Supplementary Information

CoA Adducts of 4-Oxo-4-Phenylbut-2-enoates: Inhibitors of MenB from the *M. tuberculosis* Menaquinone Biosynthesis Pathway

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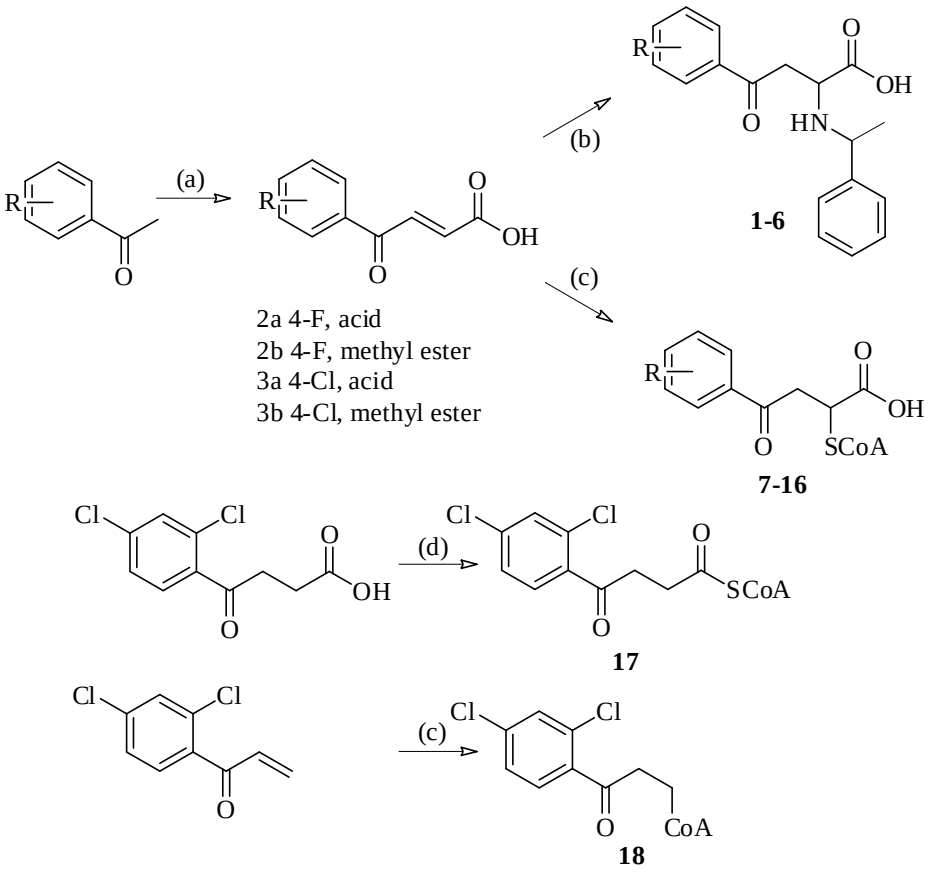
Scheme S1: Synthetic route for compounds 1-18



(a) acetophenone, glyoxylic acid monohydrate, acetic acid, reflux, overnight.[1] (b) (*S*)-(-)-methylbenzylamine, MeOH, 40°C, 3h.[2] (c) CoASH, pH 7.0 phosphate buffer, 25°C, 1h; (d) ethyl chloroformate, CoASH, pH 7.0 phosphate buffer, 25°C, 1h.

Figure S1: 2-Amino-4-aryl-4-oxo-butanoic acid analogues

These analogues were designed to modulate the stability of the parent molecules. **1S-6S** are stable in aqueous solution.

