SUPPORTING INFORMATION FOR

Dual Inhibitor of MurD and MurE Ligases from Escherichia coli and Staphylococcus aureus

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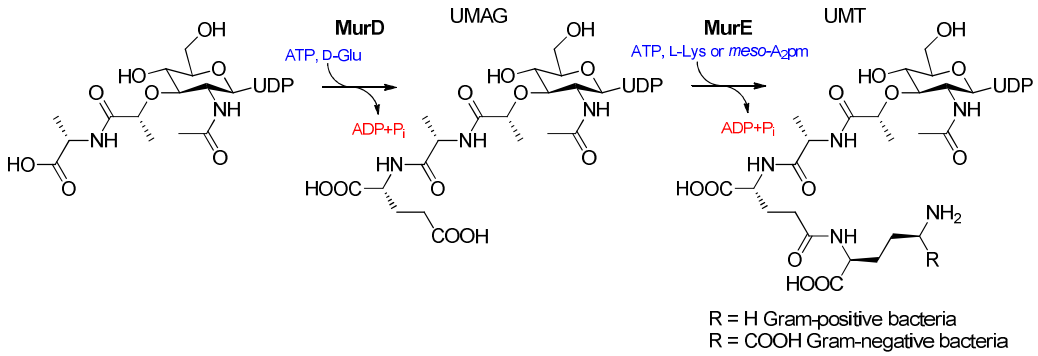


Figure S1. Enzymatic reactions catalyzed by MurD and MurE ligases.

Experimental Section

1. Chemistry

The synthesis of target compounds **9** and **10** is outlined in Scheme 1. In the first step, terephthalaldehyde (**1**) was monoprotected by using ethylene glycol and *p*-toluenesulfonic acid as a catalyst to obtain aldehyde **2**. Reductive amination of aldehyde **2** with 3-aminobenzoic acid afforded compound **3** which, after deprotection using catalytic amount of pyridinium tosylate, gave aldehyde **4**. 5-Benzylidenerhodanine **5** and 5-benzylidene-thiazolidine-2,4-dione **6**, were then prepared via Knoevenagel condensation between aldehyde **4** and rhodanine or thiazolidine-2,4-dione, respectively, using glacial acetic acid and piperidine as catalysts. The presence in the ^{1}H NMR spectrum of only one signal for the methyne proton at 7.62 ppm or 7.76 ppm, respectively, suggested, according to previous studies, exclusive formation of a thermodynamically more stable *Z* isomer. The target compounds **9** and **10** were obtained after alkaline hydrolysis of compounds **7** and **8**, respectively, which were prepared via *O*-(Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU)-promoted amide bond formation between **5** or **6** and the dimethyl ester of D-glutamic acid. The *Z* configuration of compounds **5-10** could also be inferred from the X-ray structure of the *E. coli* MurD-**7** complex.

General methods: Chemicals were obtained from Acros, Aldrich Chemical Co., and Fluka and used without further purification. Analytical thin-layer chromatography was performed on silica gel Merck 60 F_{254} precoated plates (0.25 mm), using visualization with ultraviolet light, ninhydrin and 2,4-dinitrophenylhydrazine. Flash column chromatography was carried out on silica gel 60 (particle size 0.040-0.063 mm; Merck, Germany). HPLC analysis was performed on an Agilent Technologies HP 1100 instrument with a G1365B UV-VIS detector, a G1316A

thermostat and a G1313A autosampler, using a Phenomenex Luna C18 column (4.6 × 250 mm) at flow rate 1 mL/min. The eluant was a mixture of 0.1% TFA in water (A) and acetonitrile (B). Gradient was 10% B to 80% B in 25 min. Melting points were determined on a Reichert hot stage microscope and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra for compounds **2**, **3**, **4**, **5**, **7** and **9** were recorded at 300 and 75 MHz on a Bruker AVANCE DPX300 spectrometer in CD_3OD, $CDCl_3$ or DMSO-d_6 solution, with TMS as the internal standard. ^{1}H NMR and ^{13}C NMR spectra for compounds **6**, **8** and **10** were recorded at 400 and 100 MHz on a Bruker AVANCE III 400 MHz NMR spectrometer in CD_3OD or DMSO-d_6 solution, with TMS as the internal standard. Spectra were assigned using gradient COSY, HSQC and ^{1}H-coupled ^{13}C NMR experiments. IR spectra were recorded on a Perkin-Elmer 1600 FT-IR spectrometer. Mass spectra were obtained using a VG-Analytical Autospec Q mass spectrometer. Optical rotations were measured on a Perkin-Elmer 1241 MC polarimeter. The reported values for specific rotation are average values of 10 successive measurements using an integration time of 5 s. Microanalyses were performed on a Perkin-Elmer C, H, N analyzer 240 C. Microwave-assisted reactions were performed using a focused microwave reactor (Discover, CEM Corporation, Matthews, NC). Reactions were carried out in septum-sealed glass vials (10 mL) which enable high-pressure reaction conditions (max 20 bar). The temperature of the reaction mixture was monitored using a calibrated infrared temperature controller mounted under the reaction vessel.

