N-Leucinyl benzenesulfonamides as structurally simplified leucyl-tRNA synthetase inhibitors

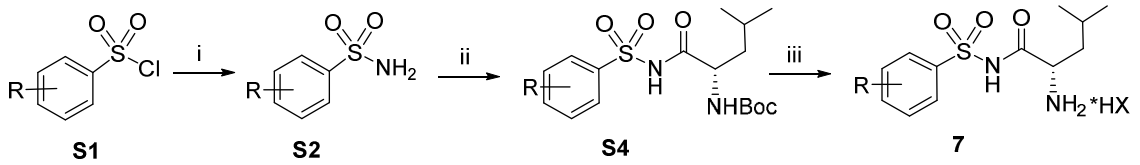
Contents

1. Synthesis 1
2. Molecular Modelling 12
3. Isothermal titration calorimetry 18
4. In vitro assay for determination of LeuRS inhibitory activity 23
5. Antibacterial susceptibility determinations 25

1. Synthesis

1.1. Synthesis description of compounds 7

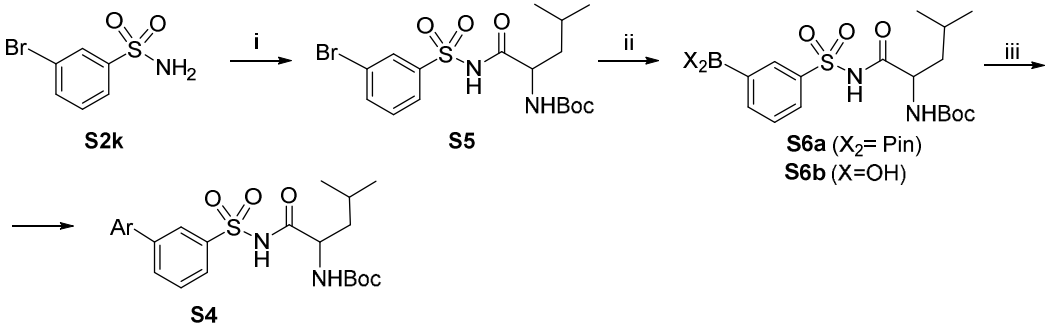
N-Leucinyl benzene sulfonamides **7** were prepared starting either from commercially available sulfonyl chlorides **S1** or sulfonamides **S2** (Scheme S1). Sulfonyl chlorides **S1** were transformed to sulfonamides **S2** (Scheme S1) in the reaction with ammonia. The sulfonamides **S2** were *N*-acylated with *N*-Boc leucine (**S3**). The resulting intermediates **S4** were deprotected to give *N*-acylsulfonamides **7** (Scheme 1).



Scheme S1. Reagents and conditions: (i) General method A: aq NH_3; (ii) General method B: BocLeu (**S3**), HBTU, TEA, cat. DMAP, DMF, r.t.; (iii) Method C1: TFA, DCM, Method C2: HCl, Method C3: HCl,

Protected intermediates **S4**, containing meta-heteroaryl substitutuent in benzenesulfonamide part were prepared starting from 3-bromobenzenesulfonamide **S2k** (Scheme S2). This was first acylated with *N*-Boc leucine (**S3**) to give intermediate **S5** which was then transformed to

a pinacolate borane **S6a** or boronic acid **S6b**. These were subjected to Suzuki-Miyaura coupling with aryl halides to give acylsulfonamides **S4** subsequently used for the synthesis of target compounds **7** according to the scheme S1.



S2k **S5** **S6a** (X_2= Pin) **S6b** (X=OH) **S4**

Scheme S2. Reagents and conditions: (i) General method B: BocLeu (**S3**), HBTU, TEA, cat. DMAP, DMF, r.t. (ii) General method D: Pin_2B_2, $PdCl_2(dppf)_2$,KOAc, Dioxane,110°C; (iii) $PdCl_2(dppf)_2$, Na_2CO_3, water, Dioxane, 110°C, PhBr or HetArBr

1.2. Experimental

1.2.1. General information

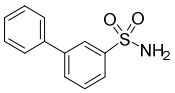
^1H and ^{13}C NMR spectra were recorded on Varian MR-400 400 MHz and Varian Inova 600 MHz spectrometers. ^1H chemical shifts were referenced to internal standard TMS (δ 0.00 ppm) or HMDSO (δ 0.055 ppm) (solvent $CDCl_3$). ^{13}C NMR spectra were referenced to the solvent ($CDCl_3$) peak at 77.0 ppm. TLC was carried out on DC Alufolien plates of Kieselgel 60. Column chromatography was carried out on Kieselgel (Acros), 0.023 – 0.070 mm, pore diameter ca 6 nm. Anhydrous solvents were obtained as follows: dichloromethane and chloroform were distilled from CaH_2; tetrahydrofuran and diethylether were distilled from Na/benzophenone. All other solvents were HPLC grade. All air-sensitive reactions were run under Ar atmosphere.

HPLC analyses were carried out on Alliance Waters 2695 Separation module (column: Waters XBridge C18, 3.5µm, 2.1x50mm, 0.6ml/min; mobile phase: gradient acetonitrile - 0.1% formic acid) or Acquity UPLC module (column: Acquity UPLC BEH C18, 1.7µm, 2.1x50mm, 0.4ml/min; mobile phase: gradient acetonitrile – 0.1% formic acid). All final compounds 1-18 were demonstrated to have ≥95% purity.

1.2.2. Synthesis of arylsulfonamides S2h,i

General method A. To a solution of sulfonyl chloride **S1** (15.94 mmol) in DCM (67 mL) was added 25% NH_4OH solution in water (3.7 ml, 57.15 mmol) at ice bath temperature. The reaction mixture was stirred at this temperature for 30 min followed by stirring for 6 h at room temperature. The precipitated solid material was filtered, washed with water, and dried *in vacuo* over P_2O_5 to give sulfonamide **S2**.

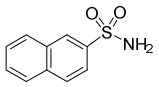
[1,1'-Biphenyl]-3-sulfonamide (**S2h**)



S2h

Prepared according to the general method A (0.284 g, 93%) as white crystals, m.p. 120-122 °C. Compound is described in the literature.[1] ^{1}H NMR ($CDCl_3$) δ: 8.16 (td, *J*=1.9, 0.5 Hz, 1H), 7.90 (ddd, *J*=7.8, 1.9, 1.1 Hz, 1H), 7.79 (ddd, *J*=7.8, 1.8, 1.1 Hz, 1H), 7.61-7.58 (m, 2H), 7.57 (td, *J*=7.8, 0.5 Hz, 1H), 7.49-7.44 (m, 2H), 7.42-7.38 (m, 1H), 4.99 (s, 2H). ^{13}C NMR ($CDCl_3$) δ: 142.7, 142.6, 139.3, 131.5, 129.8, 129.2, 128.4, 127.3, 125.1. LCMS (ESI/TOF-Q) m/z: $[M+H]^+$ Calcd for $C_{12}H_{12}NO_2S$ 234.05; Found 234.02.

Naphthalene-2-sulfonamide (**S2i**)



S2i

Prepared according to the general method A (0.425 g, 92%) as white crystals. Compound is described in the literature.[2] ^{1}H NMR (DMSO-d_6) δ: 8.43 (d, *J*=1.8 Hz, 1H), 8.14 (d, *J*=8.8 Hz, 1H), 8.12 (d, *J*=8.3 Hz, 1H), 8.07-8.00 (m, 1H), 7.89 (dd, *J*=8.8, 1.8 Hz, 1H), 7.73-7.61 (m, 2H), 7.44 (s, 2H) . LCMS (ESI/TOF-Q) m/z: $[M - H]^-$ Calcd for $C_{10}H_8NO_2S$ 206.04; Found 206.00.