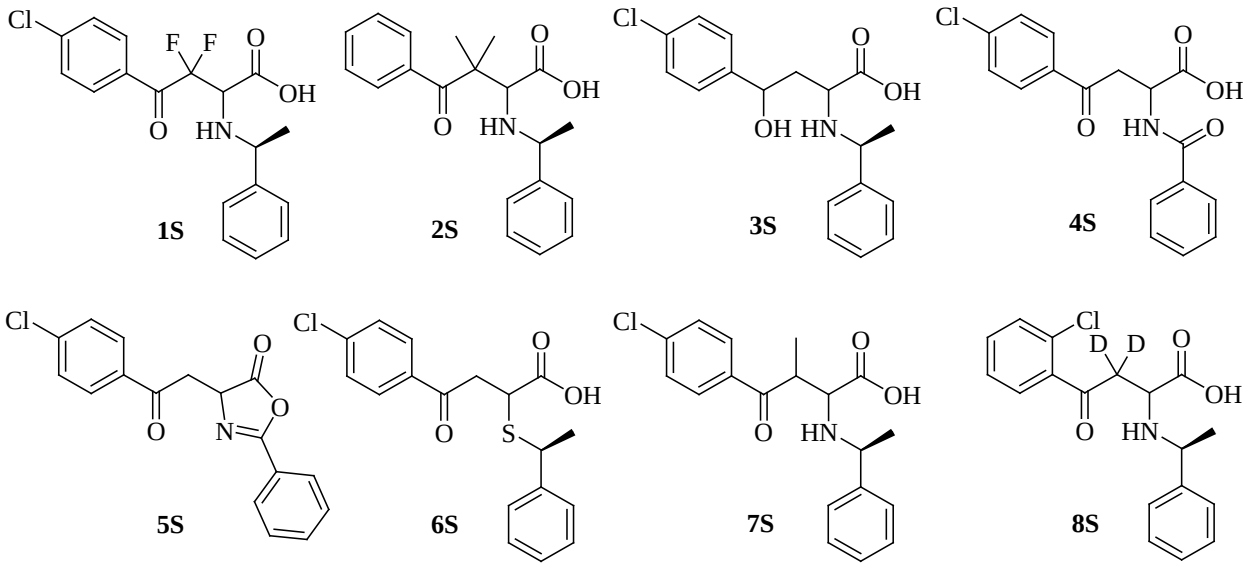


Figure S1: 2-Amino-4-aryl-4-oxo-butanoic acid analogues

A) Chemical synthesis

a) General procedures

Chemicals were purchased from commercial suppliers (Sigma-Aldrich, Acros Organics, Alfa Aesar) and used without further purification. (*E*)-4-oxo-4-phenyl-but-2-enoic acid, (*E*)-4-(4'-chlorophenyl)-4-oxo-but-2-enoic acid, and (*E*)-4-(4'-methoxylphenyl)-4-oxo-but-2-enoic acid were commercially available from Aldrich. All solvents for reaction and flash column chromatography were purchased from Fisher Scientific. ^{1}H-NMR spectra were recorded on Varian Gemini-2300 or Varian Inova-400 spectrometers. Mass spectra were recorded on an Agilent 1100 LS-MS electrospray ionization single quadrupole mass spectrometer. HPLC was performed on a Shimadzu instrument using a Phenomenex Luna 5µ C18(2) 100Å, 250×10.00 mm 5 micron column.

b) General procedure for the synthesis of (*E*)-4-aryl-4-oxo-but-2-enoic acid 1A-10A

The 1-arylethanone (1.0 mmol), glyoxylic acid monohydrate (1.3 mmol), and acetic acid (2.0 ml) were added to a round bottom flask equipped with a magnetic stir at room temperature. The mixture was heated to 130 °C and stirred under reflux overnight. After cooling to room temperature, the acetic acid was removed using a rotary evaporator. The crude product was purified by flash column chromatography on silica gel (EtOAc/Hexanes). Yields: 43-85%.

(*E*)-4-(4'-fluorophenyl)-4-oxo-but-2-enoic acid (1A)

1A was prepared according to the general procedure from 4'-fluoroacetonpheone. **1A** was obtained as light yellow liquid. ^{1}H-NMR (300MHz, Acetone-d_6) δ 8.22-8.17 (2H, m), 7.96 (1H, d, *J*=15.6Hz), 7.40-7.36 (2H, m), 6.79 (1H, d, *J*=15.6Hz). ESI-MS (Neg): found 193.0 $[M-H]^-$; calculated for $C_{10}H_7FO_3$: 194.0.

(*E*)-4-(2'-fluorophenyl)-4-oxo-but-2-enoic acid (2A)

2A was prepared according to the general procedure from 2'-fluoroacetonphenone and was obtained as a yellow solid. ^{1}H-NMR (300MHz, Acetone-d_6) δ 7.92-7.80 (1H, m), 7.80-7.62 (2H, m), 6.73 (1H, d, *J*=15.6Hz). ESI-MS (Neg): found 193.0 $[M-H]^-$; calculated for $C_{10}H_7FO_3$: 194.0.

(*E*)-4-(2'-chlorophenyl)-4-oxo-but-2-enoic acid (3A)

3A was prepared according to the general procedure from 2'-chloroacetonphenone and was obtained as a yellow solid. ^{1}H-NMR (400MHz, Chloroform-d_1) δ 7.65 (1H, d, *J*=16.0Hz), 7.56-7.52 (1H, m), 7.50-7.46 (1H, m), 7.43-7.37 (2H, m), 6.79 (1H, d, *J*=16.0Hz). ESI-MS (Neg): found 208.9 $[M-H]^-$; calculated for $C_{10}H_7ClO_3$: 211.0.

(*E*)-4-(2'-bromophenyl)-4-oxo-but-2-enoic acid (4A)

4A was prepared according to the general procedure from 2'-bromoacetonphenone and was obtained as a yellow solid. ^{1}H-NMR (300MHz, Acetone-d_6) δ 7.78-7.73 (1H, m), 7.63-7.48 (3H,

m), 7.39 (1H, d, *J*=15.6Hz), 6.53 (1H, d, *J*=15.6Hz). ESI-MS (Neg): 252.9 $[M-H]^-$; calculated for $C_{10}H_7BrO_3$: 254.0.

(*E*)-4-(2'-iodophenyl)-4-oxo-but-2-enoic acid (5A)

5A was prepared according to the general procedure from 2'-iodoacetonphenone and was obtained as a yellow solid. ^{1}H-NMR (300MHz, Acetone-d_6) δ 8.02 (1H, d, *J*=9.0Hz), 7.62-7.52 (2H, m), 7.42-7.28 (2H, m), 6.52 (1H, d, *J*=15.9). ESI-MS (Neg): found 300.9 $[M-H]^-$, calculated for $C_{10}H_7IO_3$: 301.9.

(*E*)-4-(2'-nitrophenyl)-4-oxo-but-2-enoic acid (6A)

6A was prepared according to the general procedure from 2'-nitroacetonphenone and was obtained as a yellow solid. ^{1}H-NMR (300MHz, Acetone-d_6) δ 8.25 (1H, d, *J*=9.0Hz), 8.00-7.92 (2H, m), 7.70 (1H, d, *J*=9.0Hz), 7.30 (1H, d, *J*=16.2Hz), 6.47 ((1H, d, *J*=16.2Hz). ESI-MS (Neg): found 220.0 $[M-H]^-$; calculated for $C_{10}H_7NO_5$: 221.0.

(*E*)-4-oxo-4-(2'-trifluoromethylphenyl)-but-2-enoic acid (7A)

7A was prepared according to the general procedure from 2'-trifuoromethylacetonphenone and was obtained as yellow liquids. ^{1}H-NMR (300MHz, Acetone-d_6) δ 7.92-7.66 (4H, m), 7.31 (1H, d, *J*=16.2Hz), 6.47 (1H, d, *J*=16.2Hz). ESI-MS (Neg): found 243.0 $[M-H]^-$; calculated for $C_{11}H_7F_3O_3$: 244.0.