1.1. 4-(1,3-Dioxolan-2-yl)benzaldehyde (2): To a stirred suspension of terephthalaldehyde (10.350 g, 0.77 mol) in benzene (250 mL) ethylene glycol (4.30 mL, 0.77 mol) and a catalytic amount of *p*-toluenesulfonic acid (0.500 g, 2.63 mmol) were added. A round-bottomed flask with the reaction mixture was equipped with a Dean-Stark apparatus and stirred overnight under reflux. After the reaction mixture was cooled to room temperature, the solvent was evaporated under reduced pressure and the residue was partitioned between dichloromethane and 0.1 M $NaHCO_3$. The organic phase was separated, dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash column chromatography using petrolether/dichloromethane 1:2 to methanol/dichloromethane 1:20 as eluant. Compound **2** was obtained as yellowish solidified oil (5.694 g, 41.5%): R_f= 0.11 (petrolether/CH_2Cl_2; 1:2); mp: 110-112 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.05 (s, 1 H, C*H*O), 7.92 (d, 2 H, *J*=8.2 Hz, Ar$H_{(2,6)}$), 7.67 (d, 2 H, *J*=8.2 Hz, Ar$H_{(3,5)}$), 5.90 (s, 1 H, OC*H*O), 4.17-4.05 (m, 4 H, 2 x CH_2) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 191.8, 144.3, 136.8, 129.7, 127.0, 102.7, 65.3

ppm; IR (KBr): ν = 3365, 2865, 1945, 1694, 1576, 1500, 1430, 1386, 1369, 1302, 1199, 1096, 1014, 815, 773, 730, 685, 523, 475 cm^{-1}; MS (ESI+): *m/z* (%) = 179 (18) $[M+H]^+$, 105 (100).

1.2. 3-((4-(1,3-Dioxolan-2-yl)benzyl)amino)benzoic acid (3):

A solution of aldehyde **2** (1.309 g, 7.35 mmol), 3-aminobenzoic acid (1.009 g, 7.35 mmol) and $NaCNBH_3$ (257 mg, 4.10 mmol) in methanol (70 mL) was stirred overnight at room temperature. The solvent was evaporated under reduced pressure, the residue dissolved in ethyl acetate (50 mL) and washed with water (2 × 20 mL) and brine (20 mL). The organic phase was dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The crude product was purified by flash column chromatography using dichloromethane/methanol (40:1) as eluant. Compound **3** was obtained as white solid (0.756 g, 35.0%): R_f = 0.33 (CH_2Cl_2/MeOH; 30:1); mp: 113-115 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.59 (br s, 1 H, COO*H*), 7.44-7.34 (m, 4 H, Ar*H'*), 7.17-7.09 (m, 3 H, Ar*H*), 6.79-6.75 (m, 1 H, Ar*H*), 6.54 (t, 1 H, *J*=5.9 Hz, CH_2NH), 5.69 (s, 1 H, OC*H*O), 4.31 (d, 2 H, *J*=5.9 Hz, CH_2NH), 4.06-3.90 (m, 4 H, 2 x CH_2) ppm; ^{13}C NMR (75 MHz, CD_3OD): δ = 170.6, 150.1, 142.4, 138.0, 132.4, 130.0, 128.2, 127.9, 119.1, 118.4, 114.7, 104.9, 66.3, 48.2 ppm; IR (KBr): ν = 3370, 2879, 2361, 2342, 1686, 1606, 1515, 1488, 1420, 1286, 1208, 1083, 926, 876, 832, 756, 681, 562 cm^{-1}; MS (ESI-): *m/z* (%) = 298 (100) $[M-H]^-$; HRMS (ESI-): *m/z* $[M-H]^-$ calcd for $C_{17}H_{16}NO_4$: 298.1079, found: 298.1092.

1.3. 3-((4-Formylbenzyl)amino)benzoic acid (4): A solution of compound **3** (0.719 g, 2.42 mmol) and pyridinium tosylate (0.091 g, 0.36 mmol) in a mixture of THF (20 mL) and water (3 mL) was stirred overnight at room temperature. The organic solvent was evaporated under reduced pressure and water was added (5 mL). The product was extracted with ethyl acetate (3 x 20 mL), combined organic extracts washed with brine, dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure. The crude product was purified by flash column chromatography using CH_2Cl_2/MeOH (20:1) as eluant. Compound **4** was obtained as yellowish solid (0.460 g, 74.6%): R_f= 0.10 (CH_2Cl_2/MeOH; 20:1); mp: 138-140 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.63 (br s, 1 H, COO*H*), 9.97 (s, 1 H, C*H*O), 7.87 (d, 2 H, *J*=7.9 Hz, Ar*H'*), 7.57 (d, 2 H, *J*=7.9 Hz, Ar*H'*), 7.18-7.10 (m, 3 H, Ar*H*), 6.78-6.76 (m, 1 H, Ar*H*), 6.68 (t, 1 H, *J*=6.0 Hz, CH_2N*H*), 4.42 (d, 2 H, *J*=6.0 Hz, CH_2NH) ppm; ^{13}C NMR (75 MHz, CD_3OD): δ = 192.9, 169.5, 148.9, 148.0, 135.9, 131.5, 130.0, 129.0, 127.8, 118.3, 117.3, 113.6, 41.4 ppm; IR (KBr): ν = 3371, 2850, 2360, 1682, 1603, 1519, 1480, 1436, 1295, 1208, 1167, 1096, 1016, 989, 942, 862, 832, 796, 757, 687, 559 cm^{-1}; MS (ESI+): *m/z* (%) = 256

(20) $[M+H]^+$, 278 (100) $[M+Na]^+$; HRMS (ESI+): *m/z* $[M+H]^+$ calcd for $C_{15}H_{14}NO_3$: 256.0974, found: 256.0976; Anal. calcd for $C_{15}H_{13}NO_3 \cdot 1/5H_2O$: C 69.52; H 5.18; N 5.41; found: C 69.74; H 5.24; N 5.26.

