**Supporting Information**

# Carboxylate Surrogates Enhance Antimycobacterial Activity of UDP-Galactopyranose Mutase Probes

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**Contents:**

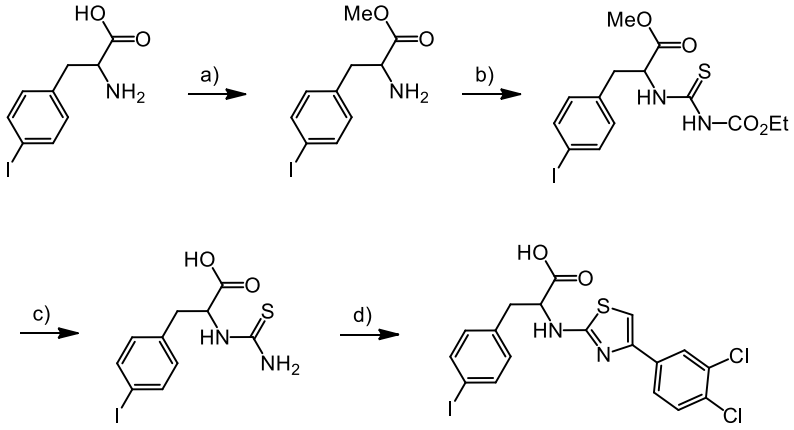
**General procedures and materials:**
All experiments were carried out in flame-dried glassware under nitrogen unless otherwise noted. Reagents were obtained via commercial sources without further purification, except for solvents. Dichloromethane (DCM) and diisopropylethylamine (DIEA) were distilled from calcium hydride. Tetrahydrofuran (THF) and toluene were distilled from sodium/benzophenone. Methanol (MeOH) was distilled from magnesium. Anhydrous dimethylformamide (DMF) was obtained from Sigma-Aldrich and used without further purification.

Analytical thin layer chromatography (TLC) was carried out on TLC silica gel 60 $F_{254}$. Visualization of the TLC plates was performed with UV-visualization and *p*-anisaldehyde stain. Flash chromatography was performed on Silacycle Silaflash P60 silica gel.

Compound identity and purity were determined using NMR analysis. All bioactive compounds were determined to have ≥ 90% purity. NMR spectra were obtained on a Bruker Avance-400 (400 MHz), or Bruker Avance-500DCH (500 MHz) instrument. Chemical shifts are reported relative to residual solvent signals ($CDCl_3$): $^1$H: 7.27, $^{13}$C: 77.23; ($CD_3OD$): $^1$H: 3.31, $^{13}$C: 49.15.

**Synthetic procedures:**

**Scheme S1**. Synthesis of inhibitor **1**:




Reagents and conditions: (a) thionyl chloride, methanol; (b) ethyl carboxyisothiocyanate; (c) 1M sodium hydroxide, methanol; (d) 2-bromo-3',4'-dichloroacetophenone, dimethylformamide

*Methyl 2-amino-3-(4-iodophenyl)propanoate*. A solution of 4-iodo-DL-phenylalanine (150 mg, 0.515 mmol) in methanol (3 mL) was cooled to 0°C and thionyl chloride (300 µL, 4.12 mmol) was added dropwise. The mixture was heated at reflux for 4 hours, then cooled and concentrated *in vacuo* to yield the hydrochloride salt in quantitative yield (176 mg, 0.515 mmol). $^1$H NMR (500 MHz, Methanol-$d_4$) δ 7.73 (d, $J$ = 7.8 Hz, 2H), 7.11 (d, $J$ = 7.9 Hz, 2H), 4.36 (dd, 1H), 3.82 (s, 3H), 3.25 (dd, $J$ = 15.7, 7.4 Hz, 2H), 3.20 (dd, $J$ = 15.7, 7.4 Hz, 1H). $^{13}$C NMR (126 MHz, MeOD) δ 168.83, 137.92, 133.87, 131.32, 92.81, 53.62, 52.45, 35.45. MS (ESI$^+$ *m/z*): (M-Cl) 305.9978 (M+H calc'd 305.9986)

*Methyl 2-(3-(ethoxycarbonyl)thioureido)-3-(4-iodophenyl)propanoate*. Methyl 2-amino-3-(4-iodophenyl)propanoate (176 mg, 0.515 mmol) was dissolved in dichloromethane (1.3 mL) and diisopropylethylamine (270 µL). The solution was cooled to 0°C and ethoxycarbonyl isothiocyanate (67 µL, 0.566 mmol) was added dropwise. After warming to room temperature, the mixture was stirred for 45 minutes. Dichloromethane (7 mL) was added, and the organic layer was washed with water (7 mL), 10% aqueous HCl (7 mL), and saturated brine (7 mL) then dried over $Na_2SO_4$ and concentrated *in vacuo* to afford the product as a yellow oil (236 mg, 0.515 mmol) in quantitative yield. $^1$H NMR (500 MHz, Chloroform-*d*) δ 10.12 (s, 1H), 7.61 (d, *J* = 8.2 Hz, 2H), 6.92 (d, *J* = 8.2 Hz, 2H), 5.27 (dd, *J* = 6.1 Hz, 1H), 4.22 (q, *J* = 7.1 Hz, 2H), 3.73 (s, 3H), 3.29 (dd, *J* = 14.0, 5.9 Hz, 1H), 3.17 (dd, *J* = 14.0, 6.0 Hz, 1H), 1.30 (t, *J* = 7.1 Hz, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 179.23, 170.55, 152.56, 137.65, 135.19, 131.22, 92.89, 77.46, 77.21 ($CDCl_3$), 76.95, 62.92, 58.82, 52.60, 36.72, 14.18. MS (ESI$^+$ *m/z*): (M+H) 437.0025 (M+H calc'd 437.0027)

