Supporting Information

Targeting of fumarate hydratase from *Mycobacterium tuberculosis* using allostERIC inhibitors with a dimeric-binding mode

Andrew J. Whitehouse,¹ M. Daben J. Libardo,‡ Monica Kasbekar,§ Paul D. Brear,¹ Gerhard Fischer,¹‡ Craig J. Thomas,§ Clifton E. Barry III,‡ Helena I. M. Boshoff,‡ Anthony G. Coyne,*,† and Chris Abell*,†

¹Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, UK. ²Tuberculosis Research Section, Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA. ³National Center for Advancing Translational Sciences, National Institutes of Health, Bethesda, MD 20850, USA. ⁴Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge, CB2 1GA, UK. ⁵Current address: Boehringer Ingelheim RCV, Dr. Boehringer-Gasse 5-11, 1121 Vienna, Austria.

Table of Contents

Synthetic Chemistry S2

Biochemical Assay Dose-response Curves S26

X-ray Crystallography S33
Synthetic Chemistry

Ethyl-(E)-2-(3-oxoisobenzofuran-1(3H)-ylidene)acetate (18). ¹ ²

A solution of (carbethoxymethylene)triphenylphosphorane (4.70 g, 13.5 mmol) in chloroform (12.5 mL) was added dropwise to a solution of phthalic anhydride 17 (2.00 g, 13.5 mmol) in chloroform (12.5 mL). The reaction mixture was heated under reflux for 3 hours, then concentrated in vacuo. Purification by flash column chromatography (5% ethyl acetate in petroleum ether) afforded 18 (1.97 g, 67% yield).

¹H NMR (500 MHz, CDCl₃) 9.05 (1H, d, J = 8.0 Hz), 7.96 (1H, dt, J = 7.5, 1.0 Hz), 7.84-7.78 (1H, m), 7.70 (1H, td, J = 7.5, 0.9 Hz), 6.15 (1H, s), 4.30 (2H, q, J = 7.2 Hz), 1.36 (3H, t, J = 7.2 Hz); ¹³C NMR (125 MHz, CDCl₃) 165.9, 165.7, 158.0, 136.3, 135.4, 132.6, 128.4, 126.7, 125.5, 102.6, 61.1, 14.4; ¹H NMR spectroscopic data consistent with literature. ²

2,2,2-Trifluoro-N-(2-methoxyphenyl)acetamide (20). ³

Trifluoroacetic anhydride (0.55 mL, 3.9 mmol) was added dropwise at 0 °C to a mixture of o-anisidine 19 (0.37 mL, 3.3 mmol), pyridine (0.39 mL, 4.9 mmol) and DCM (4 mL). The reaction mixture was warmed to room temperature and stirred over 3 days. Water (20 mL) was added dropwise at 0 °C to the reaction mixture. The product was extracted into DCM (3 x 20 mL). The combined organic extracts were dried (MgSO₄) and concentrated in vacuo.

52
Purification by flash column chromatography (0 – 20% ethyl acetate in petroleum ether) afforded 20 (0.713 g, 99% yield).

LCMS (ESI-): m/z 218.1 [M - H], rt 2.14 minutes, >99%; $^1$H NMR (400 MHz, CDCl$_3$) 8.57 (1H, br s), 8.32 (1H, d, J = 8.0 Hz), 7.17 (1H, t, J = 8.0 Hz), 7.01 (1H, t, J = 7.7 Hz), 6.94 (1H, d, J = 8.2 Hz), 3.93 (3H, s); $^{13}$C NMR (100 MHz, CDCl$_3$) 154.5 (q, J = 37 Hz), 148.4, 126.1, 125.2, 121.4, 120.3, 115.8 (q, J = 288 Hz), 110.3, 56.0; $^1$H NMR spectroscopic data consistent with literature. $^3$

4-Methoxy-3-(2,2,2-trifluoroacetamido)benzenesulfonyl chloride (21). $^3$

Chlorosulfonic acid (0.41 mL, 6.2 mmol) was added dropwise at 0 °C to a solution of 2,2,2-trifluoro-N-(2-methoxyphenyl)acetamide 20 (0.683 g, 3.12 mmol) in DCM (6 mL). The reaction mixture was warmed to room temperature and stirred over 16 hours. Water (20 mL) was added dropwise at 0 °C to the reaction mixture. The product was extracted into DCM (3 x 20 mL). The combined organic extracts were dried (MgSO$_4$) and concentrated in vacuo to afford 21 (0.767 g, 77% yield).

$^1$H NMR (400 MHz, CDCl$_3$) 9.05 (1H, s), 8.59 (1H, br s), 7.90 (1H, d, J = 8.8 Hz), 7.11 (1H, d, J = 8.9 Hz), 4.09 (3H, s); $^{13}$C NMR (100 MHz, CDCl$_3$) 154.9 (q, J = 38 Hz), 153.1, 137.1, 126.1, 125.9, 118.9, 115.5 (q, J = 289 Hz), 110.5, 57.1; $^1$H NMR spectroscopic data consistent with literature. $^3$
4-Hydroxy-3-nitrobenzenesulfonyl chloride (23).

Chlorosulfonic acid (0.96 mL, 14 mmol) was added dropwise at 0 °C to a solution of 2-nitrophenol 22 (1.00 g, 7.19 mmol) in chloroform (5 mL). The reaction mixture was heated under reflux for 90 minutes. Water (15 mL) was added dropwise at 0 °C to the reaction mixture. The product was extracted into DCM (3 x 25 mL). The combined organic extracts were dried (MgSO₄) and concentrated in vacuo to afford 23 (1.33 g, 78% yield).

LCMS (ESI-): m/z 236.0 [M - H], rt 1.89 minutes, >99%; ¹H NMR (400 MHz, CDCl₃) 11.12 (1H, s), 8.84 (1H, d, J = 2.4 Hz), 8.21 (1H, dd, J = 9.0, 2.4 Hz), 7.42 (1H, d, J = 9.0 Hz); ¹³C NMR (100 MHz, CDCl₃) 159.5, 136.2, 134.9, 132.9, 126.0, 122.4; νmax/cm⁻¹ 3249 (br, O-H), 3088, 1615, 1578, 1539 (N=O), 1328 (N=O).

4-(Azepan-1-ylsulfonyl)-2-nitrophenol (24).

Hexamethyleneimine (0.108 mL, 0.963 mmol) and N,N-diisopropylethylamine (0.305 mL, 1.75 mmol) were added to a solution of 4-hydroxy-3-nitrobenzenesulfonyl chloride 23 (0.208 g, 0.875 mmol) in DCM (2 mL). The reaction mixture was stirred over 15 hours, then water (10 mL) and aqueous HCl (37.5% w/v, 5 mL) were added. The product was extracted into DCM (3 x 25 mL). The combined organic extracts were dried (MgSO₄) and concentrated in vacuo to afford 24 (1.02 g, 78% yield).
vacuo. Purification by flash column chromatography (0 – 40% ethyl acetate in petroleum ether) afforded 24 (0.190 g, 64% yield).

LCMS (ESI+): m/z 301.2 [M + H]+, (ESI-): m/z 299.1 [M - H]-, rt 2.11 minutes, 88%; 1H NMR (400 MHz, CDCl3) 10.85 (1H, s), 8.56 (1H, d, J = 2.4 Hz), 7.96 (1H, dd, J = 8.9, 2.2 Hz), 7.29 (1H, d, J= 8.7 Hz), 3.29 (4H, t, J = 5.9 Hz), 1.79-1.69 (4H, m), 1.65-1.58 (4H, m);
13C NMR (100 MHz, CDCl3) 157.5, 135.2, 133.1, 132.5, 124.8, 121.4, 48.5, 29.3, 27.0; νmax/cm⁻¹ 2939, 1615, 1583, 1528 (N=O), 1330 (N=O); HRMS (ESI+): m/z calculated for [C₁₂H₁₆N₂O₅S + Na]⁺ = 323.0672, observed 323.0661.