1.4. (*Z*)-3-((4-((4-Oxo-2-thioxothiazolidin-5-ylidene)methyl)benzyl)amino)benzoic acid (5): A suspension of rhodanine (0.225 g, 1.69 mmol), aldehyde **4** (0.430 g, 1.69 mmol), glacial acetic acid (9.6 µL, 0.17 mmol) and piperidine (16.7 µL, 0.17 mmol) in absolute ethanol (20 mL) was stirred for 40 h under reflux, then cooled and the precipitate filtered off and dried. Compound **5** was obtained as yellow crystalline solid (0.356 g, 57.0%): R_f = 0.46 (CH_2Cl_2/MeOH/AcOH; 20:1:0.1); mp: 220-221 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 13.83 (br s, 1 H, CON*H*CS), 12.60 (br s, 1 H, COO*H*), 7.62 (s, 1 H, C=C*H*), 7.57 (d, 2 H, *J*=8.4 Hz, Ar*H'*), 7.52 (d, 2 H, *J*=8.4 Hz, Ar*H'*), 7.18-7.10 (m, 3 H, Ar*H*), 6.80-6.76 (m, 1 H, Ar*H*), 6.63 (br s, 1 H, CH_2NH), 4.37 (br s, 2 H, CH_2NH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 195.6, 169.3, 167.6, 148.4, 143.4, 131.4, 131.3, 131.2, 130.5, 128.9, 127.9, 124.7, 116.8, 116.2, 112.8, 46.0 ppm; IR (KBr): ν = 3432, 2864, 2365, 1686, 1589, 1420, 1335, 1315, 1285, 1240, 1194, 1076, 1014, 861, 809, 757, 685, 596, 551 cm^{-1}; MS (ESI-): *m/z* (%) = 369 (100) $[M-H]^-$; HRMS (ESI-): *m/z* $[M-H]^-$ calcd for $C_{18}H_{13}N_2O_3S_2$: 369.0368, found: 369.0384.

1.5. (*Z*)-3-((4-((2,4-dioxothiazolidin-5-ylidene)methyl)benzyl)amino)benzoic acid (6): To a suspension of thiazolidine-2,4-dione (0.185 g, 1.58 mmol) and aldehyde **4** (0.402 g, 1.58 mmol) in absolute ethanol (5 mL) in a 10 mL process vial, glacial acetic acid (10 µL, 0.18 mmol) and piperidine (15.5 µL, 0.16 mmol) were added. The vial was sealed, placed in a microwave reactor and heated at 140 °C for 30 minutes (max. power 30 W). The reaction mixture was cooled and the precipitate filtered off and dried. Compound **6** was obtained as yellow crystalline solid (0.285 g, 51.0%): R_f = 0.42 (CH_2Cl_2/MeOH/AcOH; 20:1:0.1); mp: 228-230 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.65 (br s, 2 H, COO*H*, CON*H*CO), 7.76 (s, 1 H, C=C*H*), 7.57 (d, 2 H, *J*=8.3 Hz, Ar*H'*), 7.51 (d, 2 H, *J*=8.3 Hz, Ar*H'*), 7.17-7.10 (m, 3 H, Ar*H*), 6.78 (ddd, 1 H, ^3J=7.8 Hz, ^4J=2.3 Hz, ^4J=1.5 Hz, Ar*H*), 6.65 (t, 1 H, *J*=5.6 Hz, CH_2NH), 4.37 (d, 2 H, *J*=5.2 Hz, CH_2NH) ppm; MS (ESI-): *m/z* (%) = 353 (100) $[M-H]^-$; HRMS (ESI-): *m/z* $[M-H]^-$ calcd for $C_{18}H_{13}N_2O_4S$: 353.0600, found: 353.0596.

1.6. (*R*,*Z*)-Dimethyl 2-(3-((4-((4-oxo-2-thioxothiazolidin-5-ylidene)methyl)benzyl)amino)-benzamido)pentanedioate (7): To a stirred suspension of compound **5** (0.306 g, 0.83 mmol)

in CH_2Cl_2 (70 mL) triethylamine (345 µL, 2.49 mmol) and TBTU (0.292 g, 0.91 mmol) were added. The solution was stirred for 15 min and then additional triethylamine (115 µL, 0.83 mmol) and H-D-Glu(OMe)-OMe·HCl (0.175 g, 0.83 mmol) were added. The reaction mixture was stirred at room temperature for 5 h and then washed successively with water (2 x 40 mL), 2 M HCl (2 x 40 mL), saturated aqueous $NaHCO_3$ solution (2 x 40 mL) and brine (40 mL), then dried over Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by flash column chromatography using CH_2Cl_2/MeOH (20:1) as eluant. Compound **7** was obtained as orange solid (0.320 g, 73.4%): R_f = 0.20 (CH_2Cl_2/MeOH; 40:1); mp: 89-91 °C; $[\alpha]_D^{20}$ = -36.6 (*c*=0.25 in DMF); ^{1}H NMR (300 MHz, DMSO-d_6): δ = 13.82 (br s, 1 H, CON*H*CS), 8.53 (d, 1 H, *J*=7.5 Hz, CON*H*), 7.62 (s, 1 H, C=C*H*), 7.57 (d, 2 H, *J*=8.2 Hz, Ar*H'*), 7.52 (d, 2 H, *J*=8.2 Hz, Ar*H'*), 7.13 (t, 1 H, *J*=7.7 Hz, Ar*H*), 7.06-7.03 (m, 2 H, Ar*H*), 6.71 (d, 1 H, *J*=7.7 Hz, Ar*H*), 5.75 (s, 1 H, CH_2NH), 4.45-4.38 (m, 3 H, CH_2NH, $CHCH_2CH_2$), 3.63 (s, 3 H, CH_3), 3.58 (s, 3 H, CH_3), 2.42 (t, 2 H, *J*=7.4 Hz, $CHCH_2CH_2$), 2.19-1.92 (m, 2 H, $CHCH_2CH_2$) ppm; ^{13}C NMR (75 MHz, CD_3OD): δ = 196.6, 175.0, 173.8, 171.1, 171.0, 150.0, 144.8, 135.9, 133.4, 132.8, 131.9, 130.3, 129.3, 126.7, 117.5, 116.7, 112.7, 53.7, 52.9, 52.3, 38.9, 31.3, 27.4 ppm; IR (KBr): ν = 3391, 2950, 2849, 2358, 1732, 1600, 1538, 1436, 1234, 1193, 1061, 1006, 809, 750, 681, 589, 544 cm^{-1}; MS (ESI-): *m/z* (%) = 526 (100) [*M*-H]$^-$; HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{25}H_{24}N_3O_6S_2$: 526.1107, found: 526.1101; Anal. calcd for $C_{25}H_{25}N_3O_6S_2$: C 56.91; H 4.78; N 7.96; found: C 57.01; H 5.18; N 7.98.

