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)butanamide (32)



To a solution of carboxylic acid **31** (18.2 mg, 0.054 mmol) in toluene (2.0 mL) was added oxalyl chloride (0.014 mL, 0.16 mmol) at rt and the solution was stirred for 1 h. The mixture was concentrated and used in the following step without further purification.
To a solution of acid chloride in toluene (0.50 mL) were added a solution of holothin hydrochloride **12** (11.3 mg, 0.054 mmol) in THF (2.0 mL) and Et_3N (0.022 mL, 0.16 mmol) at rt. After being stirred for 30 min at rt, the mixture was concentrated and the residue was purified through silica gel column chromatography to give the title compound **32** (13.8 mg, 52% from **31**) as a yellow amorphous.
IR (neat) cm^{-1}: 3249, 2922, 2850, 1715, 1639, 1556, 1247; ^1H NMR (400 MHz, DMSO-d_6): δ 11.93 (1H, br s), 10.68 (1H, s), 9.81 (1H, s), 7.03 (1H, s), 6.21 (1H, s), 5.86–5.76 (1H, m), 5.02 (2H, dd, *J* = 17, 12), 2.95 (2H, t, *J* = 7.3), 2.33 (2H, t, *J* = 7.3), 2.20–2.15 (1H, m), 2.11–2.08 (1H, m), 2.06 (2H, t, *J* = 7.2), 1.67 (2H, quint, *J* = 7.4), 1.56–1.41 (4H, m), 1.35–1.27 (4H, m); ^{13}C NMR (100 MHz, DMSO-d_6): δ 206.30, 180.59, 174.63, 172.00, 168.12, 160.96, 137.91, 134.13, 133.87, 115.70, 115.55, 110.63, 101.70, 99.79, 41.33, 34.79, 32.79, 32.41, 29.16, 28.58, 28.53, 25.17, 25.09; HRMS

(ESI) *m/z* calcd for $C_{23}H_{27}N_2O_6S_2$ $[M+H]^+$ 491.1311, found 491.1311.

Methyl 7-(4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-carboxamido)heptanoate (33)

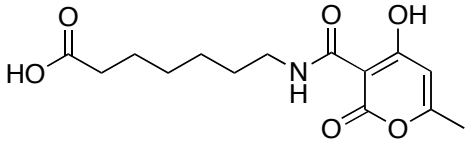


To a solution of 4-hydroxy-6-methyl-2-oxo-2*H*-pyran-3-carboxylic acid (40 mg, 0.23 mmol) in THF (1.2 mL) was added oxalyl chloride (0.06 mL, 0.69 mmol) and the mixture was stirred at rt for 1 h. The residue was concentrated and used in the following step without further purification.

The solution of acid chloride in THF (0.5 mL) were added a solution of heptanoic acid hydrochloride (27 mg, 0.14 mmol) in THF (2.5 mL) and Et_3N (0.10 mL, 0.69 mmol) at rt. After being stirred for 30 min at rt, the mixture was concentrated and the residue was purified through silica gel column chromatography to give the title compound **33** (11.0 mg) as a colorless oil.

IR (neat) cm^{-1}: 3303, 2929, 2856, 1738, 1703, 1634, 1566, 1457, 1254; ^1H NMR (400 MHz, $CDCl_3$): δ 8.95 (1H, br s), 5.97 (1H, s), 3.67 (3H, s), 3.38 (2H, dd, *J* = 13, 6.9), 2.31 (2H, t, *J* = 7.4), 2.27 (3H, s), 1.68–1.59 (4H, m), 1.38–1.36 (4H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 179.43, 174.11, 169.97, 165.57, 163.72, 102.53, 90.86, 51.48, 39.03, 33.95, 29.07, 28.69, 26.53, 24.77, 20.28; HRMS (ESI) *m/z* calcd for $C_{15}H_{21}NO_6$ $[M+H]^+$ 312.1447, found 312.1449.

7-(4-Hydroxy-6-methyl-2-oxo-2*H*-pyran-3-carboxamido)heptanoic acid (34)

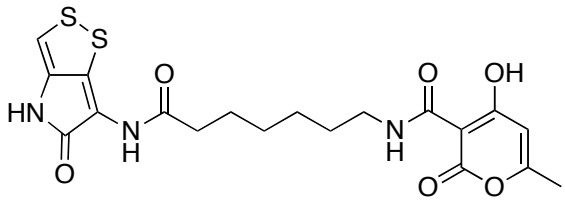


To a solution of methyl ester **33** (42.0 mg, 0.13 mmol) in H_2O (2.6 mL)/THF (1.0 mL) was added $Ba(OH)_2$ (44.5 mg, 0.26 mmol) and the mixture was stirred at 73 °C for 2 h. The reaction was diluted with $CHCl_3$ and the aqueous layer was acidified with 0.1 M HCl aq.. The aqueous layer was extracted with $CHCl_3$ and the extract was dried over Na_2SO_4, filtered and concentrated. Carboxylic acid **34** (35.0 mg) was obtained as a pale brown crystal.