1-((3-Nitrophenyl)sulfonyl)azepane (27).

Hexamethyleneimine (0.303 mL, 2.71 mmol) was added dropwise at 0 °C to a suspension of sodium hydride (60% in mineral oil, 0.271 g, 6.77 mmol) in DMF (3 mL). The reaction mixture was stirred at 0 °C over 20 minutes. A solution of 3-nitrobenzenesulfonyl chloride 25 (0.500 g, 2.26 mmol) in DMF (2 mL) was added dropwise at 0 °C to the reaction mixture. The reaction mixture was warmed to room temperature and stirred over 90 min. Water (25 mL) was added dropwise at 0 °C to the reaction mixture, followed by ethyl acetate (25 mL) with the resultant aqueous layer discarded. The organic layer was washed with water (2 x 25 mL) and brine (25 mL), dried (MgSO₄) and concentrated in vacuo. Purification by flash column chromatography (0 – 20% ethyl acetate in petroleum ether) afforded 27 (0.267 g, 42% yield).

1H NMR (400 MHz, CDCl3) 8.61 (1H, t, J = 1.9 Hz), 8.40 (1H, ddd, J = 8.2, 2.2, 1.0 Hz), 8.12 (1H, ddd, J = 7.8, 1.7, 1.1 Hz), 7.73 (1H, t, J = 8.0 Hz), 3.31 (4H, t, J = 5.9 Hz), 1.80-
1.68 (4H, m), 1.65-1.55 (4H, m); 13C NMR (100 MHz, CDCl3) 148.5, 142.0, 132.5, 130.5, 126.8, 122.1, 48.6, 29.3, 26.9; νmax/cm⁻¹ 3101, 2937, 2858, 1608, 1523 (N=O), 1338 (N=O); HRMS (ESI)+: m/z calculated for [C₁₂H₁₆N₂O₄S + H]⁺ = 285.0904, observed 285.0897.

1-((4-Methyl-3-nitrophenyl)sulfonyl)azepane (28).

Hexamethyleneimine (0.285 mL, 2.55 mmol) was added dropwise at 0 °C to a suspension of sodium hydride (60% in mineral oil, 0.110 g, 2.76 mmol) in DMF (3 mL). The reaction mixture was stirred at 0 °C over 20 minutes. A solution of 4-methyl-3-nitrobenzenesulfonyl chloride 26 (0.500 g, 2.12 mmol) in DMF (2 mL) was added dropwise at 0 °C to the reaction mixture. The reaction mixture was warmed to room temperature and stirred over 1 hour. Water (25 mL) was added dropwise at 0 °C to the reaction mixture, followed by ethyl acetate (25 mL) with the resultant aqueous layer discarded. The organic layer was washed with water (2 x 25 mL) and brine (25 mL), dried (MgSO₄) and concentrated in vacuo. Purification by flash column chromatography (0 – 20% ethyl acetate in petroleum ether) afforded 28 (0.453 g, 72% yield).

LCMS (ESI+): m/z 299.2 [M + H]⁺, rt 2.15 minutes, >99%; ¹H NMR (400 MHz, CDCl₃) 8.35 (1H, d, J = 1.9 Hz), 7.89 (1H, dd, J = 8.0, 1.9 Hz), 7.50 (1H, d, J = 8.1 Hz), 3.30 (4H, t, J = 5.9 Hz), 2.67 (3H, s), 1.81-1.68 (4H, m), 1.65-1.54 (4H, m); ¹³C NMR (100 MHz, CDCl₃) 149.3, 139.3, 137.9, 133.9, 130.8, 123.4, 48.5, 29.3, 27.0, 20.6; νmax/cm⁻¹ 2934, 2861, 1608, 1523 (N=O), 1338 (N=O); HRMS (ESI)+: m/z calculated for [C₁₃H₁₈N₂O₄S + H]⁺ = 299.1060, observed 299.1065.
5-(Azepan-1-ylsulfonyl)-2-methylaniline (29).

![Chemical Structure](image)

Sodium borohydride (83 mg, 2.2 mmol) was added portionwise at 0 °C to a suspension of NiCl$_2$ (95 mg, 0.74 mmol) in methanol (2 mL). The reaction mixture was warmed to room temperature and stirred over 30 minutes. 1-((4-Methyl-3-nitrophenyl)sulfonyl)azepane 28 (0.439 g, 1.47 mmol) was added at 0 °C to the reaction mixture, followed by further methanol (8 mL) and sodium borohydride (0.278 g, 7.36 mmol). The reaction mixture was warmed to room temperature and stirred over 45 minutes. Water (10 mL) was added at 0 °C and the mixture filtered through celite, eluted with methanol (10 mL) and water (15 mL). The filtrate was concentrated in vacuo to remove methanol, then extracted into ethyl acetate (3 x 25 mL). The combined organic extracts were washed (brine), dried (MgSO$_4$) and concentrated in vacuo. Purification by flash column chromatography (20 – 50% ethyl acetate in petroleum ether) afforded 29 (0.347 g, 88% yield).

LCMS (ESI+): m/z 269.2 [M + H]$^+$, rt 1.90 minutes, >99%; $^1$H NMR (400 MHz, CDCl$_3$) 7.13 (1H, d, J = 8.4 Hz), 7.10-7.04 (2H, m), 3.80 (2H, br s), 3.24 (4H, t, J = 5.9 Hz), 2.19 (3H, s), 1.77-1.65 (4H, m), 1.63-1.53 (4H, m); $^{13}$C NMR (100 MHz, CDCl$_3$) 145.2, 137.9, 131.0, 126.7, 116.9, 112.8, 48.4, 29.3, 27.1, 17.6; $\nu_{\text{max}}$/cm$^{-1}$ 3490 (N-H), 3377 (N-H), 2930, 2858, 1625, 1574; HRMS (ESI)+: m/z calculated for [C$_{13}$H$_{20}$N$_{2}$O$_2$S + H]$^+$ = 269.1318, observed 269.1321.
3-Amino-4-methoxy-N-methylbenzenesulfonamide (31a).

Methylamine (2 M in THF, 0.33 mL, 0.67 mmol) was added at 0 °C to a solution of 4-methoxy-3-(2,2,2-trifluoroacetamido)benzenesulfonyl chloride 21 (0.106 g, 0.334 mmol) in THF (2 mL). The reaction mixture was warmed to room temperature and stirred overnight. Further methylamine (2 M in THF, 2.0 mL, 4.0 mmol) was added, and the reaction mixture heated under reflux for 1 hour. Further methylamine (2 M in THF, 1.0 mL, 2.0 mmol) was added at room temperature, and the reaction mixture heated under reflux for 30 minutes. The reaction mixture was concentrated *in vacuo*, then ethanol (5 mL), water (5 mL) and aqueous HCl (37.5% w/v, 5 mL) were added. The reaction mixture was heated under reflux for 3 hours 30 minutes. The reaction mixture was adjusted to pH 14 by the dropwise addition of aqueous NaOH (10% w/v) at room temperature, then concentrated *in vacuo* to remove ethanol. The product was extracted into ethyl acetate (3 x 25 mL). The combined organic extracts were dried (MgSO₄) and concentrated *in vacuo*. Purification by flash column chromatography (25 – 75% ethyl acetate in petroleum ether) afforded 31a (59 mg, 82% yield).

LCMS (ESI+): m/z 217.2 [M + H]+, rt 1.23 minutes, >99%; ¹H NMR (400 MHz, CD₃CN) 7.12-7.06 (2H, m), 6.92 (1H, d, J = 8.0 Hz), 5.28-5.07 (1H, m), 4.39 (2H, br s), 3.88 (3H, s), 2.46 (3H, d, J = 5.3 Hz); ¹³C NMR (100 MHz, CD₃CN) 150.8, 138.8, 131.8, 117.8, 112.5, 110.7, 56.5, 29.6; νmax/cm⁻¹ 3388 (N-H), 3306 (N-H), 3043, 2919, 2840, 1590, 1509; HRMS (ESI+): m/z calculated for [C₈H₁₂N₂O₃S + Na]⁺ = 239.0461, observed 239.0463.
5-(Azocan-1-ylsulfonyl)-2-methoxyaniline (31b).