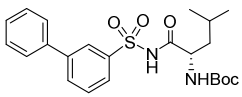
1.2.3. Synthesis of Boc-protected *N*-acyl sulfonamides S4h-j and S5

General method B. To a solution of BOC-L-leucine monohydrate (**S3**) (0.592 g, 2.37 mmol) in DMF (5 mL) successively were added sulfonamide **S2** (2.37 mmol), HBTU (0.900 g, 2.37 mmol), TEA (0.66 ml, 4.75 mmol), and a catalytic amount of DMAP (0.029 g, 0.237 mmol). The reaction mixture was stirred for 24 h and poured into water (70 mL). The mixture was extracted with EtOAc (3 × 75 mL), the combined organic extracts were washed with 1N HCl

[1] Laurent, L. G.; Jonathan, B. WO 2006002474 A1 Jan 12, 2006
[2] Aime, S.; Botta, M.; Cravotto, G.; Frullano, L.; Giovenzana, G. B.; Crich, S. G.; Palmisano, G.; Sisti, M. *Helv. Chim. Acta* **2005**, *88*, 588.

(20 mL), water (2 × 100 mL), saturated solution of NaCl (100 mL), and dried (Na_2SO_4). The volatiles were evaporated and the residue was purified by Biotage purification system (C18HS 40+M column, eluent water-methanol, gradient from 1:1 to 0:100) to afford of *N*-(Boc-leucinyl)-sulfonamide **S4** or **S5**.

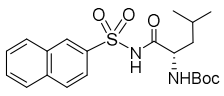
N-(Boc-leucinyl)-sulfonamide **S4h**



S4h

Prepared according to the general method B (0.200 g, 45%) as a viscous oil which solidifies upon standing in a refrigerator. ^{1}H NMR (DMSO-d_6) δ: 12.31 and 12.28 (br s and br s, altogether 1H), 8.15 and 8.10 (br s and unresolved t, *J*=1.7 Hz, altogether 1H), 7.99 (d, *J*=7.8 Hz, 1H), 7.88 (d, *J*=7.8 Hz, 1H), 7.76-7.64 (m, 3H), 7.52 (t, *J*=7.5 Hz, 2H), 7.47-7.42 (m, 1H), 7.05 and 6.65 (d and br d, *J*=7.2 Hz, altogether 1H), 4.02-3.92 and 3.85-3.77 (m and m, altogether 1H), 1.63-1.45 (m, 1H), 1.39-1.18 (m, 2H), 1.27 and 1.05 (s and s, altogether 9H), 0.81 (d, *J*=6.7 Hz, 3H), 0.79 (d, *J*=6.7 Hz, 3H). LCMS (ESI/TOF-Q) m/z: $[M-H]^-$ Calcd for $C_{23}H_{29}N_2O_5S$ 445.19; Found 445.29.

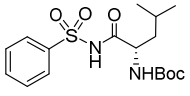
N-(Boc-leucinyl)-sulfonamide **S4i**



S4i

Prepared according to the general method B (0.158 g, 38%) as a foam. ^{1}H NMR (DMSO-d_6) δ: 12.32 (br s, 1H), 8.58 (s, 1H), 8.20 (d, *J*=7.9 Hz, 1H), 8.13 (d, *J*=8.7 Hz, 1H), 8.06 (d, *J*=7.9 Hz, 1H), 7.85 (dd, *J*=8.7, 1.4 Hz, 1H), 7.74 (t, *J*=7.5 Hz, 1H), 7.69 (t, *J*=7.5 Hz, 1H), 7.00 (unresolved d, *J*~7.5 Hz), 4.01-3.92 (m, 1H), 1.58-1.40 (m, 1H), 1.40-1.13 (m, 2H), 1.26 and 1.01 (s and s, altogether 9H), 0.80 (d, *J*=6.7 Hz, 3H), 0.78 (d, *J*=6.7 Hz, 3H). LCMS (ESI/TOF-Q) m/z: $[M-H]^-$ Calcd for $C_{21}H_{27}N_2O_5S$ 419.17; Found 419.22.

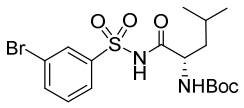
N-(Boc-leucinyl)-sulfonamide **S4j**



S4j

Prepared according to the general method B (0.161 g, 49%) as a foam. ^{1}H NMR (DMSO-d_6) δ: 12.24 (br s, 1H), 7.96-7.86 (m, 2H), 7.70 (t, *J*=7.1 Hz, 1H), 7.60 (t, *J*=7.5 Hz, 2H), 7.02 (unresolved d, *J*~7.5 Hz), 4.00-3.91 (m, 1H), 1.55-1.43 (m, 1H), 1.37-1.13 (m, 2H), 1.32 and 1.12 (s and s, altogether 9H), 0.81 (d, *J*=6.7 Hz, 3H), 0.79 (d, *J*=6.7 Hz, 3H). LCMS (ESI/TOF-Q) m/z: $[M-H]^-$ Calcd for $C_{17}H_{25}N_2O_5S$ 369.16; Found 369.22.

N-(Boc-leucinyl)-sulfonamide **S5**



S5

Prepared according to the general method B (0.66 g 61.8%) as a foam. ^{1}H NMR ($CDCl_3$) δ: 9.57 (b s, 1H), 8.16 (t, *J*=1.8z, 1H), 8.00 (d, *J*=7.9 Hz, 1H), 7.75 (ddd, *J*=7.9, 1.8, 1.0 Hz, 1H), 7.40 (t, *J*=7.9 Hz, 1H), 4.70 (unresolved d, *J*=6.4 Hz, 1H), 4.06-3.92 (m, 1H), 1.70-1.52 (m, 3H), 1.44 (s, 9H), 0.91 (d, *J*=6.3 Hz, 3H), 0.87 (d, *J*=6.3 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ: 170.3, 156.7, 140.4, 137.1, 131.2, 130.5, 127.3, 122.9, 81.9, 77.4, 53.5, 39.0, 28.3, 24.7, 22.9, 21.9 . HRMS (ESI) m/z: Calculated for $C_{17}H_{24}BrN_2O_5S$ $(M-H)^-$ 447.0595, found 447.0592.

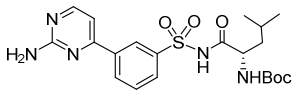
1.2.4. Synthesis of boronic acid building block S6a,b

Argon was bubbled for 10 min through a mixture of (*N*-(Boc-leucinyl)-sulfonamide (**S5)** (1.00 g, 2.23 mmol), KOAc (0.874 g, 8.9 mmol), and $PdCl_2(dppf)_2$ (0.18 g, 0.22 mmol) in dioxane (40 mL). Then bis(pinacolato)diboron (0.85 g, 3.35 mmol) was added The reaction vessel was closed, and the content was stirred at 110°C for 17 h. The mixture was cooled to room temperature and filtered through short Celite® column. The filtrate was concentrated and the dark oily residue (3.0 g) was purified by column chromatography on silica gel (eluent petroleum ether-ethyl acetate, gradient from 4:1 to 1:1) to give 1.281 g of crude product as a glass-like material. This was a mixture of pinacolate ester of boronic acid **S6a** and boronic acid **S6b**. The mixture was utilized in the next step without further purification.