*3-(4-Iodophenyl)-2-thioureidopropanoic acid*. Methyl 2-(3-(ethoxycarbonyl)thioureido)-3-(4-iodophenyl)propanoate (236 mg, 0.515 mmol) was dissolved in a 1:1 solution of 1 M NaOH and MeOH (3 mL). The solution was heated to reflux for 30 min, then cooled and acidified to a pH of 3 with 10% HCl. The solution was extracted with EtOAc (3 x 15 mL), and the combined organic layers were dried with $Na_2SO_4$. The solvent was removed *in vacuo* to yield the thiourea as a white solid in quantitative yield (191 mg, 0.515 mmol). $^1$H NMR (500 MHz, Methanol-$d_4$) δ 7.51 (d, *J* = 8.2 Hz, 2H), 6.90 (d, *J* = 8.1 Hz, 2H), 5.09 (dd, *J* = 5.3 Hz, 1H), 3.17 (dd, *J* = 13.9, 5.5 Hz, 1H), 2.94 (dd, *J* = 13.9, 6.5 Hz, 1H). $^{13}$C NMR (126 MHz, MeOD) δ 183.30, 173.18, 137.20, 136.41, 91.59, 57.97, 36.84. MS (ESI$^-$ *m/z*): (M-H) 348.9521 (M-H calc'd 348.9513)

*2-((4-(3,4-Dichlorophenyl)thiazol-2-yl)amino)-3-(4-iodophenyl)propanoic acid* (**1**). 3-(4-Iodophenyl)-2-thioureidopropanoic acid (500 mg, 1.43 mmol) and 2-bromo-3',4'-dichloroacetophenone (458 mg, 1.71 mmol) were combined and dissolved in DMF (2.9 mL). The reaction was stirred for 1.5 hrs and concentrated *in vacuo.* The reaction mixture was purified with flash chromatography over $SiO_2$ (33% EtOAc/hexanes → 50% EtOAc/hexanes + 1% AcOH) to afford **1** in 85% yield (632 mg, 1.22 mmol). $^1$H NMR (500 MHz, Methanol-$d_4$) δ 7.95 (d, *J* = 2.0 Hz, 1H), 7.70 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.61 (d, *J* = 8.3 Hz, 2H), 7.47 (d, *J* = 8.4 Hz, 1H), 7.07 (d, *J* = 8.3 Hz, 2H), 6.97 (s, 1H), 4.73 (dd, *J* = 8.0, 5.4 Hz, 1H), 3.28 (dd, *J* = 13.8, 5.2 Hz, 1H), 3.06 (dd, *J* = 13.9, 8.1 Hz, 1H). $^{13}$C NMR (126 MHz, Methanol-$d_4$) δ 175.47, 169.58, 149.43, 138.68, 136.92, 133.51, 132.75, 131.85, 131.65, 128.98, 126.63, 104.51, 92.79, 59.90, 38.45. MS (ESI$^+$ *m/z*): (M+H) 518.9183 (M+H calc'd 518.9193)

*General procedure for* N-*acylsulfonamide coupling (used to generate compounds 2-9):*
A solution of carbonyldiimidazole (31.3 mg, 0.193 mmol) and **1** (50 mg, 0.0963 mmol) was prepared in DMF (200 µL) and stirred for 1 hr at room temperature. To this was added a solution

of sulfonamide $RSO_2NH_2$ (0.963 mmol) in DMF (500 µL), followed by a solution of DBU (144 µL, 0.963 mmol) in DMF (500 µL). The reaction mixture was stirred at room temperature overnight, then diluted with EtOAc (2 mL) and quenched with 10% HCl (2 mL). The organic fraction was washed with 5% $NaHCO_3$ (2 mL), dried over $Na_2SO_4$ and concentrated *in vacuo.* Initial purification of the crude product was carried out with a Waters Sep-Pak Plus PSA cartridge (eluted with 1% triethylamine in acetonitrile) to yield the resultant *N*-acylsulfonamide as a triethylammonium salt. Excess triethylamine was removed by dissolving the product in dichloromethane and washing with a 10% HCl solution. As necessary, further purification was performed by flash chromatography over $SiO_2$ (20% EtOAc/hexanes → 20% EtOAc/hexanes + 1% AcOH).

*2-((4-(3,4-dichlorophenyl)thiazol-2-yl)amino)-3-(4-iodophenyl)-N-(methylsulfonyl)propanamide* (**2**). Using the general procedure for sulfonamide coupling, where $RSO_2NH_2$ is methanesulfonamide, **2** was isolated in 58% yield. $^1$H NMR (500 MHz, Methanol-$d_4$) δ 8.01 (d, $J$ = 2.0 Hz, 1H), 7.73 (dd, $J$ = 8.4, 2.1 Hz, 1H), 7.65 (d, $J$ = 8.3 Hz, 2H), 7.47 (d, $J$ = 8.4 Hz, 1H), 7.10 (d, $J$ = 8.3 Hz, 2H), 7.03 (s, 1H), 4.61 (dd, $J$ = 8.3, 6.2 Hz, 1H), 3.16 (dd, $J$ = 13.8, 6.2 Hz, 1H), 3.09 (s, 3H), 3.03 (dd, $J$ = 13.9, 8.3 Hz, 1H). $^{13}$C NMR (126 MHz, MeOD) δ 169.11, 149.32, 138.84, 137.90, 136.78, 133.61, 132.83, 131.95, 131.72, 129.01, 126.59, 105.16, 93.05, 61.19, 41.42, 38.33. MS (ESI$^+$ *m/z*): (M+H) 595.9110 (M+H calc'd 595.9128).