Heptamethyleneimine (95 μL, 0.76 mmol) was added dropwise at 0 °C to a suspension of sodium hydride (60% in mineral oil, 76 mg, 1.9 mmol) in DMF (2 mL). The reaction mixture was stirred at 0 °C over 20 minutes. A solution of 4-methoxy-3-(2,2,2-trifluoroacetamido)benzenesulfonyl chloride 21 (0.200 g, 0.630 mmol) in DMF (1 mL) was added dropwise at 0 °C to the reaction mixture. The reaction mixture was warmed to room temperature and stirred over 2 hours. Ethanol (5 mL) was added dropwise at 0 °C to the reaction mixture, followed by water (5 mL) and aqueous HCl (37.5% w/v, 5 mL). The reaction mixture was heated under reflux for 17 hours. The reaction mixture was adjusted to pH 9 by the dropwise addition of aqueous NaOH (10% w/v) at room temperature, then concentrated in vacuo to remove ethanol. The mixture was diluted with ethyl acetate (25 mL), and the resultant aqueous layer discarded. The organic layer was washed with water (2 x 25 mL) and brine (25 mL), dried (MgSO₄) and concentrated in vacuo. Purification by flash column chromatography (0 – 50% ethyl acetate in petroleum ether) afforded 31b (0.130 g, 69% yield).

LCMS (ESI+): m/z 299.2 [M + H]⁺, rt 1.96 minutes, >99%; ¹H NMR (400 MHz, CDCl₃) 7.16 (1H, dd, J = 8.4, 2.2 Hz), 7.09 (1H, d, J = 2.2 Hz), 6.81 (1H, d, J = 8.3 Hz), 3.97 (2H, br s), 3.89 (3H, s), 3.11 (4H, t, J = 5.8 Hz), 1.75-1.57 (10H, m); ¹³C NMR (100 MHz, CDCl₃) 150.1, 136.7, 130.8, 118.2, 112.9, 109.7, 55.8, 48.8, 28.0, 26.8, 25.3; ν_max/cm⁻¹ 3488 (N-H), 3383 (N-H), 2914, 2851, 1729, 1611, 1577, 1512; HRMS (ESI+): m/z calculated for [C₁₄H₂₂N₂O₃S + H]⁺ = 299.1424, observed 299.1429.
2-Methoxy-5-(piperidin-1-ylsulfonyl)aniline (31c).

\[
\begin{array}{c}
O=S-N\\
\hline
\end{array}
\]

Piperidine (62 µL, 0.63 mmol) was added dropwise at 0 °C to a suspension of sodium hydride (60% in mineral oil, 76 mg, 1.9 mmol) in DMF (1 mL). The reaction mixture was stirred at 0 °C over 20 minutes. A solution of 4-methoxy-3-(2,2,2-trifluoroacetamido)benzenesulfonyl chloride 21 (0.200 g, 0.630 mmol) in DMF (2 mL) was added dropwise at 0 °C to the reaction mixture. The reaction mixture was warmed to room temperature and stirred over 20 hours. Ethanol (5 mL) was added dropwise at 0 °C to the reaction mixture, followed by water (5 mL) and aqueous HCl (37.5% w/v, 5 mL). The reaction mixture was heated under reflux for 9 hours. The reaction mixture was adjusted to pH 7 by the dropwise addition of aqueous NaOH (10% w/v) at room temperature, then concentrated \textit{in vacuo}. Water (15 mL) was added to the crude residue. The product was extracted into DCM (3 x 20 mL). The combined organic extracts were dried (MgSO\textsubscript{4}) and concentrated \textit{in vacuo}. Purification by flash column chromatography (20 – 50% ethyl acetate in petroleum ether) afforded 31c (0.104 g, 61% yield).

LCMS (ESI+): m/z 271.2 [M + H]\textsuperscript{+}, rt 1.86 minutes, >99%; \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) 7.13 (1H, dd, J = 8.4, 2.1 Hz), 7.05 (1H, d, J = 2.2 Hz), 6.83 (1H, d, J = 8.4 Hz), 3.97 (2H, br s), 3.91 (3H, s), 2.95 (4H, t, J = 5.4 Hz), 1.63 (4H, quin, J = 5.7 Hz), 1.45-1.36 (2H, m); \textsuperscript{13}C NMR (125 MHz, CDCl\textsubscript{3}) 150.3, 136.6, 128.2, 118.9, 113.3, 109.7, 55.9, 47.1, 25.3, 23.7; \textsuperscript{\nu}_{\text{max}}/\text{cm}^{-1} 3475 (N-H), 3372 (N-H), 2939, 2851, 1616, 1581, 1510; HRMS (ESI+)\textsuperscript{+}: m/z calculated for [C\textsubscript{12}H\textsubscript{18}N\textsubscript{2}O\textsubscript{3}S + Na]\textsuperscript{+} = 293.0930, observed 293.0935.
1-Methylpiperazine (84 µL, 0.76 mmol) was added dropwise at 0 °C to a suspension of sodium hydride (60% in mineral oil, 76 mg, 1.9 mmol) in DMF (2 mL). The reaction mixture was stirred at 0 °C over 20 minutes. A solution of 4-methoxy-3-(2,2,2-trifluoroacetamido)benzenesulfonyl chloride 21 (0.200 g, 0.630 mmol) in DMF (1 mL) was added dropwise at 0 °C to the reaction mixture. The reaction mixture was warmed to room temperature and stirred over 2 hours. Ethanol (5 mL) was added dropwise at 0 °C to the reaction mixture, followed by water (5 mL) and aqueous HCl (37.5% w/v, 5 mL). The reaction mixture was heated under reflux for 18 hours. The reaction mixture was adjusted to pH 9 by the dropwise addition of aqueous NaOH (10% w/v) at room temperature, then concentrated in vacuo to remove ethanol. The mixture was diluted with ethyl acetate (25 mL), and the resultant aqueous layer discarded. The organic layer was washed with water (2 x 25 mL) and brine (25 mL), dried (MgSO₄) and concentrated in vacuo. Purification by flash column chromatography (0 – 10% methanol in DCM) afforded 31d (0.120 g, 65% yield).

LCMS (ESI+): m/z 286.3 [M + H]⁺, rt 0.47 minutes, 97%; ¹H NMR (500 MHz, (CD₃)₂SO) 6.99-6.95 (2H, m), 6.88 (1H, dd, J = 8.5, 2.3 Hz), 5.23 (2H, s), 3.84 (3H, s), 2.82 (4H, br s), 2.34 (4H, t, J = 4.4 Hz), 2.13 (3H, s); ¹³C NMR (125 MHz, (CD₃)₂SO) 149.5, 138.3, 126.3, 116.1, 111.5, 109.9, 55.6, 53.6, 45.7, 45.3; v_max/cm⁻¹ 3481 (N-H), 3376 (N-H), 2919, 2842, 2795, 1609, 1576, 1516; HRMS (ESI+): m/z calculated for [C₁₂H₁₉N₃O₃S + H]⁺ = 286.1220, observed 286.1225.
2-Methoxy-5-(morpholinosulfonyl)aniline (31e).