1.7. (*R*,*Z*)-dimethyl 2-(3-((4-((2,4-dioxothiazolidin-5-ylidene)methyl)benzyl)amino) benzamido)pentanedioate (8): To a stirred suspension of compound **6** (0.930 g, 2.63 mmol) in CH_2Cl_2 (70 mL) triethylamine (1.10 mL, 7.89 mmol) and TBTU (0.928 g, 2.89 mmol) were added. The solution was stirred for 15 min and then additional triethylamine (365 µL, 2.63 mmol) and H-D-Glu(OMe)-OMe·HCl (0.556 g, 2.63 mmol) were added. The reaction mixture was stirred at room temperature for 5 h and then washed successively with water (2 x 50 mL), 2 M HCl (2 x 50 mL), saturated aqueous $NaHCO_3$ solution (2 x 50 mL) and brine (50 mL), then dried over Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by flash column chromatography using CH_2Cl_2/MeOH (20:1) as eluant. Compound **8** was obtained as yellow solid (0.565 g, 42.1%): R_f = 0.18 (CH_2Cl_2/MeOH; 40:1); mp: 93-95 °C; $[\alpha]_D^{20}$ = -30.1 (*c*=0.24 in DMF); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.61 (br s, 1 H, CON*H*CO), 8.57 (d, 1 H, *J*=7.4 Hz, CON*H*), 7.77 (s, 1 H, C=C*H*), 7.57 (d, 2 H, *J*=8.1 Hz, Ar*H'*), 7.51 (d, 2 H, *J*=8.1 Hz, Ar*H'*), 7.13 (t, 1 H, *J*=7.7 Hz, Ar*H*), 7.06-7.03 (m, 2 H, Ar*H*),

6.71 (d, 1 H, *J*=7.7 Hz, Ar*H*), 6.59 (t, 1 H, *J*=6.6 Hz, CH_2NH), 4.44-4.38 (m, 3 H, CH_2NH, $CHCH_2CH_2$), 3.64 (s, 3 H, CH_3), 3.59 (s, 3 H, CH_3), 2.43 (t, 2 H, *J*=7.1 Hz, $CHCH_2CH_2$), 2.14-1.94 (m, 2 H, $CHCH_2CH_2$) ppm; ^{13}C NMR (100 MHz, CD_3OD): δ = 174.9, 173.8, 171.1, 169.4, 169.0, 150.1, 144.5, 135.8, 133.5, 133.3, 131.5, 130.2, 129.1, 124.2, 117.4, 116.6, 112.6, 53.6, 52.9, 52.3, 38.9, 31.3, 27.4 ppm; MS (ESI+): *m/z* (%) = 512 (100) $[M+H]^+$; HRMS (ESI+): *m/z* $[M+H]^+$ calcd for $C_{25}H_{26}N_3O_7S$: 512.1496, found: 512.1491.