*2-((4-(3,4-dichlorophenyl)thiazol-2-yl)amino)-3-(4-iodophenyl)-N-((trifluoromethyl)sulfonyl)propanamide* (**3**). Using the general procedure for sulfonamide coupling, where $RSO_2NH_2$ is trifluoromethanesulfonamide, **3** was isolated in 73% yield. $^1$H NMR (500 MHz, Methanol-$d_4$) δ 7.75 (d, $J$ = 1.9 Hz, 1H), 7.53 (d, $J$ = 8.3 Hz, 2H), 7.48 (dd, $J$ = 8.4, 2.0 Hz, 1H), 7.45 (d, $J$ = 8.4 Hz, 1H), 7.00 (d, $J$ = 8.3 Hz, 2H), 6.96 (s, 1H), 4.47 (dd, $J$ = 8.8, 4.9 Hz, 1H), 3.23 (dd, $J$ = 14.0, 4.9 Hz, 1H), 2.92 (dd, $J$ = 14.0, 8.8 Hz, 1H). $^{13}$C NMR (126 MHz, MeOD) δ 175.45, 170.46, 138.95, 138.80, 137.98, 134.01, 133.70, 132.83, 132.66, 132.07, 129.39, 127.08, 122.85, 120.29, 105.40, 93.22, 63.83, 38.74. $^{19}$F NMR (377 MHz, MeOD) δ -79.19. MS (ESI$^+$ *m/z*): (M+H) 649.8871 (M+H calc'd 649.8846).

*2-((4-(3,4-dichlorophenyl)thiazol-2-yl)amino)-3-(4-iodophenyl)-N-(phenylsulfonyl)propanamide* (**4**). Using the general procedure for sulfonamide coupling, where $RSO_2NH_2$ is benzenesulfonamide, **4** was isolated in 81% yield. $^1$H NMR (500 MHz, Chloroform-*d*) δ 7.98 (d, $J$ = 7.7 Hz, 2H), 7.79 (d, $J$ = 1.7 Hz, 1H), 7.63 – 7.51 (m, 4H), 7.46 (t, $J$ = 9.1 Hz, 3H), 6.87 (d, $J$ = 7.7 Hz, 2H), 6.79 (s, 1H), 4.50 (t, $J$ = 8.1, 5.7 Hz, 1H), 3.21 (dd, $J$ = 14.5, 5.7 Hz, 1H), 3.09 (dd, $J$ = 14.4, 8.1 Hz, 1H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 169.14, 167.19, 148.82, 138.33, 135.10, 134.16, 133.94, 133.09, 132.17, 131.30, 130.87, 129.40, 129.26, 129.11, 128.45, 128.07, 126.66, 125.36, 104.75, 93.21, 60.33, 35.81. MS (ESI$^+$ *m/z*): (M+H) 657.9292 (M+H calc'd 657.9285).

*2-((4-(3,4-dichlorophenyl)thiazol-2-yl)amino)-3-(4-iodophenyl)-N-((4-nitrophenyl)sulfonyl)propanamide* (**5**). Using the general procedure for sulfonamide coupling, where $RSO_2NH_2$ is 4-nitrobenzenesulfonamide, **5** was isolated in 76% yield. $^1$H NMR (500 MHz, Methanol-$d_4$) δ 8.04 (s, 4H), 7.76 (d, $J$ = 2.0 Hz, 1H), 7.58 (d, $J$ = 8.3 Hz, 2H), 7.52 (dd, $J$ = 8.4, 2.0 Hz, 1H), 7.39 (d, $J$ = 8.4 Hz, 1H), 7.02 (d, $J$ = 8.3 Hz, 2H), 6.96 (s, 1H), 4.49 (dd, $J$ = 8.1, 7.0 Hz, 1H), 3.06 (dd, $J$ = 14.0, 7.1 Hz, 1H), 3.02 (dd, $J$ = 14.0, 8.3 Hz, 1H).$^{13}$C NMR (126 MHz, Methanol-$d_4$) δ 175.34, 168.69, 151.69, 149.07, 146.38, 138.84, 137.63, 136.44, 133.57, 132.66, 132.06, 131.67, 130.37, 128.98, 126.26, 124.95, 105.27, 93.08, 61.40, 37.83. MS (ESI$^+$ *m/z*): (M+H) 702.9169 (M+H calc'd 702.9135).

*2-((4-(3,4-dichlorophenyl)thiazol-2-yl)amino)-3-(4-iodophenyl)-N-((4-methoxyphenyl)sulfonyl)propanamide* (**6**). Using the general procedure for sulfonamide coupling, where $RSO_2NH_2$ is 4-methoxybenzenesulfonamide, **6** was isolated in 42% yield. $^1$H NMR (500 MHz, Methanol-$d_4$) δ 7.87 (d, $J$ = 2.0 Hz, 1H), 7.79 (d, $J$ = 9.0 Hz, 2H), 7.60 (dd, $J$ = 8.4, 2.0 Hz, 1H), 7.54 (d, $J$ = 8.3 Hz, 2H), 7.44 (d, $J$ = 8.4 Hz, 1H), 6.97 (s, 1H), 6.96 (d, $J$ = 8.4 Hz, 2H), 6.81 (d, $J$ = 9.0 Hz, 2H), 4.50 (dd, $J$ = 8.0, 6.7 Hz, 1H), 3.76 (s, 3H), 3.05 (dd, $J$ = 13.8, 6.7 Hz, 1H), 2.96 (dd, $J$ = 13.8, 8.1 Hz, 1H).$^{13}$C NMR (126 MHz, Methanol-$d_4$) δ 173.39, 168.76, 165.18, 149.30, 138.77, 137.70, 136.68, 133.49, 132.65, 132.29, 131.83, 131.60, 131.34, 129.07, 126.57, 114.99, 105.05, 92.97, 61.17, 56.31, 38.05. MS (ESI$^+$ *m/z*): (M+H) 687.9413 (M+H calc'd 687.9390).