![Chemical structure](image)

Morpholine (66 µL, 0.76 mmol) was added dropwise at 0 °C to a suspension of sodium hydride (60% in mineral oil, 76 mg, 1.9 mmol) in DMF (2 mL). The reaction mixture was stirred at 0 °C over 20 minutes. A solution of 4-methoxy-3-(2,2,2-trifluoroacetamido)benzenesulfonyl chloride 21 (0.200 g, 0.630 mmol) in DMF (1 mL) was added dropwise at 0 °C to the reaction mixture. The reaction mixture was warmed to room temperature and stirred over 2 hours. Ethanol (5 mL) was added dropwise at 0 °C to the reaction mixture, followed by water (5 mL) and aqueous HCl (37.5% w/v, 5 mL). The reaction mixture was heated under reflux for 18 hours. The reaction mixture was adjusted to pH 9 by the dropwise addition of aqueous NaOH (10% w/v) at room temperature, then concentrated in vacuo to remove ethanol. The mixture was diluted with ethyl acetate (25 mL), and the resultant aqueous layer discarded. The organic layer was washed with water (2 x 25 mL) and brine (25 mL), dried (MgSO₄) and concentrated in vacuo. Purification by flash column chromatography (20 – 60% ethyl acetate in petroleum ether) afforded 31e (0.117 g, 68% yield).

LCMS (ESI+): m/z 273.2 [M + H]⁺, rt 1.40 minutes, >99%; ¹H NMR (500 MHz, (CD₃)₂SO) 7.01-6.96 (2H, m), 6.89 (1H, dd, J = 8.3, 2.3 Hz), 5.25 (2H, s), 3.85 (3H, s), 3.62 (4H, t, J = 4.8 Hz), 2.80 (4H, t, J = 4.7 Hz); ¹³C NMR (125 MHz, (CD₃)₂SO) 149.6, 138.4, 125.9, 116.2, 111.5, 109.9, 65.3, 55.6, 45.9; νₘₐₓ/cm⁻¹ 3486 (N-H), 3390 (N-H), 2924, 2864, 1691, 1611, 1507; HRMS (ESI)+: m/z calculated for [C₁₁H₁₆N₂O₄S + Na]⁺ = 295.0723, observed 295.0719.
2-Methoxy-5-(thiomorpholinosulfonyl)aniline (31f).

![Chemical Structure](attachment:structure.png)

Thiomorpholine (76 µL, 0.76 mmol) was added dropwise at 0 °C to a suspension of sodium hydride (60% in mineral oil, 76 mg, 1.9 mmol) in DMF (2 mL). The reaction mixture was stirred at 0 °C over 20 minutes. A solution of 4-methoxy-3-(2,2,2-trifluoroacetamido)benzenesulfonyl chloride 21 (0.200 g, 0.630 mmol) in DMF (1 mL) was added dropwise at 0 °C to the reaction mixture. The reaction mixture was warmed to room temperature and stirred over 2 hours. Ethanol (5 mL) was added dropwise at 0 °C to the reaction mixture, followed by water (5 mL) and aqueous HCl (37.5% w/v, 5 mL). The reaction mixture was heated under reflux for 17 hours. The reaction mixture was adjusted to pH 9 by the dropwise addition of aqueous NaOH (10% w/v) at room temperature, then concentrated in vacuo to remove ethanol. The mixture was diluted with ethyl acetate (50 mL), washed with water (3 x 25 mL) and brine (25 mL), dried (MgSO₄) and concentrated in vacuo. Purification by flash column chromatography (0 – 50% ethyl acetate in petroleum ether) afforded 31f (0.126 g, 67% yield).

**LCMS (ESI+):** m/z 289.2 [M + H]^+, rt 1.66 minutes, 96%; **^1H NMR (400 MHz, CDCl₃)** 7.11 (1H, dd, J = 8.4, 2.2 Hz), 7.02 (1H, d, J = 2.2 Hz), 6.83 (1H, d, J = 8.4 Hz), 4.02 (2H, br s), 3.91 (3H, s), 3.31 (4H, t, J = 4.9 Hz), 2.69 (4H, t, J = 5.2 Hz); **^13C NMR (100 MHz, CDCl₃)** 150.5, 137.0, 128.6, 118.5, 112.8, 109.8, 55.9, 48.1, 27.5; \(\nu_{\text{max}}/\text{cm}^{-1}\) 3489 (N-H), 3389 (N-H), 2970, 2914, 2852, 1730, 1611, 1577, 1512; **HRMS (ESI)+**: m/z calculated for \([C_{11}H_{16}N_{2}O_{3}S_{2} + H]^+\) = 289.0675, observed 289.0688.
4-((3-Amino-4-methoxyphenyl)sulfonyl)thiomorpholine 1,1-dioxide (31g).

![Chemical Structure](image)

Triethylamine (0.66 mL, 4.7 mmol) and DCM (20 mL) were added to a mixture of thiomorpholine 1,1-dioxide (0.153 g, 1.13 mmol), 4-methoxy-3-(2,2,2-trifluoroacetamido)benzenesulfonyl chloride 21 (0.300 g, 0.944 mmol) and DMAP (35 mg, 0.28 mmol). The reaction mixture was stirred over 30 minutes, then concentrated in vacuo. Ethanol (30 mL), water (30 mL) and aqueous HCl (37.5% w/v, 30 mL) were added, and the reaction mixture heated under reflux for 3 hours. The reaction mixture was concentrated in vacuo to remove ethanol, then adjusted to pH 14 by the dropwise addition of aqueous NaOH (10% w/v). The product was extracted into DCM (3 x 100 mL). The combined organic extracts were washed (brine), dried (MgSO₄) and concentrated in vacuo. Purification by flash column chromatography (20 – 70% ethyl acetate in petroleum ether, 0 – 7% methanol in DCM) afforded 31g (0.250 g, 80% yield).

**LCMS (ESI+):** m/z 321.2 [M + H]+, rt 1.44 minutes, 97%; ¹H NMR (400 MHz, (CD₃)₂SO) 7.03-6.92 (3H, m), 5.27 (2H, s), 3.85 (3H, s), 3.42-3.30 (4H, m), 3.22 (4H, t, J = 5.0 Hz); ¹³C NMR (100 MHz, (CD₃)₂SO) 150.1, 138.9, 127.4, 116.2, 111.0, 110.4, 55.9, 50.2, 45.3; νmax/cm⁻¹ 3458 (N-H), 3369 (N-H), 2907, 2849, 1616, 1579, 1510; HRMS (ESI)+: m/z calculated for [C₁₁H₁₆N₂O₅S₂ + Na]⁺ = 343.0393, observed 343.0394.
3-Amino-4-methoxy-N-phenylbenzenesulfonamide (31h).

Aniline (43 µL, 0.47 mmol) was added dropwise at 0 °C to a suspension of sodium hydride (60% in mineral oil, 57 mg, 1.4 mmol) in DMF (1 mL). The reaction mixture was stirred at 0 °C over 20 minutes. A solution of 4-methoxy-3-(2,2,2-trifluoroacetamido)benzenesulfonyl chloride 21 (0.150 g, 0.472 mmol) in DMF (2 mL) was added dropwise at 0 °C to the reaction mixture. The reaction mixture was warmed to room temperature and stirred over 1 hour. Ethanol (5 mL) was added dropwise at 0 °C to the reaction mixture, followed by water (5 mL) and aqueous HCl (37.5% w/v, 5 mL). The reaction mixture was heated under reflux for 10 hours. The reaction mixture was adjusted to pH 9 by the dropwise addition of aqueous NaOH (10% w/v) at room temperature, then concentrated in vacuo to remove ethanol. The product was extracted into DCM (3 x 25 mL). The combined organic extracts were washed (brine), dried (MgSO₄) and concentrated in vacuo. Purification by flash column chromatography (0 – 75% ethyl acetate in petroleum ether) afforded 31h (50 mg, 37% yield).

LCMS (ESI+): m/z 279.2 [M + H]^+; (ESI-): m/z 277.1 [M - H]^-; rt 1.77 minutes, 97%; ^1H NMR (400 MHz, CDCl₃) 7.25-7.19 (2H, m), 7.16 (1H, dd, J = 8.4, 2.3 Hz), 7.12-7.04 (4H, m), 6.82 (1H, s), 6.72 (1H, d, J = 8.5 Hz), 3.85 (3H, s), 3.54 (1H, br s); ^13C NMR (100 MHz, CDCl₃) 150.7, 136.9, 136.7, 131.0, 129.4, 125.2, 121.6, 118.7, 112.7, 109.6, 55.8; v_max/cm⁻¹ 3380 (N-H), 3250 (N-H), 1615, 1598, 1508; HRMS (ESI)+: m/z calculated for [C₁₃H₁₄N₂O₃S + H]^+ = 279.0798, observed 279.0796.
3-Amino-N-benzyl-4-methoxybenzenesulfonamide (31i).