(*E*)-4-(2'-methoxyphenyl)-4-oxo-but-2-enoic acid (8A)

8A was prepared according to the general procedure from 2'-methoxyacetonphenone and was obtained as a yellow solid. ^{1}H-NMR (300MHz, Acetone-d_6) δ 7.74 (1H, d, *J*=15.3Hz), 7.65-7.57 (2H, m), 7.22 (1H, d, *J*=9.0Hz), 7.07 (1H, t, *J*=7.5Hz), 6.62 (1H, d, *J*=15.9Hz), 3.96 (3H, s). ESI-MS (Neg): found 205.0 $[M-H]^-$; calculated for $C_{11}H_{10}O_4$: 206.1.

(*E*)-4-(3'-chlorophenyl)-4-oxo-but-2-enoic acid (9A)

9A was prepared according to the general procedure from 3'-chloroacetonphenone and was obtained as a yellow solid. ^{1}H-NMR (400MHz, Acetone-d_6) δ 8.06-8.03 (2H, m), 7.92 (1H, d, *J*=15.6Hz), 7.75-7.73 (1H, m), 7.66-7.64 (1H, m), 6.80 (1H, d, *J*=15.6Hz). ESI-MS (Neg): found 209.0 $[M-H]^-$; calculated for $C_{10}H_7ClO_3$: 210.0.

(*E*)-4-(2', 4'-dichlorophenyl)-4-oxo-but-2-enoic acid (10A)

10A was prepared according to the general procedure from 2', 4'-dichloroacetonphenone and was obtained as a yellow solid. ^{1}H-NMR (300MHz, DMSO-d_6) δ 7.79 (1H, d, *J*=2.1Hz), 7.67 (1H, d, *J*=8.1Hz), 7.62-7.56 (1H, m), 7.30 (1H, d, *J*=15.9Hz), 6.48 (1H, d, *J*=15.9Hz). ESI-MS (Neg): found 242.9 $[M-H]^-$; calculated for $C_{10}H_6Cl_2O_3$: 244.0.

c) General procedure for the synthesis of the 2-amino-4-aryl-4-oxobutanoic acids (1-6)

The (*E*)-4-aryl-4-oxo-but-2-enoic acid (1.0 mmol) was first added to a round bottom flask equipped with a magnetic stirrer bar at room temperature and under nitrogen. Primary amines (1.1 mmol) in 10 ml dry ethanol were added to the flask and the temperature was increased to 40-50^{o}C. The mixture was stirred for 4h and a white precipitate formed. The precipitate was obtained and filtration and recrystallized using 1:1 acetonitrile/H_2O (pH 1.0).

4-oxo-4-phenyl-2-((*S*)-1-phenylethylamino)butanoic acid (1)

Compound **1** was prepared according to the general procedure from commercially available (*E*)-4-oxo-4-phenyl-but-2-enoic acid and was obtained as a white solid. ^{1}H-NMR (300MHz, 1% DCl in Acetone-d_6) δ 7.99 (2H, d, *J*=9.0Hz), 7.70 (2H, d, *J*=9.0Hz), 7.63 (1H, t, *J*=7.5Hz), 7.55-7.42 (5H, m), 4.97 (1H, q, *J*=7.2Hz), 4.16 (1H, t, *J*=5.0Hz), 4.10-3.96 (2H, m), 1.92 (3H, d, *J*=7.2Hz). ESI-MS (Pos): found 298.1 $[M+H]^+$; calculated for $C_{18}H_{19}NO_3$: 297.1.

4-(4'-fluorophenyl)-4-oxo-2-((*S*)-1-phenylethylamino)butanoic acid (2)

Compound **2** was prepared according to the general procedure from (*E*)-4-(4'-fluorophenyl)-4-oxo-but-2-enoic acid (**1A**) and was obtained as a white solid. ^{1}H-NMR (300MHz, 1% DCl in Acetone-d_6) δ 8.22-8.12 (2H,m), 7.87-7.77 (2H, m), 7.54-7.42 (3H, m), 7.32-7.22 (2H, m), 4.94 (1H, q, *J*=6.8Hz), 4.20-3.98 (3H, m), 1.90 (3H, d, *J*=6.9Hz). ESI-MS (Pos): found 316.1 $[M+H]^+$; calculated for $C_{18}H_{18}FNO_3$: 315.1.

4-(4'-chlorophenyl)-4-oxo-2-((*S*)-1-phenylethylamino)butanoic acid (3)

Compound **3** was prepared according to the general procedure from commercially available (*E*)-4-(4'-chlorophenyl)-4-oxo-but-2-enoic acid and was obtained as a white solid. ^{1}H-NMR (300MHz, 1% DCl in Acetonitrile-d_3) δ 7.96 (2H, d, *J*=7.2Hz), 7.50-7.35 (5H, m), 7.08 (2H, d, *J*=7.2Hz), 4.78 (1H, q, *J*=7.2Hz), 4.13 (1H, t, *J*=6.0Hz), 3.82 (2H, d, *J*=6.9Hz), 1.87 (3H, d, 7.2Hz). ESI-MS (Pos): found 332.0 $[M+H]^+$; calculated for $C_{18}H_{18}ClNO_3$: 331.1.

4-(2'-chlorophenyl)-4-oxo-2-((*S*)-1-phenylethylamino)butanoic acid (4)

Compound **4** was prepared according to the general procedure from (*E*)-4-(2'-chlorophenyl)-4-oxo-but-2-enoic acid (**3A**) and was obtained as a white solid. ^{1}H-NMR (400MHz, 1% DCl in Acetone-d_6) δ 7.91-7.89 (1H, m), 7.83-7.81 (2H, m), 7.58-7.40 (6H, m), 4.93 (1H, q, *J*=7.2Hz), 4.12 (1H, t, *J*=5.2Hz), 4.00 (2H, d, *J*=5.6Hz), 1.91 (3H, d, *J*=6.8Hz). ESI-MS (Pos): found 332.0 $[M+H]^+$; calculated for $C_{18}H_{18}ClNO_3$: 331.1.