1.8. (*R*,*Z*)-2-(3-((4-((4-Oxo-2-thioxothiazolidin-5-ylidene)methyl)benzyl)amino)-benzamido)pentanedioic acid (9): To a stirred solution of dimethyl ester **7** (0.160 g, 0.300 mmol) in MeOH/water (1:1) (10 mL), 2 M LiOH (0.60 mL, 1.20 mmol) was added and the reaction mixture stirred overnight at room temperature. The solution was neutralized with 1 M HCl and concentrated under reduced pressure. The residual aqueous solution was acidified with 1 M HCl to pH 2 and the product extracted with ethyl acetate (3 × 15 mL). The combined organic phases were washed with brine (2 × 15 mL), dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure. The crude product was purified by flash column chromatography using CH_2Cl_2/MeOH (9:1) to CH_2Cl_2/MeOH/AcOH (7:1:0.1) as eluant. Compound **9** was obtained as yellow solid (0.130 g, 86.1%): R_f = 0.11 (CH_2Cl_2/MeOH/AcOH; 9:1:0.1); mp: 134-136 °C; $[\alpha]_D^{20}$ = -136.5 (*c*=0.26 in DMF); ^{1}H NMR (300 MHz, DMSO-d_6): δ = 13.80 (br s, 1 H, CON*H*CS), 12.32 (br s, 2 H, 2 x COOH), 8.37 (d, 1 H, *J*=7.5 Hz, CON*H*), 7.61 (s, 1 H, C=C*H*), 7.57 (d, 2 H, *J*=8.1 Hz, Ar*H'*), 7.52 (d, 2 H, *J*=8.1 Hz, Ar*H'*), 7.12-7.03 (m, 3 H, Ar*H*), 6.70 (d, 1 H, *J*=7.8 Hz, Ar*H*), 6.55 (br s, 1 H, CH_2NH), 4.38-4.32 (m, 3 H, CH_2NH, $CHCH_2CH_2$), 2.33 (t, 2 H, *J*=7.2 Hz, $CHCH_2CH_2$), 2.11-1.91 (m, 2 H, $CHCH_2CH_2$) ppm; ^{13}C NMR (75 MHz, CD_3OD): δ = 182.9, 182.6, 180.0, 170.4, 150.2, 148.6, 140.0, 137.7, 136.2, 134.2, 130.3, 127.4, 126.4, 116.3, 113.0, 68.9, 57.1, 42.3, 35.4, 30.3, 26.5 ppm; IR (KBr): ν = 3402, 2360, 1722, 1603, 1532, 1231, 1193, 812, 753, 682 cm^{-1}; MS (ESI-): *m/z* (%) = 498 (100) $[M-H]^-$; HRMS (ESI-): *m/z* $[M-H]^-$ calcd for $C_{23}H_{20}N_3O_6S_2$: 498.0794, found: 498.0790; Anal. calcd for $C_{23}H_{21}N_3O_6S_2 \cdot 3H_2O$: C 49.86; H 4.87; N 7.59; found: C 49.57; H 4.48; N 7.39. HPLC t_r = 13.672 min (95.05% at 254 nm, 95.10% at 280 nm).

1.9. (*R*,*Z*)-2-(3-((4-((2,4-dioxothiazolidin-5-ylidene)methyl)benzyl)amino)benzamido) pentanedioic acid (10): To a stirred solution of dimethyl ester **8** (0.203 g, 0.397 mmol) in MeOH/water (1:1) (10 mL), 1 M NaOH (2.00 mL, 2.00 mmol) was added and the reaction mixture stirred at room temperature for 3 h. The solution was neutralized with 1 M HCl and

concentrated under reduced pressure. The residual aqueous solution was acidified with 1 M HCl to pH 2 and the product extracted with ethyl acetate (3 × 15 mL). The combined organic phases were washed with brine (2 × 15 mL), dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure. The crude product was purified by flash column chromatography using CH_2Cl_2/MeOH (9:1) to CH_2Cl_2/MeOH/AcOH (7:1:0.1) as eluant. Compound **10** was obtained as pale yellow solid (0.085 g, 44.3%): R_f = 0.10 (CH_2Cl_2/MeOH/AcOH; 9:1:0.1); mp: 142-144 °C; $[\alpha]_D^{20}$ = -120.1 (*c*=0.21 in DMF); ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ = 12.62 (br s, 1 H, CON*H*CO), 12.32 (br s, 2 H, 2 x COOH), 8.41 (d, 1 H, *J*=7.7 Hz, CON*H*), 7.76 (s, 1 H, C=C*H*), 7.57 (d, 2 H, *J*=8.3 Hz, Ar*H'*), 7.52 (d, 2 H, *J*=8.3 Hz, Ar*H'*), 7.13 (t, 1 H, *J*=7.8 Hz, Ar*H*), 7.08-7.03 (m, 2 H, Ar*H*), 6.70 (dd, 1 H, ^3J=7.8 Hz, ^4J=1.7 Hz, Ar*H*), 6.58 (t, 1 H, *J*=6.0 Hz, CH_2NH), 4.39-4.32 (m, 3 H, CH_2NH, $CHCH_2CH_2$), 2.33 (t, 2 H, *J*=7.4 Hz, $CHCH_2CH_2$), 2.11-1.88 (m, 2 H, $CHCH_2CH_2$) ppm; ^{13}C NMR (100 MHz, CD_3OD): δ = 176.7, 175.1, 171.1, 169.5, 169.0, 150.1, 144.5, 136.0, 133.6, 133.3, 131,5, 130.2, 129.1, 124.2, 117.2, 116.6, 112.7, 53.7, 38.9, 31.5, 27.6 ppm; MS (ESI-): *m/z* (%) = 482 (100) [*M*-H]$^-$; HRMS (ESI-): *m/z* [*M*-H]$^-$ calcd for $C_{23}H_{20}N_3O_7S$: 482.1007, found: 482.1022; HPLC t_r = 14.516 min (95.21 % at 220 nm, 95.53 % at 254 nm).

2. Biology

2.1. Enzyme assays

2.1.1. Radioactivity inhibition assays.

Compounds **9** and **10** were tested for their ability to inhibit the activity of MurD and MurE, measured by the formation of radioactive product from radioactive UDP-MurNAc-L-Ala or UDP-MurNAc-dipeptide. In order that the comparison of the IC_{50} values between Mur orthologues be valid, the concentrations of the UDP-precursor and amino acid substrates were set at their respective K_m values. Reaction mixtures (final volume, 40 µL) contained:

E. coli MurD: 100 mM Tris-HCl, pH 8.6, 5 mM $MgCl_2$, 5 mM ATP, 7.5 µM UDP-MurNAc-L-[^{14}C]Ala, 55 µM D-Glu, 30 µM Tween-20, 5% DMSO, purified MurD from *E. coli*[1] (18 µL of an appropriate dilution in 20 mM potassium phosphate, pH 7.2, 1 mM DTT, 1 mg/mL BSA), and inhibitor.