1.2.5. Synthesis of Boc-protected *N*-acyl sulfonamides S4a-g

General method D. The mixture of pinacolate ester of boronic acid **S6a** and boronic acid **S6b** (0.600 g), obtained in the preceding step, was dissolved in dioxane (34 mL) and to this arylhalide (1.21 mmol), Na_2CO_3 (0.385 g, 3.63 mmol), and water (1.7 mL) were added. Argon was bubbled through the mixture for 10 min, and $PdCl_2(dppf)_2$ (0.045 g, 0.061 mmol), was added. The reaction vessel was closed, and the content was stirred at 110°C for 18 h. The mixture was cooled to room temperature and filtered through short Celite® column. The filtrate was concentrated and the residue was purified by column chromatography on silicagel (eluent chloroform-methanol, gradient from 100:1 to 100:2) to give *N*-(Boc-leucinyl)-sulfonamides **S4a-g**.

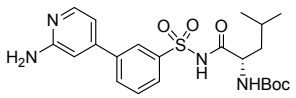
N-(Boc-leucinyl)-sulfonamide **S4a**



S4a

Prepared according to the general method D (1.06 g, 60%) as a foam. ^{1}H NMR (DMSO-d_6) δ: 12.33 (br s, 1H), 8.65 and 8.61 (br s and unresolved t, *J*=1.7 Hz, altogether 1H), 8.37 (d, *J*=5.2 Hz, 1H), 8.31 (d, *J*=7.8 Hz, 1H), 7.99 (d, *J*=7.8 Hz, 1H), 7.72 (t, *J*=7.8 Hz, 1H), 7.16 (d, *J*=5.2 hz, 1H), 6.98 and 6.61 (two unresolved d, *J*~6.3 Hz, altogether 1H), 6.81 (s, 2H), 4.00-3.89 and 3.85-3.75 (m and m, altogether 1H), 1.62-1.42 (m, 1H), 1.38-1.18 (m, 2H), 1.27 and 1.07 (s and s, altogether 9H), 0.80 (d, *J*=6.8 Hz, 3H), 0.78 (d, *J*=6.8 Hz, 3H). ^{13}C NMR (DMSO-d_6) δ: 172.7, 163.9, 161.9, 159.6, 155.4, 140.2, 137.8, 131.4, 129.6, 128.9, 125.5, 105.9, 79.2, 78.2, 53.2, 28.1, 27.6, 24.2, 22.8, 21.2. LCMS (ESI/TOF-Q) m/z: $[M+H]^+$ Calcd for $C_{21}H_{30}N_5O_5S$ 464.19; Found 464.37.

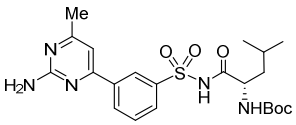
N-(Boc-leucinyl)-sulfonamide **S4b**



S4b

Prepared according to the general method D (0.082 g, 45%) as a foam. LCMS (ESI/TOF-Q) m/z: $[M-H]^-$ Calcd for $C_{22}H_{30}N_4O_5S$ 462.19; Found 461.34.

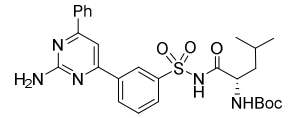
N-(Boc-leucinyl)-sulfonamide **S4c**



S4c

Prepared according to the general method D (0.246 g, 49%) as a foam. ^{1}H NMR (DMSO-d_6) δ: 12.33 (b s, 1H), 8.58 (s, 1H), 8.31 (d, *J*=7.9 Hz, 1H), 7.97 (d, *J*=7.9 Hz, 1H), 7.71 (t, *J*=7.9 Hz, 1H), 7.08 (s, 1H), 7.01 (b s, 1H), 6.70 (s, 2H), 4.00-3.90 (m, 1H), 2.32 (s, 3H), 1.63-1.19 (m, 3H), 1.28 (s, 9H), 0.80 (d, *J*=6.7 Hz, 3H), 0.78 (d, *J*=6.7 Hz, 3H). LCMS (ESI/TOF-Q) m/z: $[M+H]^+$ Calcd for $C_{22}H_{31}N_5O_5S$ 478.20; Found 478.36.

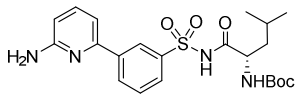
N-(Boc-leucinyl)-sulfonamide **S4d**



S4d

Prepared according to the general method D (0.088 g, 41%) as a foam. LCMS (ESI/TOF-Q) m/z: $[M+H]^+$ Calcd for $C_{27}H_{33}N_5O_5S$ 540.22; Found 540.39

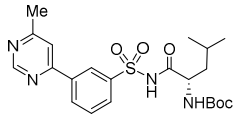
N-(Boc-leucinyl)-sulfonamide **S4e**



S4e

Prepared according to the general method D (0.100 g, 61%) as a foam. LCMS (ESI/TOF-Q) m/z: $[M-H]^-$ Calcd for $C_{22}H_{30}N_4O_5S$ 462.19; Found 461.32.

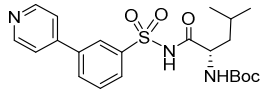
N-(Boc-leucinyl)-sulfonamide **S4f**



S4f

Prepared according to the general method D (0.127 g, 68%) as a foam. LCMS (ESI/TOF-Q) m/z: $[M+H]^+$ Calcd for $C_{22}H_{31}N_4O_5S$ 463.19; Found 463.39.

N-(Boc-leucinyl)-sulfonamide **S4g**



S4g

Prepared according to the general method D (0.118 g, 50 %) as a foam. ^{1}H NMR ($CDCl_3$) δ: 9.52 (br s, 1H), 8.72-8.69 (m, 2H), 8.31 (s, 1H), 8.11 (d, *J*=7.9 Hz, 1H), 7.88 (d, *J*=7.8 Hz, 1H), 7.65 (t, *J*=7.9 Hz, 1H), 7.55-7.52 (m, 2H), 4.71 (d, *J*=6.5 Hz, 1H), 4.06-3.93 (m, 1H), 1.71-1.53 (m, 2H), 1.53-1.26 (m, 1H), 1.39 and 1.24 (s and s, altogether 9H), 0.90 (d, *J*=6.4 Hz, 3H), 0.85 (d, *J*=6.3 Hz, 3H). LCMS (ESI/TOF-Q) m/z: $[M-H]^-$ Calcd for $C_{22}H_{28}N_3O_5S$ 446.18; Found 446.34.

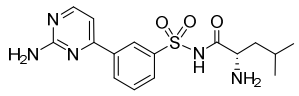
1.2.6. Synthesis of *N*-acyl sulfonamides 7a-j

General procedure C1. *N*-(Boc-leucinyl)-sulfonamide **S4** (0.515 mmol) was dissolved in a 5% TFA solution in dichloromethane (12 mL) and the solution was stirred at room temperature for 2 h. The reaction mixture was concentrated *in vacuo* and the oily brown residue was purified by Biotage purification system (C18HS 40+M column, eluent water-acetonitrile, gradient from 95:5 to 60:40) to afford of *N*-acyl sulfonamide **7**.

General method C2. To a solution of *N*-(Boc-leucinyl)-sulfonamide **S4** (0.43 mmol) in dioxane (5 mL) 4 N HCl dioxane solution (2.5 mL) was added dropwise under argon atmosphere and the resulting mixture was stirred at room temperature overnight. The reaction mixture was concentrated *in vacuo* and the residue was triturated with diethyl ether (4 × 5 mL). The solid material was filtered, washed with diethyl ether (5 mL) and dried *in vacuo* over P_2O_5 to give *N*-acyl sulfonamide **7**.