*2-((4-(3,4-dichlorophenyl)thiazol-2-yl)amino)-3-(4-iodophenyl)-N-tosylpropanamide* (**7**). Using the general procedure for sulfonamide coupling, where $RSO_2NH_2$ is 4-toluenesulfonamide, **7** was isolated in 50% yield. $^1$H NMR (500 MHz, Methanol-$d_4$) δ 7.86 (d, $J$ = 2.0 Hz, 1H), 7.73 (d, $J$ = 8.3 Hz, 2H), 7.60 (dd, $J$ = 8.4, 2.1 Hz, 1H), 7.53 (d, $J$ = 8.3 Hz, 2H), 7.44 (d, $J$ = 8.4 Hz, 1H), 7.13 (d, $J$ = 8.0 Hz, 2H), 6.96 (s, 1H), 6.95 (d, $J$ = 8.4 Hz, 2H), 4.51 (dd, $J$ = 8.0, 6.8 Hz, 1H), 3.04 (dd, $J$ = 13.8, 6.7 Hz, 1H), 2.96 (dd, $J$ = 13.8, 8.1 Hz, 1H), 2.28 (s, 3H). $^{13}$C NMR (126 MHz, Methanol-$d_4$) δ 173.39, 168.76, 149.29, 145.95, 138.76, 138.00, 137.63, 136.70, 133.49, 132.63, 131.81, 131.58, 130.46, 129.12, 129.06, 126.59, 105.06, 92.99, 61.14, 38.05, 21.69. MS (ESI$^+$ *m/z*): (M+H) 671.9437 (M+H calc'd 671.9441).

*N-((4-chlorophenyl)sulfonyl)-2-((4-(3,4-dichlorophenyl)thiazol-2-yl)amino)-3-(4-iodophenyl)propanamide* (**8**). Using the general procedure for sulfonamide coupling, where $RSO_2NH_2$ is 4-chlorobenzenesulfonamide, **8** was isolated in 49% yield. $^1$H NMR (500 MHz, Methanol-$d_4$) δ 7.84 (d, $J$ = 2.0 Hz, 1H), 7.81 (d, $J$ = 8.7 Hz, 2H), 7.58 (dd, $J$ = 8.4, 2.0 Hz, 1H), 7.54 (d, $J$ = 8.3 Hz, 2H), 7.43 (d, $J$ = 8.4 Hz, 1H), 7.28 (d, $J$ = 8.7 Hz, 2H), 6.96 (d, $J$ = 8.3 Hz, 2H), 6.95 (s, 1H), 4.50 (dd, $J$ = 8.1, 6.8 Hz, 1H), 3.04 (dd, $J$ = 13.8, 6.9 Hz, 1H), 2.98 (dd, $J$ = 13.8, 8.0 Hz, 1H). $^{13}$C NMR (126 MHz, Methanol-$d_4$) δ 173.52, 168.69, 149.23, 141.06, 139.47, 138.77, 137.53, 136.55, 133.53, 132.62, 131.96, 131.66, 130.69, 130.12, 129.02, 126.48, 105.14, 93.06, 61.16, 37.96. MS (ESI$^+$ *m/z*): (M+H) 691.8908 (M+H calc'd 691.8895).

*2-((4-(3,4-dichlorophenyl)thiazol-2-yl)amino)-3-(4-iodophenyl)-N-((2-nitrophenyl)sulfonyl)propanamide* (**9**). Using the general procedure for sulfonamide coupling, where $RSO_2NH_2$ is 2-nitrobenzenesulfonamide, **9** was isolated in 37% yield. $^1$H NMR (500 MHz, Methanol-$d_4$) δ 8.27 – 8.21 (m, 1H), 7.82 (d, $J$ = 2.0 Hz, 1H), 7.72 – 7.64 (m, 3H), 7.61 (dd, $J$ = 8.4, 2.0 Hz, 1H), 7.52 (d, $J$ = 8.3 Hz, 2H), 7.42 (d, $J$ = 8.4 Hz, 1H), 6.98 (d, $J$ = 8.3 Hz, 2H), 6.96 (s, 1H), 4.62 (dd, $J$ = 8.6, 6.0 Hz, 1H), 3.15 (dd, $J$ = 13.9, 5.9 Hz, 1H), 3.00 (dd, $J$ = 13.9, 8.6 Hz, 1H). $^{13}$C NMR (126 MHz, MeOD) δ 175.34, 168.86, 149.52, 149.21, 138.73, 137.75, 136.63, 136.02, 134.19, 133.54, 133.18, 132.66, 131.87, 131.61, 128.98, 126.59, 125.81, 105.20, 92.98, 61.28, 37.72. MS (ESI$^+$ *m/z*): (M+H) 702.9134 (M+H calc'd 702.9135).

*Methyl 2-((4-(3,4-dichlorophenyl)thiazol-2-yl)amino)-3-(4-iodophenyl)propanoate* (**10**). A solution of **1** (19 mg, 0.024 mmol) in MeOH (200 µL) was cooled to 0°C, then trimethylsilyl diazomethane (72 µL, 0.144 mmol) was added dropwise. The mixture was allowed to warm to room temperature, then stirred overnight. The reaction mixture was concentrated *in vacuo* and purified with flash chromatography over $SiO_2$ (5% EtOAc/hexanes → 10% EtOAc/hexanes) to afford **10** in 58% yield (7.7 mg, 0.014 mmol). $^1$H NMR (500 MHz, Chloroform-*d*) δ 7.90 (d, $J$ = 1.9 Hz, 1H), 7.62 (d, $J$ = 8.1 Hz, 2H), 7.59 (dd, $J$ = 8.4, 2.0 Hz, 1H), 7.43 (d, $J$ = 8.4 Hz, 1H), 6.90 (d, $J$ = 8.2 Hz, 2H), 6.74 (s, 1H), 4.85 (dd, $J$ = 11.7, 4.1 Hz, 1H), 3.77 (s, 3H), 3.32 (dd, $J$ = 13.9, 5.7 Hz, 1H), 3.14 (dd, $J$ = 14.0, 5.4 Hz, 1H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 172.19, 166.86, 149.16, 137.88, 135.77, 134.89, 132.87, 131.60, 131.55, 130.64, 128.18, 125.28, 103.38, 92.93, 58.34, 52.76, 37.58. MS (ESI$^+$ *m/z*): (M+H) 532.9324 (M calc'd 532.9349)