Benzylamine (52 µL, 0.47 mmol) was added dropwise at 0 °C to a suspension of sodium hydride (60% in mineral oil, 57 mg, 1.4 mmol) in DMF (1 mL). The reaction mixture was stirred at 0 °C over 20 minutes. A solution of 4-methoxy-3-(2,2,2-trifluoroacetamido)benzenesulfonyl chloride 21 (0.150 g, 0.472 mmol) in DMF (2 mL) was added dropwise at 0 °C to the reaction mixture. The reaction mixture was warmed to room temperature and stirred over 1 hour. Ethanol (5 mL) was added dropwise at 0 °C to the reaction mixture, followed by water (5 mL) and aqueous HCl (37.5% w/v, 5 mL). The reaction mixture was heated under reflux for 10 hours. The reaction mixture was adjusted to pH 7 by the dropwise addition of aqueous NaOH (10% w/v) at room temperature, then concentrated in vacuo to remove ethanol. The product was extracted into DCM (3 x 25 mL). The combined organic extracts were washed (brine), dried (MgSO₄) and concentrated in vacuo. Purification by flash column chromatography (0 – 10% methanol in DCM) afforded 31i (89 mg, 59% yield).

LCMS (ESI+): m/z 293.2 [M + H]+, (ESI-): m/z 291.1 [M - H]-, rt 1.80 minutes, 92%; ¹H NMR (400 MHz, CDCl₃) 7.31-7.18 (6H, m), 7.16 (1H, d, J = 2.3 Hz), 6.82 (1H, d, J = 8.5 Hz), 4.59 (1H, t, J = 6.2 Hz), 4.08 (2H, d, J = 6.3 Hz), 3.91 (3H, s); ¹³C NMR (100 MHz, CDCl₃) 150.5, 137.0, 136.6, 131.6, 128.8, 128.1, 128.0, 118.4, 112.6, 109.8, 55.9, 47.5; ¹H NMR spectroscopic data consistent with literature. ⁴
1,2,3,4-Tetrahydroisoquinoline (95 µL, 0.76 mmol) was added dropwise at 0 °C to a suspension of sodium hydride (60% in mineral oil, 76 mg, 1.9 mmol) in DMF (2 mL). The reaction mixture was stirred at 0 °C over 20 minutes. A solution of 4-methoxy-3-(2,2,2-trifluoroacetamido)benzenesulfonyl chloride 21 (0.200 g, 0.630 mmol) in DMF (1 mL) was added dropwise at 0 °C to the reaction mixture. The reaction mixture was warmed to room temperature and stirred over 1 hour. Ethanol (5 mL) was added dropwise at 0 °C to the reaction mixture, followed by water (5 mL) and aqueous HCl (37.5% w/v, 5 mL). The reaction mixture was heated under reflux for 24 hours, then further ethanol (10 mL), water (10 mL) and aqueous HCl (37.5% w/v, 10 mL) were added. The reaction mixture was heated under reflux for 24 hours. The reaction mixture was adjusted to pH 10 by the dropwise addition of aqueous NaOH (10% w/v) at room temperature, then concentrated in vacuo to remove ethanol. The mixture was diluted with ethyl acetate (50 mL), and the resultant aqueous layer discarded. The organic layer was washed with water (2 x 50 mL) and brine (50 mL), dried (MgSO₄) and concentrated in vacuo. Purification by flash column chromatography (0 – 30% ethyl acetate in petroleum ether) afforded 31j (0.146 g, 73% yield).

LCMS (ESI+): m/z 319.2 [M + H]^+, rt 1.94 minutes, >99%; ^1^H NMR (400 MHz, (CD₃)₂SO) 7.18-7.09 (4H, m), 7.07 (1H, d, J = 1.9 Hz), 7.02-6.94 (2H, m), 5.23 (2H, s), 4.10 (2H, s), 3.83 (3H, s), 3.20 (2H, t, J = 5.9 Hz), 2.86 (2H, t, J = 5.9 Hz); ^1^C NMR (100 MHz, (CD₃)₂SO) 149.5, 138.4, 133.0, 131.7, 128.7, 127.1, 126.6, 126.4, 126.1, 116.1, 111.4, 110.0,
55.6, 47.3, 43.6, 28.2; $\nu_{\text{max}}/\text{cm}^{-1}$ 3457 (N-H), 3363 (N-H), 1624, 1583, 1505; HRMS (ESI)$^+$: m/z calculated for $[C_{16}H_{18}N_2O_3S + H]^+ = 319.1111$, observed 319.1122.

5-((6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)sulfonyl)-2-methoxyaniline (31k).

6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (0.174 g, 0.756 mmol) was added portionwise at 0 °C to a suspension of sodium hydride (60% in mineral oil, 0.126 g, 3.15 mmol) in DMF (3 mL). The reaction mixture was stirred at 0 °C over 20 minutes. A solution of 4-methoxy-3-(2,2,2-trifluoroacetamido)benzenesulfonyl chloride 21 (0.200 g, 0.630 mmol) in DMF (2 mL) was added dropwise at 0 °C to the reaction mixture. The reaction mixture was warmed to room temperature and stirred over 1 hour. Ethanol (15 mL) was added dropwise at 0 °C to the reaction mixture, followed by water (15 mL) and aqueous HCl (37.5% w/v, 15 mL). The reaction mixture was heated under reflux for 20 hours. The reaction mixture was concentrated in vacuo to remove ethanol, then adjusted to pH 14 by the dropwise addition of aqueous NaOH (10% w/v). The mixture was diluted with ethyl acetate (50 mL), and the resultant aqueous layer discarded. The organic layer was washed with water (2 x 50 mL) and brine (50 mL), dried (MgSO$_4$) and concentrated in vacuo. Purification by flash column chromatography (0 – 100% ethyl acetate in petroleum ether) afforded 31k (0.194 g, 81% yield).

LCMS (ESI$^+$): m/z 379.3 [M + H]$^+$, rt 1.80 minutes, >99%; $^1$H NMR (400 MHz, CDCl$_3$) 7.21 (1H, dd, J = 8.4, 2.2 Hz), 7.13 (1H, d, J = 2.2 Hz), 6.84 (1H, d, J = 8.4 Hz), 6.55 (1H, s), 6.50 (1H, s), 4.15 (2H, s), 4.01 (2H, br s), 3.90 (3H, s), 3.82 (3H, s), 3.81 (3H, s), 3.31 (2H, t, J = 5.9 Hz), 2.84 (2H, t, J = 5.8 Hz); $^{13}$C NMR (100 MHz, CDCl$_3$) 150.5, 147.9, 147.8, 136.8,
128.1, 125.2, 123.8, 118.9, 119.8, 109.2, 56.1, 56.0, 55.9, 47.5, 44.0, 28.7; ν\text{max}/\text{cm}^{-1} 3483 (N-H), 3365 (N-H), 2966, 2930, 2842, 1661, 1611, 1578, 1512; HRMS (ESI)+: m/z calculated for [C_{18}H_{22}N_{2}O_{5}S + Na]^+ = 401.1142, observed 401.1124.

2-Methoxy-5-((1,2,4,5-tetrahydro-3H-benzo[d]azepin-3-yl)sulfonyl)aniline (31l).