General method C3. *N*-(Boc-leucinyl)-sulfonamide **S4** (0.33 mmol) was dissolved in dioxane (6.5 mL). The solution was cooled to 0 – 5 °C and 4 N HCl solution in dioxane (1.95 mL) was added dropwise. The solution was stirred at room temperature for 16 h. The reaction mixture was concentrated *in vacuo* and the residue was purified by Biotage purification system (C18HS 40+M column, eluent water-acetonitrile, gradient from 100:0 to 50:50) to afford *N*-leucinyl sulfonamide **7**.

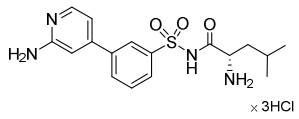
N-Leucinyl sulfonamide **7a**



7a, IK-587

Prepared according to method C1 (0.045 g, 75%) as white crystals. M.p. 224-226°C (dec.). ^{1}H NMR (DMSO-d_6, HMDSO) δ: 0.81 (3H, d, *J*=6.5 Hz), 0.83 (3H, d, *J*=6.5 Hz), 1.38 (1H, ddd, *J*=5.8, 8.3, 13.7 Hz), 1.58 (1H, ddd, *J*=5.6, 8.3, 13.7 Hz), 1.67 (1H, m), 3.32 (1H, dd, *J*=5.6, 8.3 Hz, overlapped with water), 6.75 (2H, s), 7.09 (1H, d, *J*=5.2 Hz), 7.52 (1H, t, *J*=7.8 Hz), 7.67 (3H, b s), 7.89 (1H, ddd, *J*=0.9, 1.6, 7.8 Hz), 8.06 (1H, ddd, *J*=0.9, 1.6, 7.8 Hz), 8.33 (1H, d, *J*=5.2 Hz), 8.53 (1H, t, *J*=1.6 Hz). ^{13}C NMR (DMSO-d_6) δ: 21.8, 22.7, 23.7, 40.5, 53.4, 105.8, 125.3, 125.4, 128.3, 128.9, 136.6, 146.0, 159.3, 163.0, 163.9, 172.7. LCMS (ESI/TOF-Q) m/z: $[M+H]^+$ Calcd for $C_{16}H_{22}N_5O_3S$ 364.1; Found 364.1. Anal. Calcd for $C_{16}H_{21}N_5O_3S \times 0.05\ CF_3COOH$ (1.5%) × 0.85 H_2O (4.0%): C 50.30, H 5.96, N 18.22. Found: C 50.31, H 5.98, N 18.17

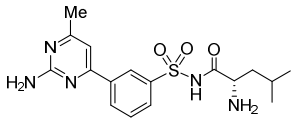
N-Leucinyl sulfonamide **7b**



7b, IK-634

Prepared according to method C3 (0.037 g, 52%) as beige crystals. M.p. 141 °C (dec). $[\alpha]_D^{22}$ +26.5° (c 1.55, CH_3OH). ^{1}H NMR (DMSO-d_6, HMDSO) δ: 13.86 (b s, 1H), 8.36-8.02 (m, 9H), 7.80 (t, *J*=7.3 Hz, 1H), 7.36-7.31 (m, 1H), 7.22 (dd, *J*=6.7, 1.6 Hz, 1H), 8.36-7.10 (b s, 1H), 3.74 (m, overlapped with water, 1H), 1.67-1.43 (m, 3H), 0.82 (d, *J*=6.2 Hz, 3H), 0.82 (d, *J*=6.3 Hz, 3H). ^{13}C NMR (DMSO-d_6) δ: 170.0, 154.4, 152.2, 141.1, 136.7, 136.1, 131.9, 130.4, 129.4, 125.9, 110.4, 110.2, 51.7, 23.4, 22.5, 21.7. LCMS (ESI/TOF-Q) m/z: $[M+H]^+$ Calcd for $C_{17}H_{23}N_4O_3S$ 363.1; Found 363.2. Anal. Calcd for $C_{17}H_{22}N_4O_3S \times 3$ HCl (22.0%) × 1.45 H_2O (5.2%): C 41.01, H 5.65, N 11.25. Found: C 41.01, H 5.62, N 11.20.

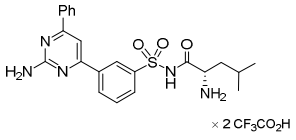
N-Leucinyl sulfonamide **7c**



7c, IK-580

Prepared according to method C1 (0.048 g, 80%) as white crystals. M.p. 241-242°C (dec.). $[\alpha]_D^{22}$ +9.8° (c 0.74, CH_3OH). ^1H NMR (DMSO-d_6, HMDSO) δ: 8.50 (t, *J*=1.7 Hz, 1H), 8.05 (ddd, *J*=7.8, 1.7, 1.2 Hz, 1H), 7.88 (ddd, *J*=7.8, 1.7, 1.2 Hz, 1H), 7.67 (b s, 3H), 7.50 (t, *J*=7.8 Hz, 1H), 7.01 (s, 1H), 6.63 (s, 2H), 3.37-3.30 (m, 1H, overlapped with water), 2.31 (s, 3H), 1.73-1.60 (m, 1H), 1.58 (ddd, *J*=13.7, 8.4, 5.6 Hz, 1H), 1.38 (ddd, *J*=13.7, 8.3, 5.8 Hz, 1H), 0.83 (d, *J*=6.3 Hz, 3H), 0.81 (d, *J*=6.3 Hz, 3H). ^{13}C NMR (DMSO-d_6) δ: 172.7, 168.4, 163.7, 163.0, 146.0, 136.8, 128.7, 128.3, 128.0, 125.4, 105.2, 53.4, 40.6, 23.7, 23.7, 22.7, 21.8. LCMS (ESI/TOF-Q) m/z: $[M+H]^+$ Calcd for $C_{17}H_{24}N_5O_3S$ 378.15; Found 378.2. Anal. Calcd for $C_{17}H_{23}N_5O_3S \times 0.09\ CF_3COOH$ (2.5%) × 1.12 H_2O (4.9%): C 50.59, H 6.26, N 17.17. Found: C 50.59, H 6.32, N 17.23.

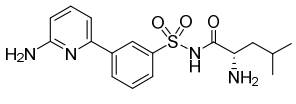
N- Leucinyl sulfonamide **7d**



7d, IK-625

Prepared according to method C1 (0.061 g, 86%) as white crystals. M.p. 186-188 °C (dec). $[\alpha]_D^{22}$ +16.6° (c 1.95, CH_3OH). ^1H NMR (DMSO-d_6, HMDSO) δ: 8.76 (s, 1H), 8.54-8.48 (m, 1H), 8.25-8.19 (m, 2H), 8.08-7.94 (m, 4H), 7.79-7.72 (m, 2H), 7.57-7.52 (m, 3H), 6.91 (b s, 2H), 3.76-3.65 (m, 1H, overlapped with water), 1.65-1.42 (m, 3H), 0.83 (d, *J*=6.2 Hz, 3H), 0.82 (d, *J*=6.2 Hz, 3H). ^{13}C NMR (DMSO-d_6) δ: 169.6, 165.3, 163.9, 163.1, 158.5, 158.1, 138.1, 136.9, 132.1, 130.8, 129.6, 129.4, 128.7, 127.0, 126.0, 102.1, 51.6, 23.4, 22.6, 21.3. LCMS (ESI/TOF-Q) m/z: $[M+H]^+$ Calcd for $C_{22}H_{26}N_5O_3S$ 440.2; Found 440.3. Anal. Calcd for $C_{22}H_{25}N_5O_3S \times 2.1\ CF_3COOH$ (34.3%) × 1.05 H_2O (2.7%): C 45.09, H 4.22, N 10.03. Found: C 45.05, H 4.26, N 9.83.