*2-(3-(4-bromobenzyl)-6-(4-chlorophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-7-yl)-N-(methylsulfonyl)acetamide* (**12**). Using the general procedure for sulfonamide coupling, with **11** as the carboxylic acid and where $RSO_2NH_2$ is methanesulfonamide, **12** was isolated in 60% yield. $^1$H NMR (500 MHz, Methanol-$d_4$) δ 7.99 (d, $J$ = 8.7 Hz, 2H), 7.55 (d, $J$ = 8.7 Hz, 2H), 7.49 (d, $J$ = 8.4 Hz, 2H), 7.27 (d, $J$ = 8.4 Hz, 2H), 5.11 (dd, $J$ = 9.9, 4.9 Hz, 1H), 4.37 (s, 2H), 2.57 (dd, $J$ = 15.7, 4.9 Hz, 1H), 2.45 (dd, $J$ = 15.7, 9.9 Hz, 1H). $^{13}$C NMR (126 MHz, MeOD) δ 175.02, 157.91, 154.47, 142.14, 139.68, 136.38, 133.09, 132.73, 131.99, 130.59, 122.14, 41.81, 40.52, 35.49, 31.09. MS (ESI$^+$ *m/z*): (M+H) 553.9717 (M calc'd 553.9718).

*2-(3-(4-bromobenzyl)-6-(4-chlorophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-7-yl)-N-(phenylsulfonyl)acetamide* (**13**). Using the general procedure for sulfonamide coupling, with **11** as the carboxylic acid and where $RSO_2NH_2$ is benzenesulfonamide, **13** was isolated in 46% yield. $^1$H NMR (500 MHz, Methanol-$d_4$) δ 7.93 – 7.87 (m, 4H), 7.58 (t, $J$ = 7.4 Hz, 1H), 7.53 – 7.46 (m, 6H), 7.25 (d, $J$ = 8.4 Hz, 2H), 5.02 (dd, $J$ = 9.9, 4.8 Hz, 1H), 4.35 (s, 2H), 2.55 (dd, $J$ = 16.0, 4.8 Hz, 1H), 2.45 (dd, $J$ = 16.0, 9.9 Hz, 1H). $^{13}$C NMR (126 MHz, MeOD) δ 175.43, 157.52, 154.47, 143.15, 141.90, 139.69, 136.34, 133.64, 133.09, 132.41, 131.96, 130.58, 130.45, 129.72, 128.79, 122.15, 40.78, 34.88, 31.08. MS (ESI$^+$ *m/z*): (M+H) 615.9876 (M calc'd 615.9874).

*Methyl 2-(3-(4-bromobenzyl)-6-(4-chlorophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-7-yl)acetate* (**14**). A solution of **11** (16.5 mg, 0.034 mmol) in MeOH (340 µL) was cooled to 0°C, and thionyl chloride (25 µL, 0.34 mmol) was added dropwise. The mixture was heated at 65°C for 6 hours. The reaction mixture was concentrated *in vacuo* and purified with flash chromatography over $SiO_2$ (2:1 hexanes/EtOAc) to afford **14** in 31% yield (5.2 mg, 0.0106 mmol). $^1$H NMR (500 MHz, Chloroform-*d*) δ 7.80 (d, *J* = 8.7 Hz, 2H), 7.49 (d, *J* = 8.7 Hz, 2H), 7.42 (d, *J* = 8.4 Hz, 2H), 7.20 (d, *J* = 8.3 Hz, 2H), 4.78 (dd, *J* = 10.0, 4.5 Hz, 1H), 4.34 (s, 2H), 2.63 (dd, *J* = 16.8, 10.0 Hz, 1H), 2.54 (dd, *J* = 16.8, 4.5 Hz, 1H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 169.04, 154.13, 152.70, 139.25, 138.97, 134.44, 131.90, 130.61, 130.57, 129.74, 128.60, 121.21, 52.75, 36.84, 32.85, 30.49. MS ($ESI^+$ *m/z*): (M+H) 490.9932 (M calc'd 490.9939)

**Compound characterization by dynamic light scattering:**

A representative subset of compounds (1, 2, and 4) were evaluated for aggregation by Dynamic Light Scattering (DLS). Samples for DLS analysis were prepared in 50 mM sodium phosphate buffer from DMSO inhibitor stocks, at a final DMSO concentration of 1%, and a range of final concentrations of inhibitor. Samples that displayed a scattering of less than 5 x $10^4$, which is the lower detection limit of the instrument, were considered not to aggregate.

| Compound | Conc. (uM) | Aggregates observed? |
|---|---|---|
| **1** | 100 | Yes |
| | 50 | No |
| | 25 | No |
| | 10 | No |
| **2** | 100 | No |
| | 50 | No |
| | 25 | No |
| | 10 | No |
| **4** | 100 | Yes |
| | 50 | No |
| | 25 | No |
| | 10 | No |

**Table S1**. DLS evaluation of inhibitors **1**, **2**, and **4**.

**Evaluation of Inhibitory Activity:**

MtbUGM Activity Assay

Recombinant MtbUGM was produced according to published protocols, and enzyme activity was evaluated using a previously published HPLC assay.[31] 20 nM MtbUGM was incubated in 50 mM sodium phosphate buffer (pH 7.0) with 20 mM sodium dithionite and 20 μM UDP-Gal*f* in the absence or presence of an inhibitor (added as a DMSO stock at a final concentration of 1% DMSO). After a 40 second incubation period, the reaction was quenched with 1:1 chloroform/methanol. The aqueous portion was separated and analyzed on an Agilent 1260 Infinity HPLC with a Dionex Carbopac PA-100 column to quantify conversion of UDP-Gal*f* to UDP-Gal*p*. Relative enzyme activity was derived by normalizing activity in the presence of inhibitors against the activity of the enzyme alone.

Microplate Alamar Blue Assay

*M. smegmatis* was grown to saturation over 48 hours at 37°C in Middlebrook 7H9 media with Albumin Dextrose Catalase (ADC) enrichment and 0.05% Tween80. The culture was diluted to $OD_{600}$ = ~0.02 in LB liquid media and added to 96-well plates at a final volume of 100 μL, with added inhibitor concentrations in two-fold dilutions. Perimeter wells were filled with sterile water. After 24 hours at 37°C in a shaking incubator, 10 μL of the Alamar Blue reagent was added to each well. The plate was incubated for an additional 4 hours at 37°C, at which point fluorescence emission at 585 nm was measured using a plate reader. The lowest inhibitor concentration at which 90% or greater growth inhibition was observed was determined to be the MIC.