A solution of 2,3,4,5-tetrahydro-1H-benzo[d]azepine (0.167 g, 1.13 mmol) in DMF (1 mL) was added dropwise at 0 °C to a suspension of sodium hydride (60% in mineral oil, 0.113 g, 2.83 mmol) in DMF (2 mL). The reaction mixture was stirred at 0 °C over 20 minutes. A solution of 4-methoxy-3-(2,2,2-trifluoroacetamido)benzenesulfonyl chloride 21 (0.300 g, 0.944 mmol) in DMF (2 mL) was added dropwise at 0 °C to the reaction mixture. The reaction mixture was warmed to room temperature and stirred over 30 minutes. Ethanol (30 mL) was added dropwise at 0 °C to the reaction mixture, followed by water (30 mL) and aqueous HCl (37.5% w/v, 30 mL). The reaction mixture was heated under reflux for 15 hours. The reaction mixture was concentrated in vacuo to remove ethanol, then adjusted to pH 8 by the dropwise addition of aqueous NaOH (10% w/v). The mixture was diluted with ethyl acetate (100 mL), and the resultant aqueous layer discarded. The organic layer was washed with water (2 x 100 mL) and brine (100 mL), dried (MgSO\textsubscript{4}) and concentrated in vacuo. Purification by flash column chromatography (0 – 50% ethyl acetate in petroleum ether) afforded 31l (0.131 g, 42% yield).

LCMS (ESI+): m/z 333.2 [M + H]^+, rt 2.07 minutes, >99%; \textsuperscript{1}H NMR (500 MHz, (CD\textsubscript{3})\textsubscript{2}SO) 7.10 (4H, s), 7.00-6.96 (1H, m), 6.93-6.88 (2H, m), 5.17 (2H, s), 3.80 (3H, s), 3.17-3.06 (4H, m), 2.96-2.87 (4H, m); \textsuperscript{13}C NMR (125 MHz, (CD\textsubscript{3})\textsubscript{2}SO) 149.3, 140.5, 138.3, 129.1, 129.0,
126.5, 115.5, 110.9, 109.9, 55.5, 48.2, 35.6; $\nu_{\text{max}}/\text{cm}^{-1}$ 3459 (N-H), 3367 (N-H), 2906, 2850, 1615, 1579, 1509; HRMS (ESI)+: m/z calculated for [C$_{17}$H$_{20}$N$_2$O$_3$S + Na]$^+$ = 355.1087, observed 355.1085.

2-Methoxy-5-((7-methoxy-1,2,4,5-tetrahydro-3H-benzo[d]azepin-3-yl)sulfonyl)aniline (31m).

Triethylamine (0.29 mL, 2.1 mmol) and DCM (10 mL) were added to a mixture of 7-methoxy-2,3,4,5-tetrahydro-1H-benzo[d]azepine hydrochloride (96 mg, 0.45 mmol), 4-methoxy-3-(2,2,2-trifluoroacetamido)benzenesulfonyl chloride 21 (0.130 g, 0.409 mmol) and DMAP (10 mg, 0.082 mmol). The reaction mixture was stirred over 1 hour, then concentrated in vacuo. Ethanol (30 mL), water (30 mL) and aqueous HCl (37.5% w/v, 30 mL) were added, and the reaction mixture heated under reflux for 3 hours. The reaction mixture was adjusted to pH 10 by the dropwise addition of Na$_2$CO$_3$ solution at 0 °C and extracted into DCM (3 x 100 mL). The combined organic extracts were washed (brine), dried (MgSO$_4$) and concentrated in vacuo. Purification by flash column chromatography (20 – 50% ethyl acetate in petroleum ether) afforded 31m (0.111 g, 75% yield).

LCMS (ESI+): m/z 363.3 [M + H]$^+$, rt 2.01 minutes, >99%; $^1$H NMR (500 MHz, (CD$_3$)$_2$SO) 7.00 (1H, d, J = 8.3 Hz), 6.97 (1H, d, J = 1.9 Hz), 6.93-6.87 (2H, m), 6.69 (1H, d, J = 2.7 Hz), 6.64 (1H, dd, J = 8.3, 2.7 Hz), 5.17 (2H, s), 3.80 (3H, s), 3.67 (3H, s), 3.16-3.02 (4H, m), 2.91-2.77 (4H, m); $^{13}$C NMR (125 MHz, (CD$_3$)$_2$SO) 157.8, 149.3, 141.8, 138.4, 132.5, 130.2, 129.0, 115.6, 115.0, 111.2, 110.9, 109.9, 55.6, 55.0, 48.7, 48.2, 35.8, 34.7; $\nu_{\text{max}}/\text{cm}^{-1}$ 3474 (N-
H), 3443, 3367 (N-H), 2944, 2906, 2845, 1613, 1579, 1506; HRMS (ESI)+: m/z calculated for [C\textsubscript{18}H\textsubscript{22}N\textsubscript{2}O\textsubscript{4}S + Na]\textsuperscript{+} = 385.1192, observed 385.1192.

2-Methoxy-5-((1,2,4,5-tetrahydro-3H-1,5-methanobenzo[d]azepin-3-yl)sulfonyl)aniline (31n).

![Chemical Structure]

Triethylamine (0.33 mL, 2.4 mmol) and DCM (10 mL) were added to a mixture of 2,3,4,5-tetrahydro-1H-1,5-methan-3-0-benzazepine hydrochloride (0.102 g, 0.519 mmol), 4-methoxy-3-(2,2,2-trifluoroacetamido)benzenesulfonyl chloride 21 (0.150 g, 0.472 mmol) and DMAP (12 mg, 0.094 mmol). The reaction mixture was stirred over 1 hour, then concentrated in vacuo. Ethanol (30 mL), water (30 mL) and aqueous HCl (37.5% w/v, 30 mL) were added, and the reaction mixture heated under reflux for 16 hours. The reaction mixture was adjusted to pH 10 by the dropwise addition of Na\textsubscript{2}CO\textsubscript{3} solution at 0 °C and extracted into DCM (3 x 100 mL). The combined organic extracts were washed (brine), dried (MgSO\textsubscript{4}) and concentrated in vacuo. Purification by flash column chromatography (0 – 50% ethyl acetate in petroleum ether) afforded 31n (0.118 g, 65% yield).

LCMS (ESI+): m/z 345.2 [M + H]\textsuperscript{+}, rt 2.05 minutes, >99%; \textsuperscript{1}H NMR (500 MHz, (CD\textsubscript{3})\textsubscript{2}SO) 7.25-7.14 (4H, m), 6.88 (1H, d, J = 8.5 Hz), 6.84 (1H, d, J = 2.3 Hz), 6.68 (1H, dd, J = 8.3, 2.3 Hz), 5.14 (2H, s), 3.83 (3H, s), 3.46-3.38 (2H, m), 3.23-3.16 (2H, m), 2.74 (2H, dd, J = 10.7, 1.3 Hz), 2.14-2.04 (1H, m), 1.50 (1H, d, J = 10.8 Hz); \textsuperscript{13}C NMR (125 MHz, (CD\textsubscript{3})\textsubscript{2}SO) 149.2, 144.4, 138.1, 128.0, 126.9, 122.4, 115.6, 111.2, 109.8, 55.6, 49.3, 41.5 (1 peak missing); \textit{v}_{max}/\text{cm}^{-1} 3455 (N-H), 3364 (N-H), 2950, 2854, 1733, 1621, 1578, 1509; HRMS (ESI)+: m/z calculated for [C\textsubscript{18}H\textsubscript{20}N\textsubscript{2}O\textsubscript{3}S + Na]\textsuperscript{+} = 367.1087, observed 367.1092.
Azepan-1-yl(4-hydroxy-3-nitrophenyl)methanone (33).

T3P® (50 wt. % in DMF, 3.3 mL, 5.5 mmol) and N,N-diisopropylethylamine (0.95 mL, 5.5 mmol) were added to a solution of 4-hydroxy-3-nitrobenzoic acid 32 (0.500 g, 2.73 mmol) and hexamethylenimine (0.61 mL, 5.5 mmol) in DMF (2 mL). The reaction mixture was stirred over 1 day, then diluted with water (15 mL), adjusted to pH 2 and extracted into DCM (3 x 20 mL). The combined organic extracts were dried (MgSO₄) and concentrated in vacuo. Purification by flash column chromatography (0 – 10% methanol in DCM) afforded 33 (0.174 g, 24% yield).