4-oxo-2-((*S*)-1-phenylethylamino)-4-(2'-(trifluoromethyl)phenyl)butanoic acid (5)

Compound **6** was prepared according to the general procedure from (*E*)-4-oxo-4-(2'-trifluoromethylphenyl) -but-2-enoic acid (**7A**) and was obtained as a white solid. ^{1}H-NMR (300MHz, 1% DCl in Acetone-d_6) δ 8.25 (1H, d, *J*=7.5Hz, 7.92-7.68 (5H, m), 7.56-7.40 (3H, m),

4.97 (1H, q, *J*=7.2Hz), 4.20 (1H, dd, *J*=4.5, 9.0Hz), 4.17 (1H, t, *J*=2.7Hz), 3.90 (1H, dd, *J*=4.5, 9.0Hz), 1.93 (3H, d, *J*=7.5Hz). ESI-MS (Pos): found 366.1 $[M+H]^+$; calculated for $C_{19}H_{18}F_3NO_3$: 365.1.

4-(2'-methoxyphenyl)-4-oxo-2-((*S*)-1-phenylethylamino)butanoic acid (6)

Compound **6** was prepared according to the general procedure from (*E*)-4-(2'-methoxyphenyl)-4-oxo-but-2-enoic acid (**8A**) and was obtained as a white solid. ^{1}H-NMR (300MHz, 1% DCl in Acetone-d_6) δ 7.85-7.74 (3H, m), 7.60-7.40 (4H, m), 7.17 (1H, d, *J*=7.8Hz), 7.02 (1H, t, *J*=7.5Hz), 4.94 (1H, q, 6.9Hz), 4.13 (1H, t, *J*=6.3Hz), 4.03-3.98 (2H, m), 3.95 (3H, s), 1.92 (3H, d, *J*=7.2Hz). ESI-MS (Pos): found 328.1 $[M+H]^+$; calculated for $C_{19}H_{21}NO_4$: 327.1.

d) General procedure for the synthesis of the 2-CoA-4-aryl-4-oxobutanoic acids (7-16, 18)

The (*E*)-4-aryl-4-oxo-but-2-enoic acid (1.0 mmol) was first added to a round bottom flask equipped with a magnetic stirrer bar at room temperature and under nitrogen. CoA (1.1 mmol) in 10 ml dd-H_2O was added to the flask and the mixture was stirred for 2h at room temperature. The product was purified by HPLC using 20 mM NH_4OAc in water as solvent A and acetonitrile as solvent B. The HPLC gradient was: 0-40min, B% 0-40%; 40-50min, B% 40-100%.

4-(4'-chlorophenyl)-2-CoA-4-oxo-butanoic acid (7)

Compound **7** was prepared according to the general procedure from commercially available (*E*)-4-(4'-chlorophenyl)-4-oxo-but-2-enoic acid and was obtained as a white solid. ^{1}H-NMR (300MHz, in D_2O) δ 8.36 (1H, s), 8.09 (1H, s), 7.96 (2H, d, *J*=7.2Hz), 7.08 (2H, d, *J*=7.2Hz), 6.01 (1H, d, *J*=6.0Hz), 4.42-4.40 (1H, m), 4.11-4.09 (2H, m), 3.88 (1H, s), 3.74-3.62 (1H, m), 3.56-3.50 (1H, m), 3.47-3.04 (9H, m), 2.75-2.50 (2H, m), 2.35 (2H, t, *J*=6.9Hz). 0.74 (3H,s), 0.62 (3H, s). ESI-MS (Neg): 976.2 $[M-H]^-$; calculated for $C_{31}H_{43}ClN_7O_{19}P_3S$: 977.1.

2-CoA-4-(4'-methoxyphenyl)-4-oxo-butanoic acid (8)

Compound **8** was prepared according to the general procedure from commercially available (*E*)-4-(4'-methoxyphenyl)-4-oxo-but-2-enoic acid and was obtained as a white solid. ^{1}H-NMR (300MHz, in D_2O) δ 8.30 (s, 1H), 7.98 (s, 1H), 7.67 (2H, d, *J*=8.1Hz), 6.75 (2H, d, *J*=8.1Hz), 5.91 (1H, d, *J*=6.0Hz), 4.40 (1H, s), 4.07 (2H, s), 3.82 (1H, s), 3.68 (3H, s), 3.67-3.62 (1H, m), 3.53 (1H, t, *J*=6.6Hz), 3.42-3.04 (9H, m), 2.64-2.54 (2H, m), 2.25 (2H, t, *J*=6.0Hz), 0.69 (3H, s), 0.56 (3H, s). ESI-MS (Neg): 972.2 $[M-H]^-$; calculated for $C_{32}H_{46}N_7O_{20}P_3S$: 973.1.

2-CoA-4-(2'-fluorophenyl)-4-oxo-butanoic acid (9)

Compound **9** was prepared according to the general procedure from (*E*)-4-(2'-fluorophenyl)-4-oxo-but-2-enoic acid (**2A**) and was obtained as a white solid. ^{1}H-NMR (300MHz, in D_2O) δ 8.33 (1H, s), 8.02 (1H, s), 7.70-7.40 (2H, m), 7.20-7.00 (2H, m), 5.95 (1H, q, *J*=3.0Hz), 4.44-4.38 (1H, m), 4.10-4.02 (2H, m), 3.83 (1H, s), 3.70-3.60 (1H, m), 3.58-3.50 (1H, m), 3.44-3.06 (9H,

m), 2.64-2.56 (2H, m), 2.27 (2H, d, *J*=6.6Hz), 0.71 (3H, s), 0.57 (3H, s). ESI-MS (Neg): 960.1 [M-H]$^-$; calculated for $C_{31}H_{43}FN_7O_{19}P_3S$: 961.1.