Agar Disk Diffusion Assay

*M. smegmatis* was grown to saturation over 48 hours at 37°C in Middlebrook 7H9 media with Albumin Dextrose Catalase (ADC) enrichment and 0.05% Tween80. The culture was diluted to $OD_{600}$ = ~0.2 in LB liquid media and 100 μL of dilute culture was spread onto LB agar plates, then allowed to soak into the agar for 1 hour at room temperature.. Sterile disks (3 mm diameter) were impregnated with a solution of inhibitor in DMSO (1.5 μL) and placed on top of the bacterial lawn. Compounds **1**-**10** were tested using a 10 mM inhibitor stock (final amount 15 nmols). The plates were incubated for 72 hours at 37°C, at which point a lawn of bacteria was visible. Zones of inhibition were measured as the average diameter of the region around a cloning disk where bacterial growth was not visible. For each disk, three diameter measurements were taken and averaged.

The same procedure was followed for evaluation of inhibition of *E. coli* BL21, grown in LB liquid media. Plates were incubated overnight at 37°C. Images are shown below:

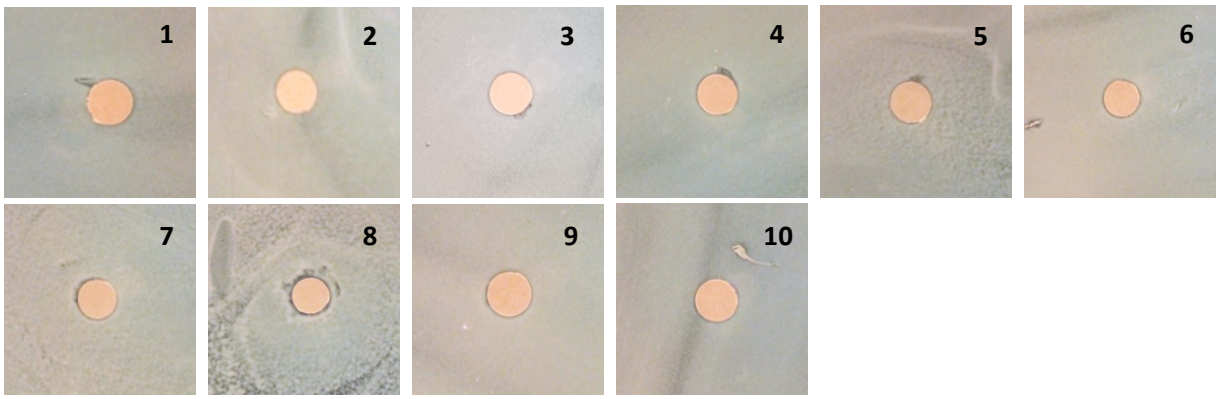



**Figure S1.** Evaluation of compounds **1-10** in disk diffusion assay for antibacterial activity against *E. coli* BL21.

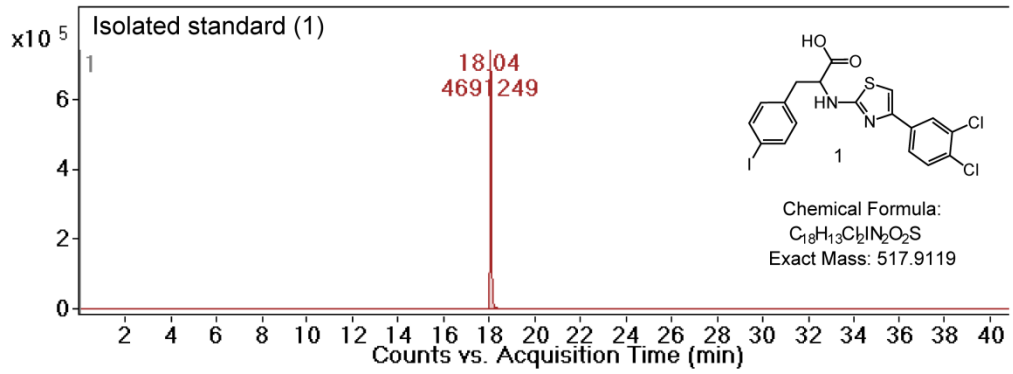
**LC-MS Quantification of Compound Accumulation:**

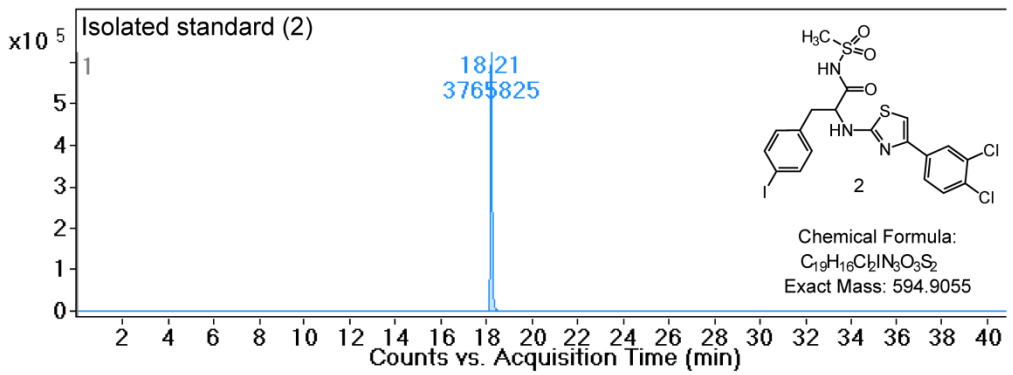
Compounds **1** and **2** were evaluated for accumulation in *M. smegmatis* using the method reported by Chatterji and coworkers.[41] Specifically, *M. smegmatis* $mc^2155$ was grown at 37°C in Middlebrook 7H9 media with Albumin Dextrose Catalase (ADC) enrichment and 0.05% Tween80, to a density of $OD_{600}$ = 5.0. For each sample, 1 mL of culture was pelleted then resuspended in 1 mL of PBS buffer (pH 7.4). The bacteria were treated with compound **1** or **2** at a final concentration of 25 µM, and incubated at room temperature for 4 hours. Samples were centrifuged and washed three times with 1 mL PBS buffer, to remove any extracellular inhibitor. Cell pellets were then resuspended in 1400 µL of 12:5:3 MeOH/$CHCl_3$/$H_2O$, heated to 65°C for 20 minutes, then stored at -20°C overnight. Samples were centrifuged for 15 min at 12,600 rcf, then 1000 µL supernatant was removed and 250 µL of water was added. The samples were vortexed, then centrifuged for 20 minutes at 12,600 rcf to achieve phase separation. From the top (aqueous) phase, 1000 µL was removed and concentrated to dryness. Samples were then reconstituted in 300 µL of 10 mM ammonium formate and extracted with 600 µL of ethyl acetate. Of the top (organic) layer, 400 µL was removed, filtered through a 0.22 uM PTFE syringe filter, and concentrated. Samples were reconstituted in 1:1 $H_2O$/acetonitrile.