mp 118.8–120.1 °C; IR (neat) cm^{-1}: 2925, 2853, 1702, 1646, 1564, 1457, 1259; ^1H NMR (400 MHz, DMSO-d_6): δ 11.97 (1H, br s), 9.00 (1H, br s), 6.27 (1H, s), 3.30–3.26

(2H, m), 2.24 (3H, s), 2.17 (2H, t, J = 7.3), 1.54–1.46 (4H, m), 1.29–1.24 (4H, m); ^{13}C NMR (100 MHz, DMSO-d_6): δ 179.28, 174.56, 169.71, 166.92, 162.76, 102.08, 90.00, 38.71, 33.71, 28.68, 28.26, 26.13, 24.51, 19.84; HRMS (ESI) *m/z* calcd for $C_{14}H_{20}NO_6$ $[M+H]^+$ 298.1291, found 298.1280.

4-Hydroxy-6-methyl-2-oxo-*N*-(7-oxo-7-((5-oxo-4,5-dihydro-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)amino)heptyl)-2*H*-pyran-3-carboxamide (35)

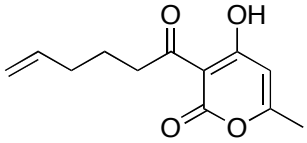


To a solution of carboxylic acid **34** (20.2 mg, 0.068 mmol) in toluene (2.0 mL) was added oxalyl chloride (0.017 mL, 0.20 mmol) at rt and the solution was stirred for 1 h. The mixture was concentrated and used in the following step without further purification.

To a solution of acid chloride in toluene (0.50 mL) were added a solution of holothin hydrochloride **12** (14.2 mg, 0.068 mmol) in THF (2.0 mL) and Et_3N (0.028 mL, 0.20 mmol) at rt. After being stirred for 30 min at rt, the mixture was concentrated and the residue was purified through silica gel column chromatography to give the title compound **35** (11.7 mg, 38% from **34**) as a yellow amorphous.

IR (neat) cm^{-1}: 2920, 2853, 1697, 1644, 1559, 1261; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.96 (1H, br s), 7.94 (1H, br s), 7.56 (1H, br s), 6.73 (1H, s), 5.97 (1H, s), 3.38 (2H, dd, J = 13, 6.9 Hz), 2.35 (2H, t, J = 7.5 Hz), 2.26 (3H, s), 1.75–1.56 (4H, m), 1.42–1.38 (4H, m); ^{13}C NMR (150 MHz, $CDCl_3$): δ 179.41, 171.38, 169.96, 168.02, 165.59, 163.75, 135.75, 132.77, 114.50, 111.07, 102.54, 90.88, 38.99, 36.23, 29.69, 28.83, 26.50, 25.11, 20.29; HRMS (ESI) *m/z* calcd for $C_{19}H_{22}N_3O_6S_2$ $[M+H]^+$ 452.0950, found 452.0942.

3-(Hex-5-enoyl)-4-hydroxy-6-methyl-2*H*-pyran-2-one (36)

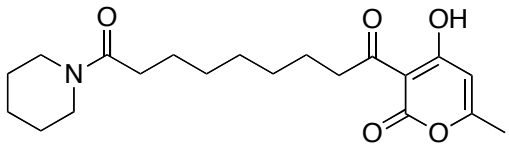


To a solution of **8** (300 mg, 2.38 mmol) in toluene (10 mL) were added hexenoic acid (0.28 mL, 2.38 mmol), DMAP (58.2 mg, 0.48 mmol) and DIC (0.37 mL, 2.38 mmol). After being stirred for 1 h at rt, the solution was stirred at 100 °C for additional 14 h.

The mixture was filtered and concentrated. The residue was purified through silica gel column chromatography to give the title compound **36** (439 mg, 83 %) as a white powder.

mp 42.1–42.6 °C; IR (neat) cm^{-1}: 3446, 1742, 1643, 1611, 1559, 1457; ^1H NMR (400 MHz, $CDCl_3$): δ 5.95 (1H, s), 5.87–5.77 (1H, m), 5.02 (2H, dd, *J* = 17, 10 Hz), 3.08 (2H, t, *J* = 8.0 Hz), 2.28 (3H, s), 2.14 (2H, dd, *J* = 14, 6.8 Hz), 1.76 (2H, quint, *J* = 7.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 207.41, 181.02, 168.75, 160.70, 137.80, 114.94, 101.29, 99.29, 40.77, 32.92, 22.89, 20.45; HRMS (ESI) *m/z* calcd for $C_{12}H_{15}O_4$ $[M+H]^+$ 223.0970, found 223.0970.