4-(2'-chlorophenyl)-2-CoA-4-oxo-butanoic acid (10)

Compound **10** was prepared according to the general procedure from (*E*)-4-(2'-chlorophenyl)-4-oxo-but-2-enoic acid (**3A**) and was obtained as a white solid. ^{1}H-NMR (300MHz, in D_2O) δ 8.38 (1H, s), 8.08 (1H, s), 7.45-7.40 (1H, m), 7.31-7.20 (3H, m), 5.99 (1H, d, *J*=6.0Hz), 4.45-4.42 (1H, m), 4.12-4.09 (2H, m), 3.86 (1H, s), 3.69 (1H, q, *J*=4.8Hz), 3.54 (1H, dd, *J*=2.4, 6.0Hz), 3.40-3.10 (9H, m), 2.63-2.52 (2H, m), 2.29 (2H, d, *J*=6.6Hz), 0.73 (3H, s), 0.59 (3H, s). ESI-MS (Neg): 976.1 [M-H]$^-$; calculated for $C_{31}H_{43}ClN_7O_{19}P_3S$: 977.1.

4-(2'-bromophenyl)-2-CoA-4-oxo-butanoic acid (11)

Compound **11** was prepared according to the general procedure from (*E*)-4-(2'-bromophenyl)-4-oxo-but-2-enoic acid (**4A**) and was obtained as a white solid. ^{1}H-NMR (300MHz, in D_2O) δ 8.38 (1H, s), 8.07 (1H, s), 7.46 (1H, d, *J*=7.8Hz), 7.38 (1H, d, *J*=6.0Hz), 7.30-7.15 (2H, m), 5.99 (1H, d, *J*=5.4Hz), 4.44 (1H, s), 4.10 (2H, s), 3.86 (1H, s), 3.74-3.62 (1H, m), 3.58-3.48 (1H, m), 3.44-3.06 (9H, m), 2.65-2.50 (2H, m), 2.29 (2H, t, *J*=6.9Hz), 0.73 (3H, s), 0.59 (3H, s). ESI-MS (Neg): 1020.1 [M-H]$^-$; calculated for $C_{31}H_{43}BrN_7O_{19}P_3S$: 1021.0.

2-CoA-4-(2'-iodophenyl)-4-oxo-butanoic acid (12)

Compound **12** was prepared according to the general procedure from (*E*)-4-(2'-iodophenyl)-4-oxo-but-2-enoic acid (**5A**) and was obtained as a white solid. ^{1}H-NMR (300MHz, in D_2O) δ 8.31 (1H, s), 7.99 (1H, s), 7.70 (1H, d, *J*=7.5Hz), 7.38-7.32 (1H, m), 7.30-7.20 (1H, m), 7.04-6.94 (1H, m), 5.94 (1H, d, *J*=6.3Hz), 4.40 (1H, s), 4.06 (2H, s), 3.82 (1H, s), 3.64 (1H, q, *J*=4.8Hz), 3.48 (1H, dd, *J*=2.1, 6.3Hz), 3.42-2.98 (9H, m), 2.62-2.50 (2H, m), 2.25 (2H, t, *J*=6.3), 0.69 (3H, s), 0.54 (3H, s). ESI-MS (Neg): 1068.0 [M-H]$^-$; calculated for $C_{31}H_{43}IN_7O_{19}P_3S$: 1069.0.

2-CoA-4-(2'-nitrophenyl)-4-oxo-butanoic acid (13)

Compound **13** was prepared according to the general procedure from (*E*)-4-(2'-nitrophenyl)-4-oxo-but-2-enoic acid (**6A**) and was obtained as a brown solid. ^{1}H-NMR (300MHz, in D_2O) δ 8.36 (1H, s), δ 8.25-8.20 (1H, m), 8.08 (1H, s), 8.00-7.92 (2H, m), 7.70-7.62 (1H, m), 6.01 (1H, d, *J*=6.3Hz), 4.46 (1H, s), 4.10 (2H, s), 3.85 (1H, s), 3.68-3.64 (1H, m), 3.52-3.46 (1H, m), 3.42-3.06 (9H, m), 2.62-2.52 (2H, m), 2.27 (2H, d, *J*=6.6Hz), 0.72 (3H, s), 0.60 (3H, s). ESI-MS (Neg): 987.1 [M-H]$^-$; calculated for $C_{31}H_{43}N_8O_{21}P_3S$: 988.1.

2-CoA-4-(2'-methoxyphenyl)-4-oxo-butanoic acid (14)

Compound **14** was prepared according to the general procedure from (*E*)-4-(2'-methoxyphenyl)-4-oxo-but-2-enoic acid (**8A**) and was obtained as a white solid. ^{1}H-NMR (300MHz, in D_2O) δ 8.36 (1H, s), 8.05 (1H, s), 7.50-7.35 (2H, m), 7.00-6.80 (2H, m), 6.97 (1H, d, *J*=6.0Hz), 4.44 (1H, s), 4.11 (2H, s), 3.85 (1H, s), 3.72 (3H, s), 3.70-3.62 (1H, m), 3.56-3.46

(1H, m), 3.44-3.04 (9H, m), 2.70-2.50 (2H, m), 2.27 (2H, t, *J*=6.6Hz), 0.72 (3H, s), 0.58 (3H, s). ESI-MS (Neg): 972.1 $[M-H]^-$; calculated for $C_{32}H_{46}N_7O_{20}P_3S$: 973.1.