LCMS (ESI+): m/z 265.3 [M + H]^+, (ESI-): m/z 263.2 [M - H]^-, rt 1.84 minutes, >99%; ¹H NMR (400 MHz, CD₃CN) 10.34 (1H, br s), 8.10 (1H, d, J = 2.0 Hz), 7.65 (1H, dd, J = 8.7, 2.2 Hz), 7.20 (1H, d, J = 8.6 Hz), 3.58 (2H, t, J = 5.7 Hz), 3.37 (2H, t, J = 5.4 Hz), 1.83-1.71 (2H, m), 1.68-1.51 (6H, m); ¹³C NMR (100 MHz, CD₃CN) 169.3, 155.8, 136.8, 134.5, 130.8, 124.4, 120.9, 50.5, 46.9, 30.0, 28.4, 28.0, 27.0; v max/cm⁻¹ 2927, 2857, 1623 (C=O), 1531 (N=O), 1350 (N=O); HRMS (ESI+): m/z calculated for [C₁₃H₁₆N₂O₄ + H]^+ = 265.1183, observed 265.1184.

Azepan-1-yl(4-methoxy-3-nitrophenyl)methanone (34).
Dimethyl sulfate (0.115 mL, 1.21 mmol) was added to a suspension of azepan-1-yl(4-hydroxy-3-nitrophenyl)methanone 33 (0.160 g, 0.605 mmol) and potassium carbonate (0.167 g, 1.21 mmol) in acetone (5 mL). The reaction mixture was heated under reflux for 2 hours. The reaction mixture was diluted with water (15 mL) at 0 °C, then concentrated \textit{in vacuo} to remove acetone. The product was extracted into DCM (3 x 20 mL). The combined organic extracts were dried (MgSO$_4$) and concentrated \textit{in vacuo}. Purification by flash column chromatography (40 – 100% ethyl acetate in petroleum ether) afforded 34 (0.129 g, 77% yield).

LCMS (ESI+): m/z 279.3 [M + H]$^+$, rt 1.90 minutes, >99%; $^1$H NMR (400 MHz, CD$_3$CN) 7.83 (1H, d, J = 2.1 Hz), 7.62 (1H, dd, J = 8.6, 2.1 Hz), 7.27 (1H, d, J = 8.7 Hz), 3.96 (3H, s), 3.58 (2H, t, J = 5.7 Hz), 3.37 (2H, t, J = 5.5 Hz), 1.83-1.71 (2H, m), 1.67-1.50 (6H, m); $^{13}$C NMR (100 MHz, CD$_3$CN) 169.4, 153.9, 140.1, 133.6, 130.7, 124.7, 114.9, 57.6, 50.5, 46.8, 30.0, 28.5, 28.0, 27.0; $\nu_{\text{max}}$/cm$^{-1}$ 2928, 2854, 1615 (C=O), 1530 (N=O), 1350 (N=O); HRMS (ESI)+: m/z calculated for [C$_{14}$H$_{18}$N$_2$O$_4$ + H]$^+$ = 279.1339, observed 279.1345.

(3-Amino-4-methoxyphenyl)(azepan-1-yl)methanone (35).

Sodium borohydride (22 mg, 0.59 mmol) was added portionwise at 0 °C to a suspension of NiCl$_2$·6H$_2$O (47 mg, 0.20 mmol) in methanol (1 mL). The reaction mixture was warmed to room temperature and stirred over 20 minutes. A solution of azepan-1-yl(4-methoxy-3-nitrophenyl)methanone 34 (0.109 g, 0.392 mmol) in methanol (1 mL) was added at 0 °C to the reaction mixture, followed by sodium borohydride (52 mg, 1.4 mmol). The reaction
mixture was warmed to room temperature and stirred over 1 hour, then further sodium borohydride (30 mg, 0.78 mmol) was added at 0 °C. The reaction mixture was warmed to room temperature and stirred over 30 minutes. Water (15 mL) was added at 0 °C, and the reaction mixture filtered through celite. The filtrate was extracted into DCM (3 x 20 mL). The combined organic extracts were dried (MgSO₄) and concentrated in vacuo. Purification by flash column chromatography (20 – 100% ethyl acetate in petroleum ether) afforded 35 (62 mg, 64% yield).

LCMS (ESI+): m/z 249.3 [M + H]⁺, rt 1.61 minutes, >99%; ¹H NMR (500 MHz, CD₃CN) 6.80 (1H, d, J = 8.1 Hz), 6.65 (1H, d, J = 2.0 Hz), 6.62 (1H, dd, J = 8.1, 2.1 Hz), 4.16 (2H, br s), 3.83 (3H, s), 3.61-3.48 (2H, m), 3.44-3.29 (2H, m), 1.82-1.67 (2H, m), 1.66-1.48 (6H, m); ¹³C NMR (125 MHz, CD₃CN) 172.2, 148.3, 138.0, 131.5, 116.6, 113.3, 110.8, 56.2, 50.4, 46.6, 30.2, 28.5, 28.1, 27.0; νmax/cm⁻¹ 3466 (N-H), 3330 (N-H), 2922, 2853, 1608 (C=O), 1584, 1516; HRMS (ESI)+: m/z calculated for [C₁₄H₂₀N₂O₂ + H]⁺ = 249.1598, observed 249.1600.

References


Biochemical Assay Dose-response Curves

Figure S1: Dose-response curves for 1 and 15g, with data points representing an average of replicates (n = 6) and error bars indicating standard errors of the mean.
**Figure S2:** Dose-response curves for 16a and 16b, with data points representing an average of replicates (n = 6) and error bars indicating standard errors of the mean.
Figure S3: Dose-response curves for 16c and 16d, with data points representing an average of replicates (n = 6) and error bars indicating standard errors of the mean.
Figure S4: Dose-response curves for 16e and 16f, with data points representing an average of replicates (n = 6) and error bars indicating standard errors of the mean.
Figure S5: Dose-response curves for 16g and 16h, with data points representing an average of replicates (n = 6) and error bars indicating standard errors of the mean.
Figure S6: Dose-response curves for 16j and 16k, with data points representing an average of replicates (n = 6) and error bars indicating standard errors of the mean.
Figure S7: Dose-response curves for 16l and 16m, with data points representing an average of replicates (n = 6) and error bars indicating standard errors of the mean.
**X-ray Crystallography**

**Figure S8:** Stereo views of the X-ray crystal structure of *Mtb* fumarase in complex with 1 (PDB code 5F91, subunit A = white, subunit B = green, subunit C = cyan, subunit D = yellow, 1 = lilac), illustrating the interactions (yellow dashed lines) of 1 in the allosteric site with a focus on a) the whole allosteric site and b) the interactions of the methoxy group of 1.
**Figure S9:** a) X-ray crystal structure of *Mtb* fumarase in complex with **16h** (PDB code 6S7S, subunit A = white, subunit B = green, subunit C = cyan, subunit D = yellow, **16h** = orange), illustrating the interactions (yellow dashed lines) of the ligand in the allosteric site. b) Overlay of the X-ray crystal structure of *Mtb* fumarase in complex with **1** (PDB code 5F91, subunit A = white, subunits B to D not shown, **1** = lilac) with the structure in complex with **16a** (PDB code 6S7K, subunit A = cyan, subunits B to D not shown, **16a** = light pink).
**Figure S10:** Overlays of the X-ray crystal structure of *Mtb* fumarase in complex with 1 (PDB code 5F91, subunit A = white, subunits B to D not shown, 1 = lilac) with the structures of *Mtb* fumarase (subunit A = cyan, subunits B to D not shown) in complex with a) 16b (beige) (PDB code 6S43) and b) 16h (orange) (PDB code 6S7S).
Figure S11: Omit F_o-F_c electron density maps (gray mesh) contoured to 2σ for ligands a) 15a and b) 15g from the X-ray crystal structures in complex with Mtb fumarase.
Figure S12: Omit Fₒ-Fₑ electron density maps (gray mesh) contoured to 2σ for ligands a) 16a and b) 16b from the X-ray crystal structures in complex with *Mtb* fumarase.
Figure S13: Omit F₀-F₆ electron density maps (gray mesh) contoured to 2σ for ligands a) 16h and b) 16j from the X-ray crystal structures in complex with *Mtb* fumarase.

a)

b)
**Figure S14:** Omit $F_\sigma - F_c$ electron density map (gray mesh) contoured to $2\sigma$ for ligand 16l from the X-ray crystal structure in complex with *Mt b* fumarase.