N- Leucinyl sulfonamide **7e**

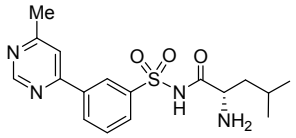


7e, IK-635

Prepared according to method C3 (0.027 g, 35%) as slightly beige crystals. M.p. 148 °C (dec). $[\alpha]_D^{22}$ +8.2° (c 0.75, CH_3OH). ^1H NMR (DMSO-d_6, HMDSO) δ: 8.42 (t, *J*=1.7 Hz, 1H), 7.96 (ddd, *J*=7.8, 1.7, 1.1 Hz, 1H), 7.77 (ddd, 7.7, 1.7, 1.1 Hz, 1H), 7.65 (b s, 3H), 7.47 (dd, *J*=8.1, 7.5 Hz, 1H), 7.43 (t, *J*=7.7 Hz, 1H), 7.01 (dd, *J*=7.5, 0.7 Hz, 1H), 6.43 (dd, *J*=8.1, 0.7 Hz, 1H), 6.05 (b s, 2H), 3.35-3.29 (m, overlapped with water, 1H), 1.75-1.61 (m, 1H), 1.59 (ddd,

J=13.8, 8.4, 5.4 Hz, 1H), 1.38 (ddd, *J*=13.8, 8.5, 5.8 Hz, 1H), 0.83 (d, *J*=6.7 Hz, 3H), 0.81 (d, *J*=6.7 Hz, 3H). ^{13}C NMR (DMSO-d_6) δ: 172.6, 159.6, 153.7, 146.7, 138.9, 138.0, 127.7, 127.7, 126.8, 125.0, 108.3, 107.3, 53.4, 40.6, 23.7, 22.7, 21.8. LCMS (ESI/TOF-Q) m/z: $[M+H]^+$ Calcd for $C_{17}H_{23}N_4O_3S$ 363.1; Found 363.2. Anal. Calcd for $C_{17}H_{22}N_4O_3S \times 0.36$ HCl (3.5%): C 54.37, H 6.00, N 14.92. Found: C 54.39, H 6.10, N 14.82.

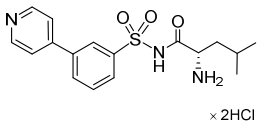
N-Leucinyl sulfonamide **7f**



7f, IK-621

Prepared according to method C1 (0.033 g, 42%) as white crystals. M.p. 246 °C. $[\alpha]_D^{22}$ +4.2° (c 0.75, CH_3OH). ^{1}H NMR (DMSO-d_6, HMDSO) δ: 9.11 (d, *J*=1.1 Hz, 1H), 8.61 (t, *J*=1.6 Hz, 1H), 8.21 (ddd, *J*=7.8, 1.6, 1.1 Hz, 1H), 7.98 (s, 1H), 7.94 (ddd, *J*=7.8, 1.5, 1.1 Hz, 1H), 7.67 (b s, 3H), 7.58 (t, *J*=7.8 Hz, 1H), 3.36-3.34 (m, 1H, overlapped with water), 2.55 (s, 3H), 1.67 (nonet, *J*=6.6 Hz, 1H), 1.59 (ddd, *J*=13.7, 8.1, 5.7 Hz, 1H), 1.38 (ddd, *J*=13.7, 8.1, 5.9 Hz, 1H), 0.83 (d, *J*=6.6 Hz, 3H), 0.81 (d, *J*=6.6 Hz, 3H). ^{13}C NMR (DMSO-d_6) δ: 173.2, 168.4, 162.1, 158.8, 146.8, 136.1, 129.8, 128.9, 128.9, 126.0, 116.8, 53.8, 41.0, 24.2, 24.1, 23.1, 22.2. LCMS (ESI/TOF-Q) m/z: $[M+H]^+$ Calcd for $C_{17}H_{23}N_4O_3S$ 363.1; Found 363.1. Anal. Calcd for $C_{17}H_{22}N_4O_3S \times 0.3$ H_2O (1.5%): C 55.51, H 6.19, N 15.23. Found: C 55.51, H 6.25, N 15.19.

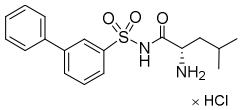
N-Leucinyl sulfonamide **7g**



7g IK-636

Prepared according to method C3 (0.057 g, 61%) as slightly beige crystals. M.p. 151 °C (dec). $[\alpha]_D^{22}$ +35.1° (c 3.41, CH_3OH). ^{1}H NMR (DMSO-d_6, HMDSO) δ: 9.01 (b s, 2H), 8.49 (b s, 3H), 8.44 (s, 1H), 8.39 (b s, 2H), 8.33 (d, *J*=7.2 Hz, 1H), 8.16 (d, *J*=7.6 Hz, 1H), 7.89 (t, *J*=7.4 Hz, 1H), ~9.4-7.2 (b s, 1H), 3.93 (b s, 1H), 1.68-1.51 (m, 3H), 0.83 (d, *J*=5.7 Hz, 3H), 0.82 (d, *J*=6.0 Hz, 3H). ^{13}C NMR (DMSO-d_6) δ: 169.9, 153.2, 143.7, 140.9, 136.2, 133.6, 131.1, 130.3, 127.2, 124.7, 51.8, 23.9, 22.9, 22.2. LCMS (ESI/TOF-Q) m/z: $[M+H]^+$ Calcd for $C_{17}H_{22}N_3O_3S$ 348.1; Found 348.2. Anal. Calcd for $C_{17}H_{21}N_3O_3S \times 2$ HCl (15.9%) $\times$ 2.1 H_2O (8.3%): C 44.56, H 5.98, N 9.17. Found: C 44.54, H 5.57, N 9.06.

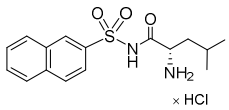
N-Leucinyl sulfonamide **7h**



7h, IK-681

Prepared according to method C2 (0.108 g, 76%) as white crystals. M.p. 149°C (dec.). $[\alpha]_D^{22}$ +20.2° (c 0.44, CH_3OH). ^{1}H NMR (DMSO-d_6) δ: 8.19 (b s, 3H), 8.16 (q, *J*=1.5 Hz, 1H), 8.01 (d, *J*=7.5 Hz, 1H), 7.93 (d, *J*=8.0 Hz, 1H), 7.73 (t, *J*=7.8 Hz, 1H), 7.72-7.68 (m, 2H), 7.56-7.51 (m, 2H), 7.48-7.43 (m, 1H), 3.86-3.74 (m, 1H), 1.64-1.45 (m, 3H), 0.82 (d, *J*=6.0 Hz, 3H), 0.81 (d, *J*=6.0 Hz, 3H). ^{13}C NMR (DMSO-d_6) δ: 169.9, 140.8, 140.7, 138.6, 131.5, 129.7, 129.3, 128.4, 126.8, 126.3, 125.6, 51.9, 23.4, 22.6, 21.5. LCMS (ESI/TOF-Q) m/z: $[M+H]^+$ Calcd for $C_{18}H_{23}N_2O_3S$ 346.14; Found 347.10. Anal. Calcd. for $C_{18}H_{22}N_2O_3S \times HCl$ (8.3%) × 0.12 H_2O (0.5%) × 0.6 $C_4H_8O_2$ (12.1%)): C 55.95, H 6.45, N 6.40, S 7.32. Found: C 55.94, H 6.45, N 6.61, S 7.52.