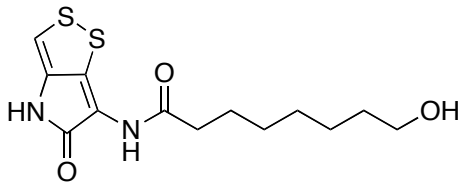
1-(4-Hydroxy-6-methyl-2-oxo-2*H*-pyran-3-yl)-9-(piperidin-1-yl)nonane-1,9-dione (37)



To a solution of **26** (45.0 mg, 0.15 mmol) in CH_2Cl_2 (2.0 mL) were added piperidine (0.030 mL, 0.30 mmol), HATU (114 mg, 0.30 mmol) and $^iPr_2NEt$ (0.052 mL, 0.30 mmol) at 0 °C. After being stirred for 14 h at rt, the mixture was quenched by H_2O and extracted with $CHCl_3$. The extract was dried over Na_2SO_4, filtered and concentrated. The residue was purified through silica gel column chromatography to give the title compound **37** (48.7 mg, 89%) as a colorless oil.

IR (neat) cm^{-1}: 3003, 2935, 2856, 1725, 1644, 1557, 1456, 1217; ^1H NMR (400 MHz, $CDCl_3$): δ 5.93 (1H, s), 3.54 (2H, dd, *J* = 5.5, 5.2), 3.39 (2H, dd, *J* = 5.5, 5.0), 3.06 (2H, t, *J* = 7.3), 2.31 (2H, t, *J* = 7.5), 2.26 (3H, s), 1.67–1.36 (16H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 207.96, 181.29, 171.45, 168.79, 160.98, 101.54, 99.50, 46.72, 42.59, 41.59, 33.44, 29.37, 29.24, 29.08, 26.59, 25.60, 25.44, 24.61, 23.91, 20.65; HRMS (ESI) *m/z* calcd for $C_{20}H_{30}NO_5$ $[M+H]^+$ 364.2124, found 364.2126.

***N*-(4,5-Dihydro-5-oxo-[1,2]dithiolo[4,3-*b*]pyrrol-6-yl)-8-hydroxyoctanamide (38)**



To a solution of 8-((*tert*-butyldimethylsilyl)oxy)octanoic acid (26.3 mg, 0.10 mmol) in

toluene (1.0 mL) was added oxalyl chloride (0.025 mL, 0.30 mmol) at rt and the solution was stirred for 1 h. The mixture was concentrated and used in the following step without further purification.

To a solution of acid chloride in toluene/THF (2.0 mL) were added a solution of **12** (20.9 mg, 0.10 mmol) in THF (2.0 mL) and Et_3N (0.042 mL, 0.30 mmol). After being stirred for 30 min, the mixture was concentrated and the residual oil was purified through silica gel column chromatography to give the acylated holothin derivative (24.0 mg, 56%) as a yellow powder.

To a solution of the acylated holothin derivative (23.9 mg, 0.06 mmol) in THF (2.0 mL) was added conc. HCl aq. (0.050 mL) at rt and the mixture was stirred for 30 min at the same temperature. The reaction was dried over $MgSO_4$, filtered and concentrated. The residual oil was purified through silica gel column chromatography to give the title compound **38** (16.2 mg, 92%) as a yellow powder.

IR ($CHCl_3$) cm^{-1}: 2957, 2928, 2857, 1731, 1467, 1375, 1250; ^1H NMR (400 MHz, $CDCl_3$): δ 7.82 (1H, br s), 7.46 (1H, br s), 6.74 (1H, s), 3.64 (2H, t, J = 6.4 Hz), 2.34 (2H, t, J = 7.2 Hz), 1.71–1.67 (2H, m), 1.58 (2H, br s), 1.37 (6H, br s); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.84, 167.93, 133.95, 133.68, 115.35, 110.48, 60.66, 34.65, 32.47, 28.60, 28.58, 25.34, 25.01; HRMS (ESI) *m/z* calcd for $C_{13}H_{18}N_2O_3NaS_2$ $[M+Na]^+$ 337.0657, found 337.0659.