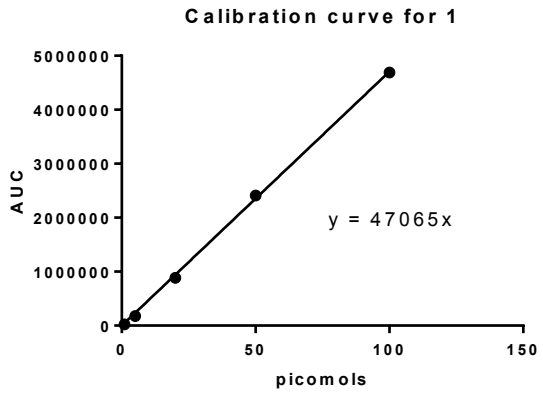
Cell lysate extracts were separated on an Agilent 1200 SL HPLC system (binary pump; heated column compartment at 35°C, thermostated autosampler at 4°C), using a Phenomenex Synergi Polar RP column (2.0mm x 150mm, 4µm particles, 80Å pore size) at a flow rate of 0.25 mL/min. Initial conditions were 90% water containing 0.1% formic acid (solvent A) and 10% acetonitrile containing 0.1% formic acid (solvent B) from 0 to 1 min. Solvent B was increased from 10% to 100% over 24 min and held at 100% for 7 min, then returned to 10% over 1 min and re-equilibrated at 10% for 9 min. Mass spectrometry analysis was performed using an Agilent model 6210 ESI-TOF with dual spray ESI source. Analytes were measured in positive mode with an electrospray voltage of 3700V, nebulizing gas at 45 psi, drying gas at 10L/min, gas temperature at 350°C, and fragmentor at 160V. Mass spectra were collected at 10,000

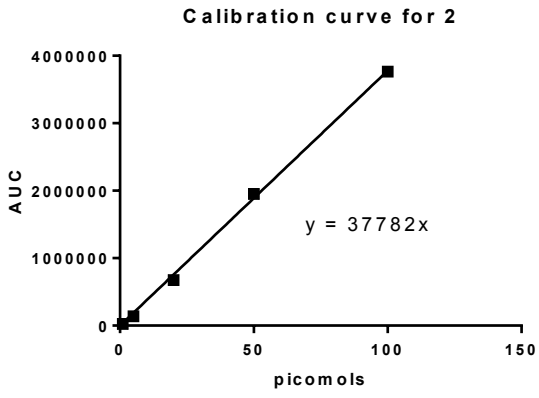
transients/scan at a frequency of 0.9 spectra/sec, over an m/z range of 100-3200. Data was analyzed with Agilent MassHunter Qualitative Analysis software.

Calibration curves were generated for **1** and **2** and found to be linear across a range of 1 to 100 picomols.




To quantify compound accumulation, extracted ion chromatograms for the expected mass of each compound were integrated. The compound of interest was easily identified based on comparison to the retention time and characteristic isotope pattern of the isolated standard. The

accumulation of **1** and **2** were compared in Prism 6 using a ratio t-test (n = 6), and **2** was found to accumulate at approximately 14-fold higher concentration compared to **1** ($p < 0.05$).

**Protein-Ligand Docking Experiments:**

Molecular docking was performed for compounds **11**, **12**, and **13** with *C. diphtheriae* UGM using AutoDock4. Predicted poses in a variety of conformations were generated; shown below are representative poses.

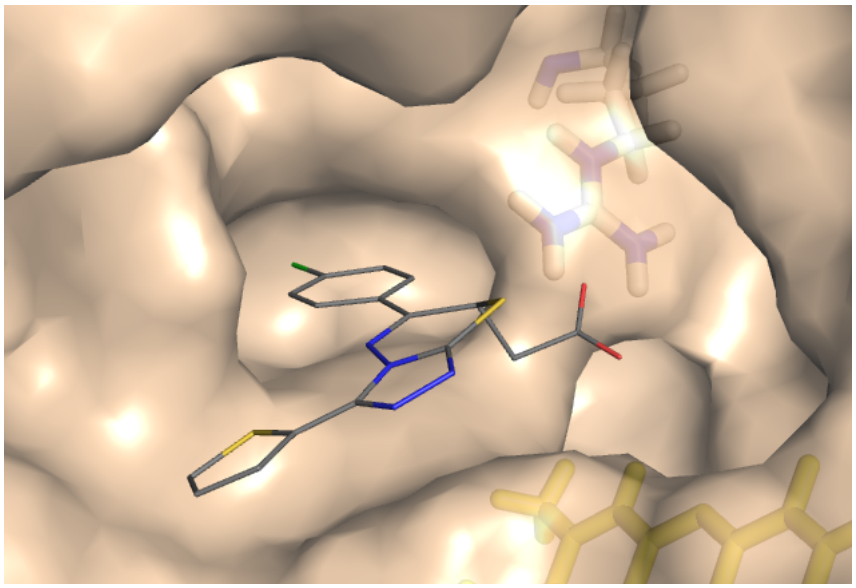


**Figure S2.** CdUGM (wheat) in complex with triazolothiadiazine analog (gray); binding pose determined by x-ray crystallography. Arg289 is highlighted. FAD cofactor shown in yellow.