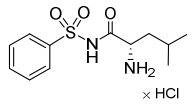
N-Leucinyl sulfonamide **7i**



7i, IK-665

Prepared according to method C2 (0.107 g, 89%) as white crystals. M.p. 215 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 13.08 (bs, 1H), 8.65 (d, *J*=1.9 Hz, 1H), 8.31 (b s, 3H), 8.24 (d, *J*=8.1 Hz, 1H), 8.17 (d, *J*=8.8 Hz, 1H), 8.07 (d, *J*=8.1 Hz, 1H), 7.92 (dd, *J*=8.8, 1.9 Hz, 1H), 7.75 (ddd, *J*=8.1, 6.9, 1.4 Hz, 1H), 7.70 (ddd, *J*=8.1, 6.9, 1.4 Hz, 1H), 3.91-3.81 (m, 1H), 1.64-1.44 (m, 3H), 0.81 (d, *J*=6.1 Hz, 6H). ^{13}C NMR (DMSO-d_6) δ: 169.2, 136.0, 134.7, 131.4, 129.6, 129.5 (2), 129.3, 129.2, 127.8, 122.5, 51.4, 40.0, 23.4, 22.6, 21.5. LCMS (ESI/TOF-Q) m/z: $[M+H]^+$ Calcd for $C_{16}H_{21}N_2O_3S$ 321.12; Found 321.20. Anal. Calcd. for $C_{16}H_{20}N_2O_3S \times HCl$ (9.5%) × 0.3 $C_4H_8O_2$ (6.9%) × 0.1 H_2O (0.5%)): C 53.65, H 6.18, N 7.27, S 8.33. Found: C 53.65, H 6.21, N 7.34, S 8.56.

N- Leucinyl sulfonamide **7j**



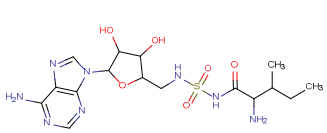
7j, IK-698

Prepared according to method C1 (0.076 g, 57%) as white crystals. M.p. 192 °C, $[\alpha]_D^{22}$ +26.9° (c 1.19, CH_3OH). ^{1}H NMR (DMSO-d_6) δ: (DMSO-d_6) δ: 13.05 (b s, 1H), 8.43 (b s, 3H), 7.98-7.94 (m, 2H), 7.76-7.70 (m, 1H), 7.67-7.61 (m, 2H), 3.93-3.83 (m, 1H), 1.67-1.48 (m, 3H), 0.82 (d, *J*=6.0 Hz, 3H), 0.81 (d, *J*=6.1 Hz, 3H). ^{13}C NMR (DMSO-d_6) δ: 169.1, 138.9, 133.9, 129.2, 127.6, 51.3, 23.4, 22.6, 21.7. LCMS (ESI/TOF-Q) m/z: $[M+H]^+$ Calcd for $C_{12}H_{19}N_2O_3S$ 271.10; Found 271.2.

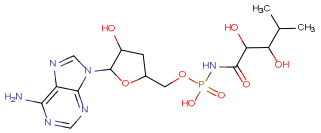
Anal. Calcd. for $C_{12}H_{18}N_2O_3S \times HCl$ (11.8%) × 0.06 H_2O (0.4%)): C 46.81, H 6.26, N 9.10. Found: C 46.81, H 6.21, N 9.02.

2. Molecular Modelling

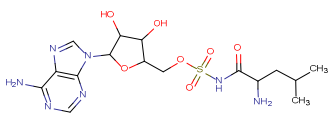
A search of the PDB (2016-03-01) using enzyme classification 6.1.1.4 as a query yields 30 LeuRS structures. Two of these are reported as asparaginyl-tRNA synthetases. Of the remainder, 12 are *E. coli* structures, of which three are solely of the editing domain. The remaining nine structures contain one of three ligands : 2-amino-N-({[5-(6-amino-9H-purin-9-yl)-3,4-dihydroxyoxolan-2-yl]methyl}sulfamoyl)-3-methylpentanamide (**1**, 4cqn), {[5-(6-amino-9H-purin-9-yl)-4-hydroxyoxolan-2-yl]methoxy}(2,3-dihydroxy-4-methylpentanamido)phosphinic acid (**2**, 3zgz) and 2-amino-1-[({[5-(6-amino-9H-purin-9-yl)-3,4-dihydroxyoxolan-2-yl]methoxy}sulfonyl)amino]-4-methylpentan-1-one (**3**, 4aq7).



(1)



(2)



(3)

Sequence-based overlay of the crystal structures superposes these ligands on one another. The binding in 3zgz is representative of the group as a whole and is shown in figure M1 and represented schematically in figure M2. The ligand is almost completely enclosed by the protein, with the adenine buried in a largely hydrophobic pocket, donating hydrogen bonds to Val569 and Met620 and accepting a hydrogen bond from Val569. The sugar ring donates a hydrogen bond to Glu532.

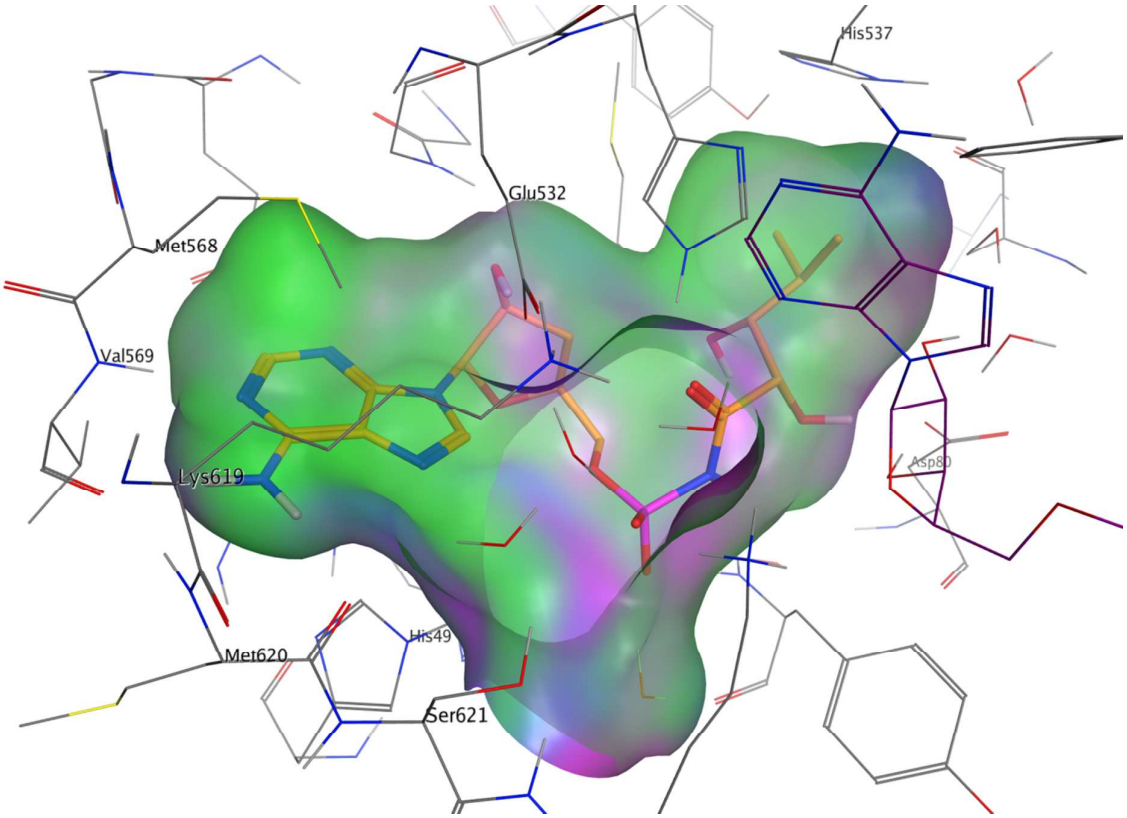


Figure M1. Binding of (2) to LeuRS (crystal structure 3zgz)