4-(3'-chlorophenyl)-2-CoA-4-oxo-butanoic acid (15)

Compound **14** was prepared according to the general procedure from (*E*)-4-(3'-chlorophenyl)-4-oxo-but-2-enoic acid (**9A**) and was obtained as a white solid. ^{1}H-NMR (300MHz, in D_2O) δ 8.29 (1H, s), 7.97 (1H, s), 7.66-7.56 (2H, m), 7.40-7.33 (1H, m), 7.28-7.16 (1H, m), 5.91 (1H, d, *J*=6.0Hz), 4.39 (1H, s), 4.06 (2H, s), 3.82 (1H, s), 3.68-3.60 (1H, m), 3.58-3.48 (1H, m), 3.44-3.06 (9H, m), 2.66-2.56 (2H, m), 2.30-2.20 (2H, m), 0.68 (3H, s), 0.55 (3H, s). ESI-MS (Neg): 976.1 $[M-H]^-$; calculated for $C_{31}H_{43}ClN_7O_{19}P_3S$: 977.1.

2-CoA-4-(2',4'-dichlorophenyl)-4-oxo-butanoic acid (16)

Compound **16** was prepared according to the general procedure from (*E*)-4-(2',4'-dichlorophenyl)-4-oxo-but-2-enoic acid (**10A**) and was obtained as a white solid. ^{1}H-NMR (300MHz, in D_2O) δ 8.35 (1H, s), 8.04 (1H, s), 7.41-7.38 (1H, m), 7.33-7.30 (1H, m), 7.22-7.16 (1H, m), 5.97 (1H, d, *J*=6.0Hz), 4.43 (1H, s), 4.09 (1H, s), 3.85 (1H, s), 3.72-3.62 (1H, m), 3.56-3.47 (1H, m), 3.44-3.06 (9H, m), 2.64-2.54 (2H, m), 2.28 (2H, t, *J*=6.6Hz), 0.72 (3H, s), 0.58 (3H, s). ESI-MS (Neg): 1010.1 $[M-H]^-$; calculated for $C_{31}H_{42}Cl_2N_7O_{19}P_3S$: 1011.1.

3-CoA-1-(2,4-dichlorophenyl)propan-1-one (18)

Compound **18** was prepared according to the general procedure from 1-(2,4-dichlorophenyl)prop-2-en-1-one[3] and was obtained as a white solid. ^{1}H-NMR (300MHz, in D_2O) δ 8.34 (1H, s), 8.02 (1H, s), 7.40-7.10 (4H, m), 5.96 (1H, d, *J*=5.7Hz), 4.42 (1H, s), 4.09 (2H, s), 3.86 (1H, s), 3.67 (1H, q, *J*=5.1Hz), 3.39 (1H, q, *J*=5.1Hz), 3.29 (2H, t, *J*=6.6Hz), 3.17 (2H, t, *J*=6.6Hz), 3.11 (2H, t, *J*=6.6Hz), 2.59 (2H, t, *J*=6.6Hz), 2.50 (2H, t, *J*=6.6Hz), 2.27 (2H, t, *J*=6.6Hz). ESI-MS (Neg): 962.1 $[M-H]^-$; calculated for $C_{30}H_{42}Cl_2N_7O_{17}P_3S$: 963.1.

e) General procedure for the synthesis OSB-CoA thioester analogues

The 4-aryl-4-oxo-butanoic acid (0.5mmol), triethylamine (1.0mmol), and 10mL anhydrous THF were added to a round bottom flask equipped with a magnetic stirrer bar at room temperature and under nitrogen. Ethyl chloroformate (0.55mmol) was added into the mixture drop by drop over 30min on ice bath. The mixture was stirred for 2h and the formed precipitate was removed by filtration. The CoA (1.05mmol) in pH 7.0 phosphate buffer was added into the filtrate and the mixture was stirred at room temperature for 45min. The product was purified by HPLC using 20 mM NH_4OAc in water as solvent A and acetonitrile as solvent B. The HPLC gradient was: 0-40min, B% 0-40%; 40-50min, B% 40-100%.

4-(2,4-dichlorophenyl)-4-oxo-butanote-CoA thioester (17)

Compound **17** was prepared according to the general procedure from 4-(2,4-dichlorophenyl)-4-oxo-butanoic acid. Compound **17** was obtained as a white powder. ^{1}H-NMR (300MHz, in D_2O)

δ 8.33 (1H, s), 8.00 (1H, s), 7.50-7.15 (3H, m), 5.94 (1H, d, *J*=6.3Hz), 4.42 (1H, s), 4.06 (2H, s), 3.86 (1H, s), 3.72-64 (1H, m), 3.51-3.45 (1H, m), 3.41-3.35 (2H, m), 3.30-3.22 (2H, t, *J*=6.0Hz), 3.18-3.10 (2H, m), 3.06 (2H, t, *J*=6.6Hz), 2.92-2.80 (2H, m), 2.40 (4H, t, *J*=6.6Hz), 2.24 (2H, t, *J*=6.6Hz), 0.72 (3H, s), 0.57 (3H, s). ESI-MS (Neg): 994.1 $[M-H]^-$; calculated for $C_{31}H_{42}Cl_2N_7O_{18}P_3S$: 995.1.

f) General procedure for the synthesis of the methyl (*E*)-4-aryl-4-oxo-but-2-enoates

The (*E*)-4-aryl-4-oxo-but-2-enoic acid (1.0 mmol), K_2CO_3 (2.0 mmol), MeI (2.0 mmol), and 10 ml anhydrous DMF were added to a round bottom flask equipped with a magnetic stirrer bar at room temperature and under nitrogen. The mixture was stirred at room temperature for 2h and subsequently washed with saturated $NaHCO_3$ solution. The crude product was purified by flash chromatography on silica gel (EtOAc/Hexanes). Yields: >95%.