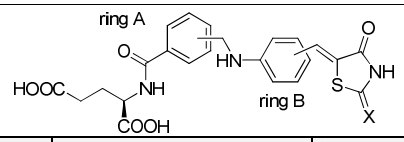
S. aureus MurD: 100 mM Tris-HCl, pH 8.6, 15 mM $MgCl_2$, 5 mM ATP, 40 µM UDP-MurNAc-L-[^{14}C]Ala, 140 µM D-Glu, 30 µM Tween-20, 5% DMSO, purified MurD from *S. aureus*[2] (18 µL of an appropriate dilution in 20 mM potassium phosphate, pH 7.2, 1 mM DTT), and inhibitor.

E. coli MurE: 100 mM Tris-HCl, pH 8.6, 40 mM $MgCl_2$, 5 mM ATP, 40 μM UDP-MurNAc-L-Ala-D-[^{14}C]Glu, 80 μM *meso*-A_2pm, 30 μM Tween-20, 5% DMSO, purified MurE from *E. coli*[3] (10 μL of an appropriate dilution in 20 mM potassium phosphate, pH 7.2, 1 mM DTT), and inhibitor.

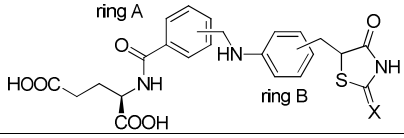
S. aureus MurE: 100 mM Tris-HCl, pH 8.6, 15 mM $MgCl_2$, 5 mM ATP, 100 μM UDP-MurNAc-L-Ala-D-[^{14}C]Glu, 500 μM L-Lys, 30 μM Tween-20, 5% DMSO, purified MurE from *S. aureus*[2] (10 μL of an appropriate dilution in 20 mM potassium phosphate, pH 7.2, 1 mM DTT), and inhibitor.

In all cases, the mixtures were incubated for 30 min at 37 °C, and the reaction was terminated by the addition of glacial acetic acid (8 μL) followed by lyophilization. Radioactive substrate and product were then separated by HPLC on a Nucleosil 100C18 5U column (150 × 4.6 mm; Alltech France) using 50 mM ammonium formate, pH 3.9 (*E. coli* and *S. aureus* MurD, *E. coli* MurE), or 50 mM sodium phosphate and 7.2 mM sodium hexanephosphonate, pH 2.5/acetonitrile (98.5:1.5, v/v)[4] (*S. aureus* MurE), at a flow rate of 0.6 mL/min. Radioactivity was detected with a flow detector (model LB506-C1, Berthold) using the Quicksafe Flow 2 scintillator (Zinsser Analytic) at 0.6 mL/min. Quantification was performed with the Radiostar software (Berthold). IC_{50} values were determined by measuring the residual activities at 7 different compound concentrations; values ± standard deviations at 95% of confidence were calculated from the fitted regression equations using the logit-log plot.

Table S1. Inhibitory activities of 5-benzylidenethiazolidin-4-ones[5] against *S. aureus* MurE.



compound	X	substitution		MurE RA[a] (%)
		ring A	ring B	
(*R*)-**20**	O	1,4	1,3	76
(*R*)-**24**	O	1,4	1,4	76
(*R*)-**32 or I**	O	1,3	1,4	64
(*R*)-**33**	S	1,3	1,4	67



compound	X	substitution		MurE
		ring A	ring B	RA[a] (%)
(*R*)-**62**	S	1,3	1,4	35

[a]Residual activity of the enzyme at 500 μM compound.

2.2. Determination of antibacterial activity

The susceptibility of five bacterial strains (*E. coli* ATCC 25922, *P. aeruginosa* 27853, *S. aureus* ATCC 29213, methicillin resistant *S. aureus* ATCC 43300 and *E. faecalis* ATCC 29212) to compounds **9** and **10** was tested with the macrodilution method.

Compounds **9** and **10** (5 mg) were dissolved in 2.5 mL of DMSO to give a stock solution of 2 mg/mL. Working solutions were made by serially diluting stock solution in cation-adjusted Mueller-Hinton broth (CAMHB) as described by Amsterdam and Barry.[6,7] 34 mL of CAMHB was added to 5 mL stock to give 256 μg/mL concentration, and the solution was filter sterilized. The compound was further serially diluted to give 14 dilutions down to 0.031 μg/mL[7] and stored frozen for a maximum of two weeks. Just prior to bacterial inoculation, 0.5 mL dilutions in range of the compounds were pipetted into 13 × 100 mm screw cap tubes.

The inoculum was prepared in such a way that four colonies of a fresh overnight culture on a nonselective agar plate were inoculated into saline. The turbidity was adjusted to match that of 0.5 McFarland standard (approx. 10^8 CFU/mL). A portion of a standardized suspension was diluted approximately 1:1000 (10^5 CFU/mL), 0.5 mL of which was added within 30 min to each tube containing 0.5 mL of the tested compound diluted in CAMHB and incubated at 35 °C for 18 to 24 hours. After inoculum was added, dilutions 0.016 to 128 μg/mL of the compound were achieved. Broth not containing any compound was inoculated as a growth control. If the compound inhibited bacterial growth, it was considered a potential antimicrobial agent. The lowest concentration of antimicrobial agent that resulted in complete inhibition of visible growth represented the minimal inhibitory concentration (MIC). Quality control of the method was done by testing *S. aureus* ATCC 29213 and gentamicin. Dilutions of antibiotic were made in the same way as for tested compound and the MICs obtained were in the range proposed by the Clinical Laboratory Standards Institute.[8]