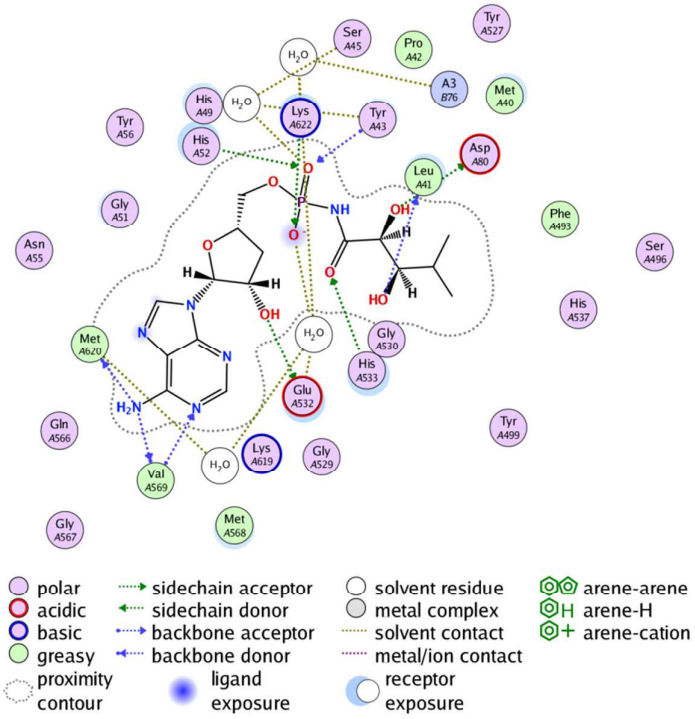


Figure M2. Schematic representation of the binding of (2) to LeuRS

The phosphoramidate sits in an anion pocket and makes strong interactions with Tyr43, His52, and Lys622. The amino acid mimic sits in a largely hydrophobic pocket and accepts a hydrogen bond at the carbonyl (His533) and donates two hydrogen bonds from the hydroxy groups (Leu41 and Asp80). The amino acid subpocket is partly comprised of the terminal adenosine of the tRNA. The 3-hydroxy group of this terminal adenosine moiety is particularly close to the position of the Cα (3.25Å), allowing for facile transfer of the amino acid to the tRNA in the non-inhibited case.

In all the structures, the loop Met620, Ser621, Lys622 (part of the larger, conserved KMSKSK loop) serves to close the pocket, leading to tight binding of the adenine. In contrast, the available T. thermophilus structures (2bte and 1h3n) are more open (figure M3). This is partly because of the absence of the tRNA in the structures but also due to the different conformation of the adenine-binding loop (Met638, Ser639, Lys640). In particular, Lys640 does not form part of the anion pocket. Comparison of these structures with those of Ile-RS (such as 1ffy, binding Mupirocin) shows an even more open pocket in the latter. A similarly-open active site can be observed in the structures that are liganded in the editing site but apo in the synthetic site (e.g. 3zjt). Here, neither the amino acid nor the anion pockets are fully formed and there is little structure to the adenine pocket. Figure M4 shows examples of the loop conformations overlaid on the 3zgz structure (pink) for 3zjt (green) and 1h3n (cyan) relative to the synthetic active site. It is clear that ligand and tRNA binding have considerable impact on the conformation of this part of the active site.