![Figure S14](image)

**Table S1:** Protein Data Bank accession numbers and X-ray crystallographic data collection and refinement statistics for crystal structures described in this study.
<table>
<thead>
<tr>
<th>Ligand</th>
<th>15a</th>
<th>15g</th>
<th>16a</th>
<th>16b</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDB Code</td>
<td>657U</td>
<td>657W</td>
<td>657K</td>
<td>6641</td>
</tr>
<tr>
<td>Synchrotron</td>
<td>Diamond Light Source</td>
<td>Diamond Light Source</td>
<td>Diamond Light Source</td>
<td>Diamond Light Source</td>
</tr>
<tr>
<td>Beamline</td>
<td>003</td>
<td>003</td>
<td>003</td>
<td>003</td>
</tr>
<tr>
<td>Collection Date</td>
<td>24/09/2018</td>
<td>21/10/2018</td>
<td>24/09/2018</td>
<td>24/09/2018</td>
</tr>
<tr>
<td>Wavelength (Å)</td>
<td>0.9783</td>
<td>0.9782</td>
<td>0.9783</td>
<td>0.9783</td>
</tr>
<tr>
<td>Resolution range</td>
<td>85.47 - 1.48 (1.593 - 1.48)</td>
<td>48.97 - 1.44 (1.491 - 1.44)</td>
<td>48.97 - 1.55 (1.083 - 1.05)</td>
<td>58.61 - 1.42 (1.475 - 1.426)</td>
</tr>
<tr>
<td>Space group</td>
<td>C 1 2 1</td>
<td>C 1 2 1</td>
<td>C 1 2 1</td>
<td>C 1 2 1</td>
</tr>
<tr>
<td>Unit cell</td>
<td>175.25b 96.962 124.299 90 102.746 90</td>
<td>175.25b 96.962 124.299 90 102.746 90</td>
<td>175.469 96.388 124.736 90 102.655 90</td>
<td>175.315 96.507 124.349 90 102.701 90</td>
</tr>
<tr>
<td>Total reflections</td>
<td>2395134 (364843)</td>
<td>1830514 (780083)</td>
<td>1955561 (307235)</td>
<td>2627362 (403102)</td>
</tr>
<tr>
<td>Unique reflections</td>
<td>322623 (33323)</td>
<td>348985 (19551)</td>
<td>269929 (28745)</td>
<td>359188 (17498)</td>
</tr>
<tr>
<td>Multiplicity</td>
<td>7.4 (1.75)</td>
<td>5.2 (0.52)</td>
<td>7.2 (7.5)</td>
<td>7.3 (7.4)</td>
</tr>
<tr>
<td>Completeness (%)</td>
<td>96.17 (99.80)</td>
<td>91.55 (86.26)</td>
<td>93.67 (95.90)</td>
<td>95.48 (99.97)</td>
</tr>
<tr>
<td>Mean (σ)/t</td>
<td>8.4 (1.1)</td>
<td>6.8 (0.9)</td>
<td>9.5 (1.1)</td>
<td>9.6 (1.1)</td>
</tr>
<tr>
<td>Wilson B-factor</td>
<td>16.95</td>
<td>12.88</td>
<td>19</td>
<td>16.99</td>
</tr>
<tr>
<td>Rmerge</td>
<td>0.181 (1.858)</td>
<td>0.134 (1.201)</td>
<td>0.118 (1.626)</td>
<td>0.105 (1.692)</td>
</tr>
<tr>
<td>Rfree</td>
<td>0.151 (1.593)</td>
<td>0.145 (2.248)</td>
<td>0.127 (1.753)</td>
<td>0.114 (1.811)</td>
</tr>
<tr>
<td>R-factor</td>
<td>0.055 (0.713)</td>
<td>0.064 (1.004)</td>
<td>0.047 (0.647)</td>
<td>0.042 (0.685)</td>
</tr>
<tr>
<td>CC1/2</td>
<td>0.958 (0.442)</td>
<td>0.959 (0.406)</td>
<td>0.958 (0.449)</td>
<td>0.958 (0.465)</td>
</tr>
<tr>
<td>Reflections used in refinement</td>
<td>322588 (33313)</td>
<td>333014 (13025)</td>
<td>268350 (28716)</td>
<td>359347 (17494)</td>
</tr>
<tr>
<td>Reflections used for R-free</td>
<td>16113 (1588)</td>
<td>16825 (1562)</td>
<td>13407 (1433)</td>
<td>17870 (1773)</td>
</tr>
<tr>
<td>R-work</td>
<td>0.1700 (0.2912)</td>
<td>0.2009 (0.5136)</td>
<td>0.1905 (0.3211)</td>
<td>0.1609 (0.3075)</td>
</tr>
<tr>
<td>R-free</td>
<td>0.1841 (0.2813)</td>
<td>0.2256 (0.3159)</td>
<td>0.1934 (0.3277)</td>
<td>0.1878 (0.3182)</td>
</tr>
<tr>
<td>Number of non-hydrogen atoms</td>
<td>15777</td>
<td>15336</td>
<td>15611</td>
<td>15622</td>
</tr>
<tr>
<td>Macromolecules</td>
<td>2095</td>
<td>13882</td>
<td>14069</td>
<td>13888</td>
</tr>
<tr>
<td>Ligands</td>
<td>67</td>
<td>68</td>
<td>61</td>
<td>73</td>
</tr>
<tr>
<td>Solvent</td>
<td>1596</td>
<td>1386</td>
<td>1490</td>
<td>1661</td>
</tr>
<tr>
<td>Protein residues</td>
<td>1805</td>
<td>1807</td>
<td>1808</td>
<td>1805</td>
</tr>
<tr>
<td>RMSD (bonds)</td>
<td>0.016</td>
<td>0.016</td>
<td>0.015</td>
<td>0.016</td>
</tr>
<tr>
<td>RMSD (angles)</td>
<td>1.88</td>
<td>1.9</td>
<td>1.85</td>
<td>1.88</td>
</tr>
<tr>
<td>Ramachandran favored (%)</td>
<td>97.04</td>
<td>97.67</td>
<td>97.60</td>
<td>97.70</td>
</tr>
<tr>
<td>Ramachandran allowed (%)</td>
<td>2.35</td>
<td>2.13</td>
<td>2.23</td>
<td>2.24</td>
</tr>
<tr>
<td>Ramachandran outliers (%)</td>
<td>0</td>
<td>0</td>
<td>0.11</td>
<td>0</td>
</tr>
<tr>
<td>Ramachandran outliers (%)</td>
<td>0.11</td>
<td>1.18</td>
<td>1.3</td>
<td>0.97</td>
</tr>
<tr>
<td>Ramachandran outliers (%)</td>
<td>2.37</td>
<td>2.42</td>
<td>3.3</td>
<td>3.06</td>
</tr>
<tr>
<td>Average B-factor</td>
<td>24.36</td>
<td>23.58</td>
<td>25.41</td>
<td>24.16</td>
</tr>
<tr>
<td>Macromolecules</td>
<td>32.23</td>
<td>22.83</td>
<td>24.63</td>
<td>23.15</td>
</tr>
<tr>
<td>Ligands</td>
<td>28.42</td>
<td>23.87</td>
<td>21.93</td>
<td>24.72</td>
</tr>
<tr>
<td>Solvent</td>
<td>35.11</td>
<td>30.82</td>
<td>32.97</td>
<td>32.57</td>
</tr>
</tbody>
</table>