(*E*)-methyl 4-(4-fluorophenyl)-4-oxobut-2-enoate 2b

Compound **2b** was prepared according to the general procedure from (*E*)-4-(4-fluorophenyl)-4-oxobut-2-enoic acid. Compound **2b** was obtained as a light yellow powder. ^{1}H-NMR (300MHz, in $CDCl_3$) δ 8.35 (2H, d, *J*=9.0Hz), 8.13 (2H, d, *J*=9.0Hz), 7.87(1H, d, *J*=15.6Hz), 6.93 (1H, d, *J*=15.6Hz), 3.85 (3H, s).

(*E*)-methyl 4-(4-chlorophenyl)-4-oxobut-2-enoate 3b

Compound **3b** was prepared according to the general procedure from (*E*)-4-(4-chlorophenyl)-4-oxobut-2-enoic acid. Compound **3b** was obtained as a light yellow powder. ^{1}H-NMR (300MHz, in $CDCl_3$) δ 7.65 (1H, d, *J*=15.9Hz), 7.45 (2H, d, *J*=8.7Hz), 7.27 (2H, d, *J*=8.7Hz), 6.70 (1H, d, *J*=15.9Hz), 3.84 (3H, s).

B) Biological assays

a) Expression and purification of *M. tuberculosis* MenB and *E. Coli* MenE

MenB and MenE were expressed and purified essentially as described previously.[4] Briefly, BL21(DE3)pLysS cells transformed with the relevant plasmids were grown in 800 ml LB media containing 0.03 mg/ml ampicillin at 37 ^{o}C. Once the OD value at 600 nm reached 0.8-1.2, protein expression was induced by addition of 0.15 g isopropyl β-D-1-thiogalactopyranoside. After incubating for a further 12 h at 25^{o}C, cells were harvested by centrifugation at 5000 rpm for 15 min at 4 ^{o}C, and proteins were purified using His-Tag column chromatography. Cells were first resuspended in 30 ml of His-bind buffer (20 mM Tris:HCl, 5 mM imidazole, 500 mM NaCl, pH 7.90), lysed by 4-5 passages through a French Press cell, and cell debris removed by centrifuging at 8600 rpm for 2h at 4 ^{o}C. The supernatant was loaded onto a His-Tag resin column (1x5 cm) which was subsequently washed with 30 ml His-bind buffer, and then with 50 ml His-wash buffer (20 mM Tris:HCl, 60 mM imidazole, 500 mM NaCl, pH 7.90). Proteins were then eluted using His-elute buffer (20 mM Tris:HCl, 500 mM imidazole, 500 mM NaCl, pH 7.90). Fractions

containing MenB or MenE were collected and imidazole was removed by chromatography on G-25 (1x50 cm) using 20 mM Na_2HPO_4, 100 mM NaCl pH 7.0 buffer as the eluent. The concentration of MenB and MenE were determined by measuring the absorption at 280 nm and by using extinction coefficients of 41,370 $M^{-1}cm^{-1}$ and 104,770 $M^{-1}cm^{-1}$, respectively.

b) The MenE-MenB coupled-assay

The MenB-catalyzed formation of DHNA-CoA was evaluated using a coupled assay in which OSB-CoA, the substrate for MenB, was synthesized *in situ* using MenE.[4] Reactions were performed at 25 ^oC in 20 mM NaH_2PO_4 pH 7.0 buffer containing 150 mM NaCl and 1 mM $MgCl_2$. Reactions were initiated by addition of MenB (100-150 nM) to solutions containing 60 μM OSB, 120 μM ATP, 120 μM CoA and 5 μM MenE. Product formation was monitored at 392 nm using a Cary-100 spectrophotometer.

c) Inhibition kinetics

A coupled assay was used to determine IC_{50} values for MenB inhibition. Inhibitors were first dissolved in 0.5% HCl/H_2O and then in acetonitrile. Compound inhibition was evaluated either without preincubation or after preincubating the compounds with MenB, CoA, ATP and OSB for 1 h. In each case the reaction was initiated by addition of MenE. The dependence of the observed initial velocities on the concentration of inhibitor were analyzed using equation **1**, where [I] is the inhibitor concentration and y is percent activity.

$$y = 100\%/[1 + (I/IC_{50})] \qquad \mathbf{1}$$

Inhibition constants were determined as a function of OSB-CoA concentration as follows. Initial velocities were obtained from reaction mixtures containing ATP (120 μM), CoA (120 μM), MenE (2 μM) and MenB (150 nM) at several fixed concentrations of inhibitor (0-4350 nM) and by varying the concentration of OSB from 7.5 to 90 μM. Under these conditions it is assumed that the concentration of OSB-CoA formed through the action of MenE is equal to the OSB concentration. For compound **7** where K_i > [E] data were analyzed by the standard double reciprocal plots clearly showing that inhibition was noncompetitive (**Figure S2**).

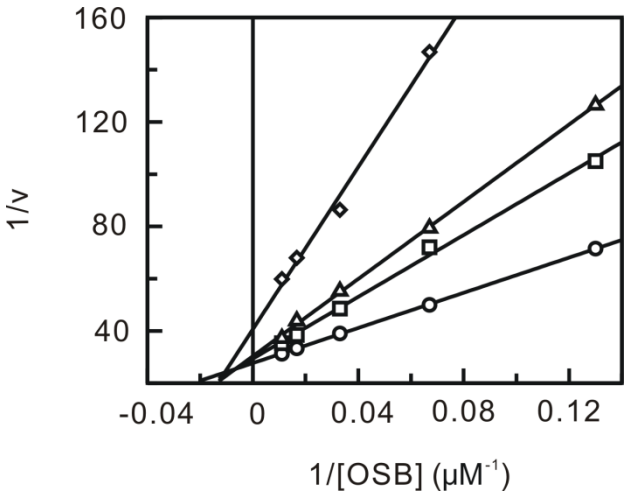


Figure S2: Lineweaver-Burk plot for the inhibition of MenB by 7.