2: Biological Evaluation

2–1) Paper disk diffusion assay

To a solution of H_2O (50 mL) were added nutrient broth (OXOID CM0001) (0.65 g) and agar bacteriological (AGAR No. 1) (OXOID LP0011) (0.4 g). Then the mixture was sterilized by autoclaving at 121 °C for 15 min. The solution of *Bacillus subtilis* ATCC6633 (Eiken chemical Co., Ltd: E–MN11, 10^9 cfu/mL) (10 μL) and the autoclaved culture medium solution (10 mL) were dispensed into each sterilized flat–bottomed dishes (90ϕ x 15 mm) respectively. As soon as the culture medium had gelled, 8 mm filter paper disks (ADVANTEC®, Tokyo Roshi Kaisha, Ltd.), which included 30 μL of sample solutions in MeOH (1.0 mg/mL), were spaced upon the agar. Rifampicin (SIGMA, Lot#109K1417) was used as a standard antibiotic. The dishes were incubated at 37 °C for 12 h. The diameters of zones of inhibition were measured.

2–2) *In vitro* MIC Assay

As a bacterial strain, Gram–positive cocci: *Staphylococcus aureus* ATCC29213, *Enterococcus faecalis* ATCC29212 and *Kocuria rizophila* (*Micrococcus luteus*) ATCC9341, Gram–positive bacillus: *Bacillus subtilis* ATCC6633, Gram–negative bacillus: *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853 and *Serratia marcescens* ATCC13880 were used. As a fungus, *Candida albicans* ATCC10231 was used. The antimicrobial susceptibility was determined by the broth microdilution method of Clinical and Laboratory Standards Institute (CLSI, M7–A8). The colony, which was cultivated at 35 °C for 24 h on the Muller–Hinton nutrient agar (Oxoid), was suspended to the Muller–Hinton liquid medium (MHB, Oxoid) at the same level of the McFarland standard (bioMérieux) 0.5 (1.5×10^8 cells/mL). The suspension was diluted 10–fold in MHB and then dispensed into 96 well microplate (Stem) by each 100 μL. Test samples were dissolved in MHB (128 μg/mL ~ 0.125 μg/mL) and 100 μL of this solution was dispensed into 96 well microplate (Stem). All of tested compounds dissolved in each concentration without precipitation becoming a problem. Next, 1 μL (10^5 cells/well) of microbial suspension was inoculated to the test sample solution with the MIC2000 inoculator (Dynatech). After the inoculation, the medium was cultivated at 35 °C for 24 h and 48 h. Then the growth of microorganisms was detected with ILLUMINATED VIEWER MIC–2000 (Dynatech). The test sample's minimum concentration for the inhibition of the microbial growth was determined as the minimum inhibitory concentration (MIC).

2–3) *In vitro* inhibitory activity against *Escherichia coli* RNA polymerase

The Assay of *in vitro* inhibitory activity against *Escherichia coli (E. coli)* RNA polymerase was performed using RNA Polymerase Assay Kit Plus (ProFoldin Catalog No. RPA100KE). This kit included the assay buffer, DNA template, NTP mix, *E. coli* RNA polymerase and fluorescence dye. Reagents were prepared according to the

protocol of the commercially available kit, 10 × DNA: dilute the 100 × DNA 10–fold with water, 10 × enzyme: dilute the 100 × RNA polymerase 10–fold with the 1 × assay buffer, 10 × NTP mix: dilute the 100 × NTP mix (50 mM) 10–fold with water and 1 × fluorescence dye: dilute the 10 × fluorescence dye 10–fold with water. To a 96–well assay plate (Costar®, flat bottom, non–treated) were added 18 μL of H_2O, 3 μL of 10 × buffer, 3 μL of 10 × DNA template, 3 μL of 10 × enzyme, 3 μL of 10 × NTP mix, and 0.5 μL of sample solution in DMSO, respectively. After being incubated for 1 h at rt, 30 μL of 1 × fluorescence dye was added to the reaction mixture. The solution was incubated for additional 5 min at rt. The resultant mixture was measured the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm.

RFP and compound **29** were examined three independent measurements and compound **14** and compound **27** were examined two independent measurements, in which each measurement was done in duplicate, with the following final concentrations: 0, 0.0001, 0.001, 0.01, 0.1, 1, 10, 100 and 1000 μg/mL, respectively. All of tested compounds dissolved in each concentration without precipitation becoming a problem. The data was analyzed after curve fitting by Microsoft Excel and the IC_{50} was determined as the compound concentrations needed to produce a 50% reduction of fluorescence intensity relative to the negative control. The range to determine the IC_{50} value for RFP was in 0.01–0.1 μg/mL and for compound **29** was in 1–100 μg/mL.