2.3. Cytotoxicity assay

Cytotoxicity of **9** was measured by the MTT reduction assay.[9] The HepG2 cells were seeded onto 96-well microplates at a density of 8000 cells/well and incubated 24 hours at 37 °C to

attach. The medium was then replaced with fresh complete medium containing graded concentrations of **9** (6.25 μM to 200 μM) and incubated for 24 hours. MTT (final concentration 0.5 mg/mL) was then added and the plates incubated for an additional 3 hours. At the end of the incubation with MTT the medium was removed and the formazan crystals dissolved in DMSO. Optical density (OD) was measured at 570 nm (reference filter 690 nm) using a microplate reading spectrofluorimeter (Tecan, Genios). Viability was determined by comparing the OD of the wells containing the **9** treated cells with those of the vehicle (0.1% (v/v) DMSO) treated cells. Student's t test was used to evaluate the statistical significance between exposed and control cells; $P < 0.05$ was considered significant.

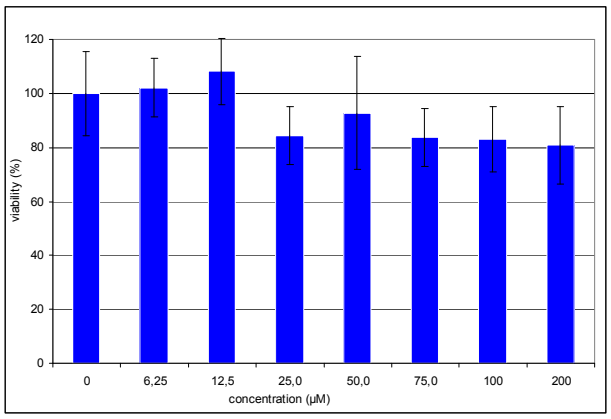


Figure S2. Viability of human HepG2 cells at different concentrations of compound **9**.

3. Crystallization, data collection, structure solution and model refinement

Crystals of the conformationally closed *E. coli* MurD ligase were obtained by co-crystallizing the 6 × His-tagged enzyme at 293 K with UMA and a non-hydrolyzable analogue of ATP (AMP-PNP)[10-12] by employing the vapor-diffusion method and hanging-drop system in Linbro plates. 2 μL of protein solution drops (6.0 mg/mL of purified enzyme, 20 mM HEPES, pH 7.4, 200 mM NaCl, 5 mM dithiothreitol, 0.05% (w/v) NaN_3, 1 mM UMA and 5 mM AMP-PNP) were mixed with 2 μL of reservoir solution (0.1 M HEPES, pH 7.5, 1.9 M ammonium sulfate, 7% (w/v) PEG 400 and 50 mM NaCl). Crystals appeared in 4 to 6 days. They were subsequently incubated with 50 μM of compound **9.** The concentration of compound was gradually raised to 1 mM over 12 hours. The final concentration of DMSO was 5% (v/v). After soaking the crystals were rapidly flash-cooled in liquid nitrogen using Paratone oil as a cryoprotectant. The data set was collected at the European Synchrotron Radiation Facility (ESRF, Grenoble, France) ID29 beamline.

Statistics on data collection and refinement are summarized in Table S2. Data were indexed and scaled with XDS[13] and solved by employing the structure of *E. coli* MurD (1UAG)[10] as a search model in a molecular replacement approach using the PHASER[14] program. The model was then rebuilt *de novo* to remove bias, as implemented in ARP/wARP 7.1.1[15] and Lafire 3.02[16]. COOT[17] was used for manual correction of the model and cycles of refinement were performed with REFMAC 5.5[18] as implemented in the CCP4-6.1.1[19] suite of programs. In addition, water molecules were added to the residual electron density map using ARP/wARP.[15] After several cycles of refinement R_{work} and R_{free} converged. The stereochemical quality of the refined models was verified with PROCHECK.[20] Secondary structure assignment was performed by DSSP[21] and STRIDE.[22] All crystals tested were in space group $P4_1$ and had one molecule per asymmetric unit. A detailed representation of the binding mode of **9** in the *E. coli* MurD active site was made by Ligplot[23] (Figure S3). The atomic coordinates and the related experimental data were deposited at the Protein Data Bank under PDB ID Code 2Y1O.

Table S2. Data collection, molecular replacement and structure refinement statistics.

Data Collection	
X-ray source	ID29
detector	ADSC Q315r
wavelength (Å)	0.972
scan-range (deg)	200
oscillation (deg)	1.0
space group	$P4_1$
cell dimensions *a*, *b*, *c* (Å)	65.84, 65.84, 135.75
mosaicity (^{o})	0.26
resolution (Å) (last shell)	1.49 (1.49-1.58)
no. observed/unique reflections	412,118/91,441
completeness (%) (last shell)	82.8 (52.2)
R_{sym} (last shell)	4.4 (47.4)
I/(I) (last shell)	27.7 (3.0)
mean B factor (Å^2)	25.64
Molecular replacement	
Mol/ASU	1
Phaser RFZ/TFZ/LLG, LLG	23.9/21.6/1645 – 16.5/62.0/3825, 5955
Refinement	
R_{work}/ R_{free} (%)	20.4/23.11
no. of atoms (protein/water)	3,350/415
mean B factor (Å^2)	19.06
compound **7** mean B factor (Å^2)	21.92
rmsd bonds (Å)	0.013
rmsd angles (deg)	1.613
residues in most favored/allowed region of Ramachandran plot (%)	100