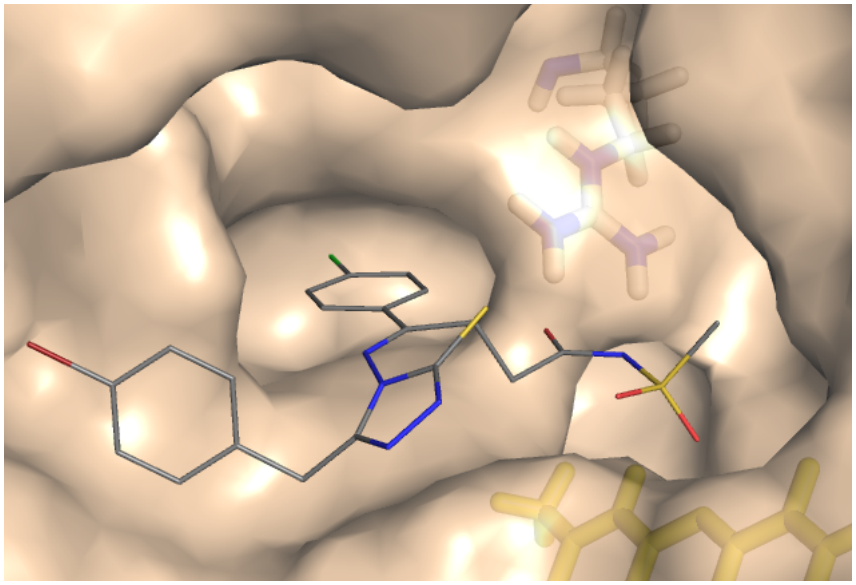


**Figure S3**. CdUGM (wheat) in complex with compound **12** (gray); binding pose predicted by molecular docking. Arg289 is highlighted. FAD cofactor shown in yellow.

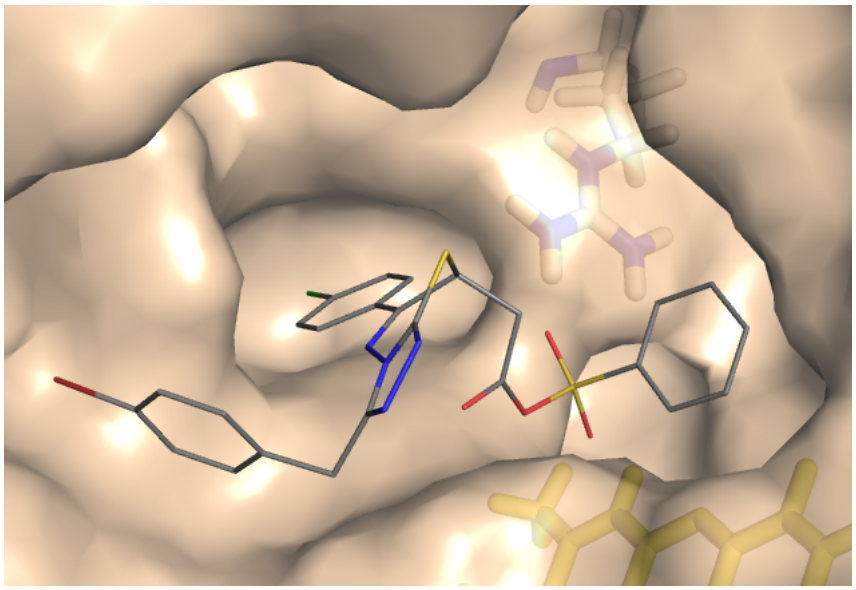


**Figure S4**. CdUGM (wheat) in complex with compound **13** (gray); binding pose predicted by molecular docking. Arg289 is highlighted. FAD cofactor shown in yellow.

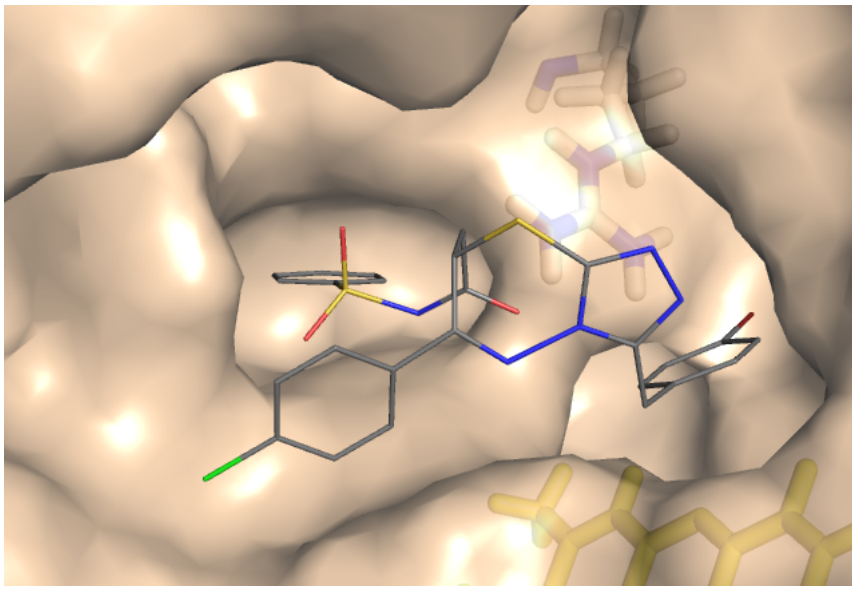


**Figure S5**. CdUGM (wheat) in complex with compound **13** (gray); binding pose predicted by molecular docking. Arg289 is highlighted. FAD cofactor shown in yellow.