Inhibitor concentrations 0 (○), 380 nM (□), 570 nM (Δ) and 1275 nM (◊).

Data were also fit to equation **5** for comparison with **16** (**Figure S3**). For **16**, which is a tight binding inhibitor, K_i^{app} and [E] were determined by fitting the data to equation **2** using Grafit 4.0, where v_i and v_0 are the initial velocities in the presence and absence of inhibitor, and K_i^{app} is the apparent dissociation constant.

$$\frac{v_i}{v_0} = 1 - \frac{([E]+[I]+K_i^{app}) - \sqrt{([E]+[I]+K_i^{app})^2 - 4[E][I]}}{2[E]} \qquad \mathbf{2}$$

Values of K_i^{app} and [E] obtained from equation **2** were then fit to equations **3**, **4** and **5** which describe the relationship between K_i^{app} and the inhibition constants for competitive, uncompetitive, and noncompetitive inhibition.[5]

$$K_i^{app} = K_i\left(1 + \frac{[S]}{K_m}\right) + \frac{1}{2}[E] \qquad \mathbf{3}$$

$$K_i^{app} = K_i'\left(1 + \frac{K_m}{[S]}\right) + \frac{1}{2}[E] \qquad \mathbf{4}$$

$$K_i^{app} = \frac{[S]+K_m}{\left(\frac{K_m}{K_i}\right)+([S]/K_i')} + \frac{1}{2}[E] \qquad \mathbf{5}$$

Results of data fitting for **16** are shown in **Figure S4** where it can be seen that the best fit is obtained with equation **5** with values of K_i and K_i‘ of 49±6 and 286±7 nM, respectively and [E]= 125 nM, close to [E] we added to the assay (150 nM). The data can also be fit using equation **3** for competitive inhibition, but this gave [E] = 250 nM. In addition, **7** is clearly a noncompetitive inhibitor of MenB and so we conclude that **16** is also a noncompetitive inhibitor.

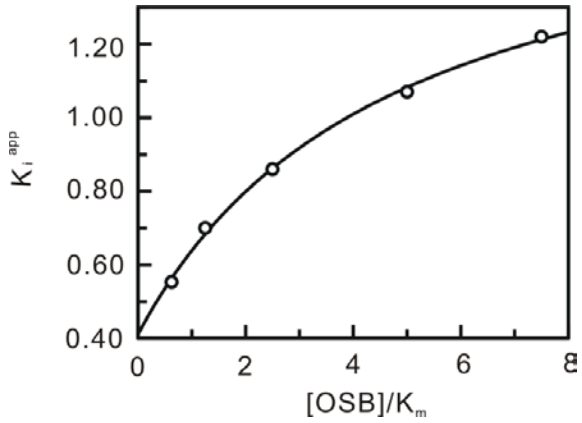


Figure S3: Effect of [OSB] on K_i^{app} for 7. Data fit to equation **5** with K_i = 350±85 µM, K_i' = 1660±50 nM and [E]=120 nM.

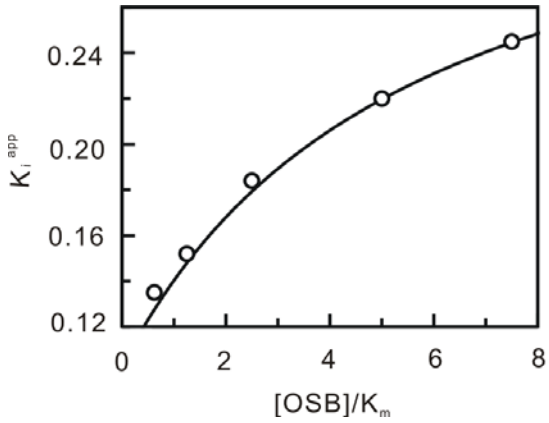


Figure S4: Effect of [OSB] on K_i^{app} for 16. Data have been it to equation **5** with K_i = 49±6 nM, K_i' = 286±7 nM and [E]=125 nM.

d) Antibacterial and Cytotoxicity Assays

MIC data were acquired using *M. tuberculosis* H37Rv essentially as described previously using the microplate dilution assay.[6] Briefly, bacterial cells were grown to early-mid log phase in Middlebrook 7H9 liquid medium containing 10% OADC enrichment and 0.05% Tween-80. Fifty µl of bacteria were added to the test wells and compounds were added individually to a final volume of 100 µL per well in 2 fold serial dilutions. Each drug dilution series was performed in triplicate. Plates were incubated at 37°C for 5-7 days and each well was evaluated for growth. AlamarBlue® was used as a growth indicator. The MIC was the lowest drug concentration that maintained a blue color in all replicates. A blue color in the alamarBlue® Assay indicates no bacterial growth whereas a red color in the assay was indicative of cell growth (BioSource International, Inc). Similar experiments were performed in the presence of varying concentrations of 1,4-dihydroxy-2-naphthoic acid (DHNA). DHNA is known to be unstable in aerated liquid media and 100 µg/ml of DHNA was required to rescue growth in the presence of 2xMIC compounds **2b** and **3b** (1.2 µg/ml).[7] Cytotoxicity was determined using African green monkey kidney cells (Vero cells) exactly as described in Boyne et al.[6] The low oxygen recovery assay for NRP-MTB was performed on *M. tuberculosis* H37Rv at the Institute for Tuberculosis Research, Chicago (Dr. S. Franzblau).[8]

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