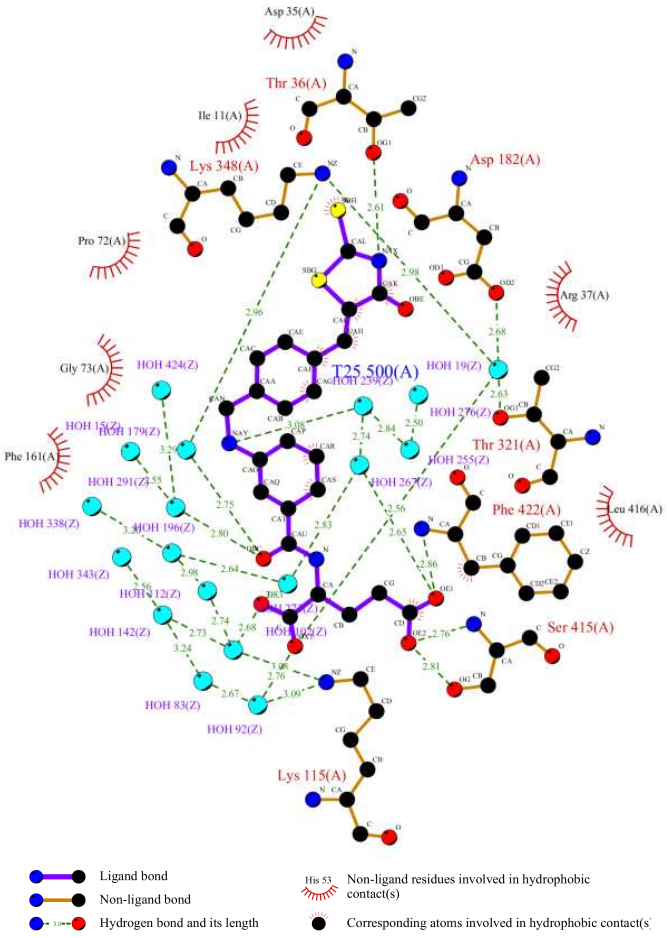


Figure S3. Ligplot[23] representation of interactions of **9** in the *E. coli* MurD active site.

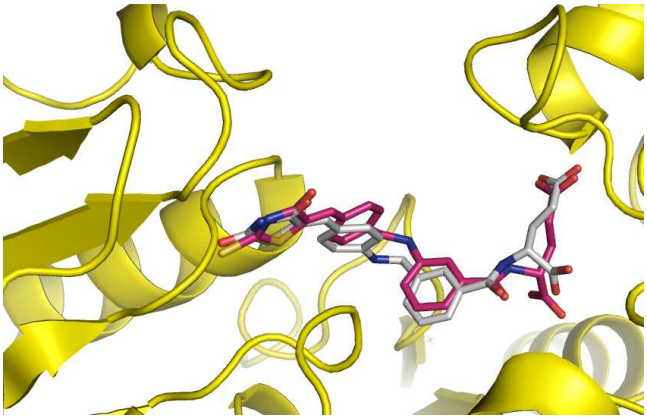


Figure S4. Superposition of MurD inhibitors **I** (PDB code: 2X5O) (in grey) and **9** (PDB code: 2Y1O) (in magenta) in the *E. coli* MurD active site. Detailed binding mode of compound **9** in the *E. coli* MurD active site is shown in Figure 2. Figure was prepared by Pymol.[24]

The α-carboxylate group of D-Glu is rotated by approximately 75°, which results in a different hydrogen bonding network. Because of a different overall conformation, the α-

carboxylate group loses direct contact with the N^{ξ} of Lys348 and forms hydrogen bonds with three water molecules (W19, W92 and W102). One of the oxygen atoms interacts with water molecule W92, which is hydrogen-bonded to N^{ξ} of Lys115, and water molecule W19, which forms a hydrogen bond network with residues Asp182, Thr321 and Lys348. The other oxygen atom of the α-carboxylate group interacts with water molecule W102, which interacts with N^{ξ} of Lys115 and water molecule W112 that is further hydrogen-bonded to the carboxyl group of Glu157. The carboxyl group of the D-Glu side chain is held in place by hydrogen bonds with Ser415 and Phe422. One of the D-Glu side chain carboxylic oxygen atoms in **9** forms hydrogen bonds with both the backbone nitrogen and the O^{γ} of Ser415, whereas the other oxygen atom interacts with the backbone nitrogen of Phe422 and also with water molecule W269. The latter is in direct contact with the amide nitrogen atom of the D-Glu moiety of the inhibitor, forming an extended ring-type system, and with water molecule W239, which interacts with the amino group of the linker between the two phenyl rings of **9**. Water molecule W239 is hydrogen-bonded also to water molecule W255 which, through interaction with the backbone carbonyl oxygen atom of Lys420, further stabilizes the inhibitor binding in the active site. The described linker modification of **I** to give **9** results in the different conformation of the middle part of the inhibitors (Figure S4). The benzylidene moiety of **9** is held in place by the hydrophobic interaction of the phenyl ring with Gly73, while the phenyl ring of the phenylamino moiety contributes to recognition of the inhibitor within the active site, with hydrophobic interactions to Leu416 and possible π-π interactions to Phe161. As observed in the case of the previously reported crystal structure of inhibitor **I** in complex with MurD,[5] the rhodanine moiety of **9** occupies the uracil-binding pocket and the rhodanine NH group forms a hydrogen bond with the O^{γ} of Thr36. Rhodanine moiety participates in inter-plane stacking, with a salt bridge formed between Asp35 and Arg37.

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