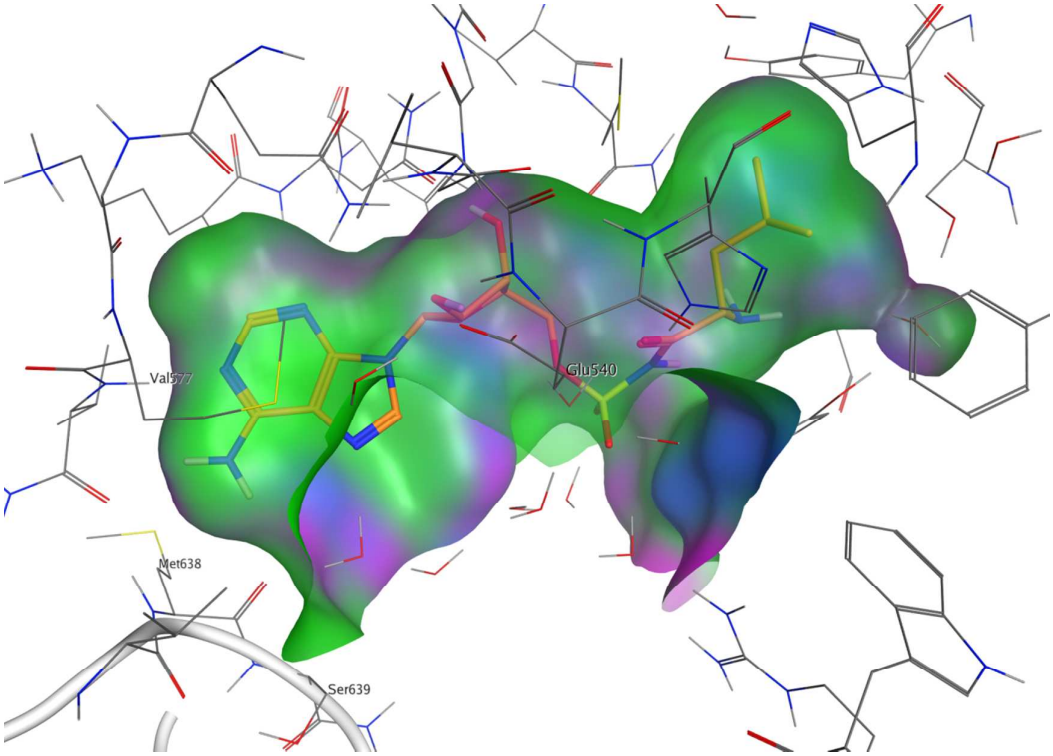


Figure M3. Binding of (3) in 1h3n showing the more-open active site

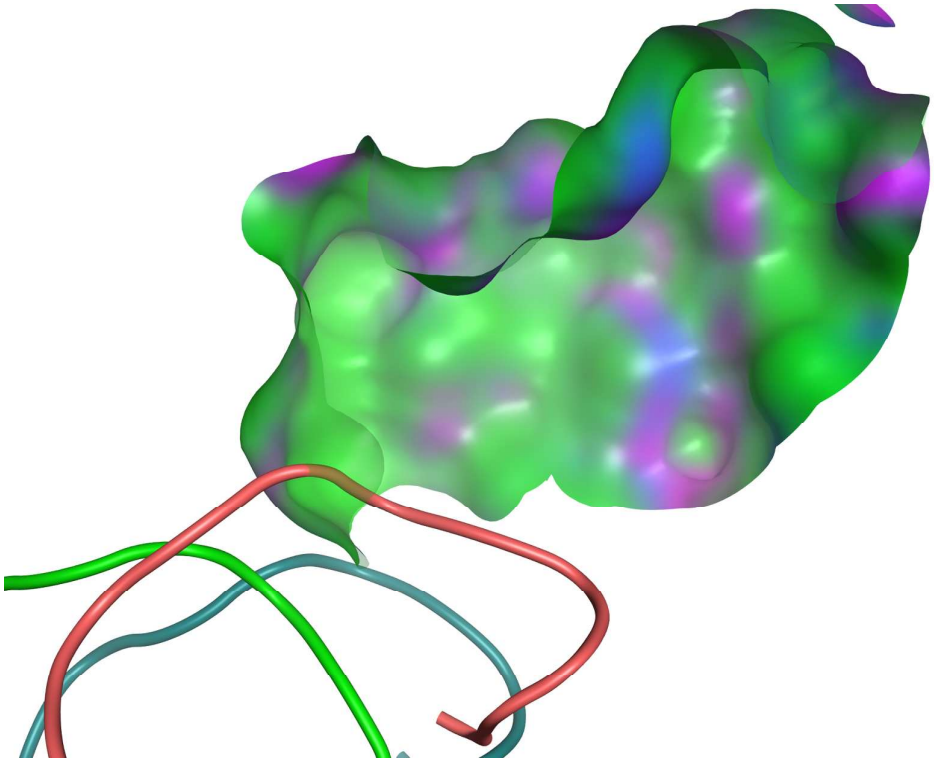


Figure M4. Loop conformations of 3zgz (pink) for 3zjt (green) and 1h3n

Docking modes of acylsulfonamides 7.

Docking has been performed into the synthetic active site of Leu-RS using GOLD v5.2 (CCDC). 3zgz was chosen because it is in an aminoacylation-like conformation and hence more relevant to inhibitor design. However, the docking to 4aq7 yields similar binding modes. The protein structure was read into MOE (CCG Inc.), protein chain 2 and associated RNA, ligand and water molecules were deleted to leave a single solvated RNA-protein-ligand complex. Hydrogen atoms were added to the structure using MOE's Protonate-3D tool.

The resultant structure was exported as a mol2 file and read into the Hermes module of GOLD for set up. All water molecules and the ligand were removed from the system. The binding site was defined to include all residues within 6Å of the position of TM84 (the ligand in the 3zgz crystal structure). Free ring corners were allowed to flip, matching commonly-observed templates from the CSD. Pyramidal nitrogens were also allowed to flip. The early termination criterion was turned off. The search criteria were set automatically by GOLD and the autoscale variable was set to 1. Initial studies of the docking of known ligands using the default ChemPLP scoring function did not always produce viable binding modes. Switching to the GOLDScore function improved the results and so this was used for all dockings.

Acyl sulfonamides are reasonably acidic with a pKa of around 4[3] Thus in aqueous solution the molecule would be expected to be in a zwitterionic form. The protonation state of the acylsulfonamides bound to LeuRS is not known because of the resolution of the available crystal structures. Interestingly, there are neither donor nor acceptor groups close to the putatively-negative nitrogen that might serve to stabilise one or other form. Docking of the *N*-form tends to give poses for the acylsulfonamide that are twisted and in which the carbonyl fails to accept a hydrogen bond from His533, such as is observed in the crystal structures. Neutralising the sulfonamide nitrogen whilst maintaining a protonated form of the amino group typically reproduces the hydrogen bonding and torsions observed in the crystal structures and hence this form has been used for the docking. Figure M5 shows the docking of the acylsulfonamide ligand from 4aq7 (3) in the 3zgz crystal structure. The best-ranked pose (orange) mimics the observed binding (cyan) of the 4aq7 ligand (generated by protein

[3] Organic Chemistry Concepts and Applications for Medicinal Chemistry. Joseph E. Rice, Elsevier, 2014

superposition with 3zgz). The purple pose was generated by docking the zwitterionic form into 3zgz. Both predicted poses mimic the observed binding well.

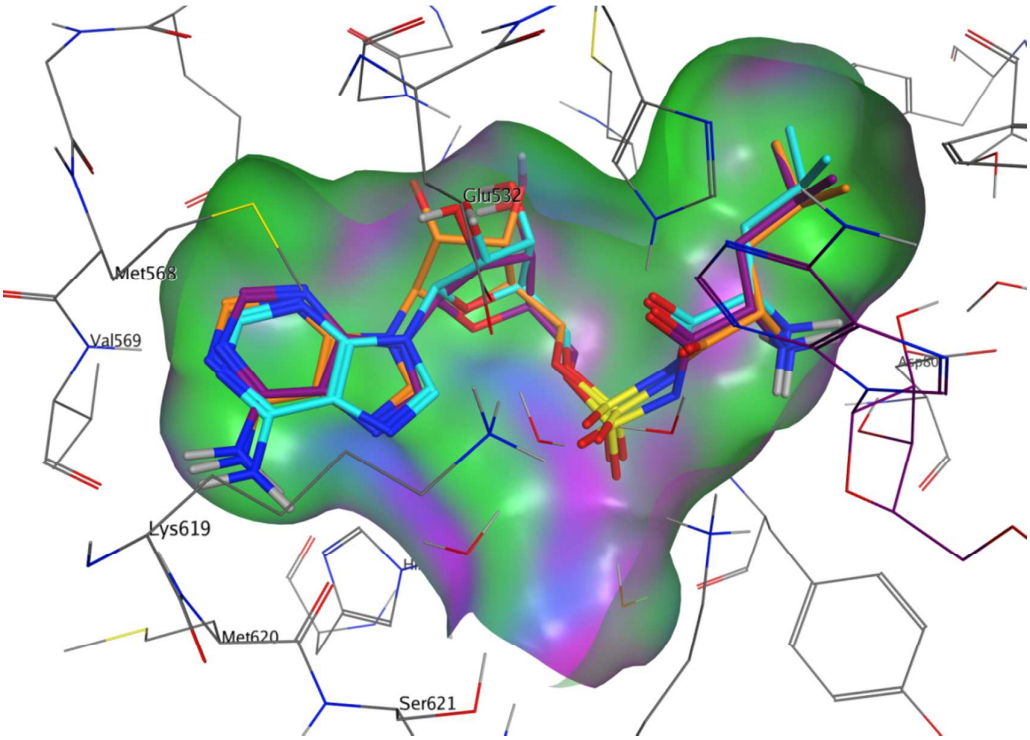


Figure M5. Predicted and experimental binding of (3) in 3zgz

Figure M6 shows the docking of of **7c** (purple), **7h** (orange) and **7j** (cyan) in 3zgz. The input structure for **7h** was generated by minimising with the MMFF94s forcefield in MOE. Use of the Amber10-EHT forcefield led to pyramidalisation of the amino group attached to the pyrimidine ring. This caused a rotation of the pyrimidine about the biaryl bond and failure of the amino group to interact with the carbonyls of Val569 and Met620. In this pose, the aminopyrimidine reproduces the interactions see in 4aq7 and of TM84 in 3zgz. The poses generated from MMFF04s and A10-EHT minimised starting structures for the other two molecules were essentially the same.

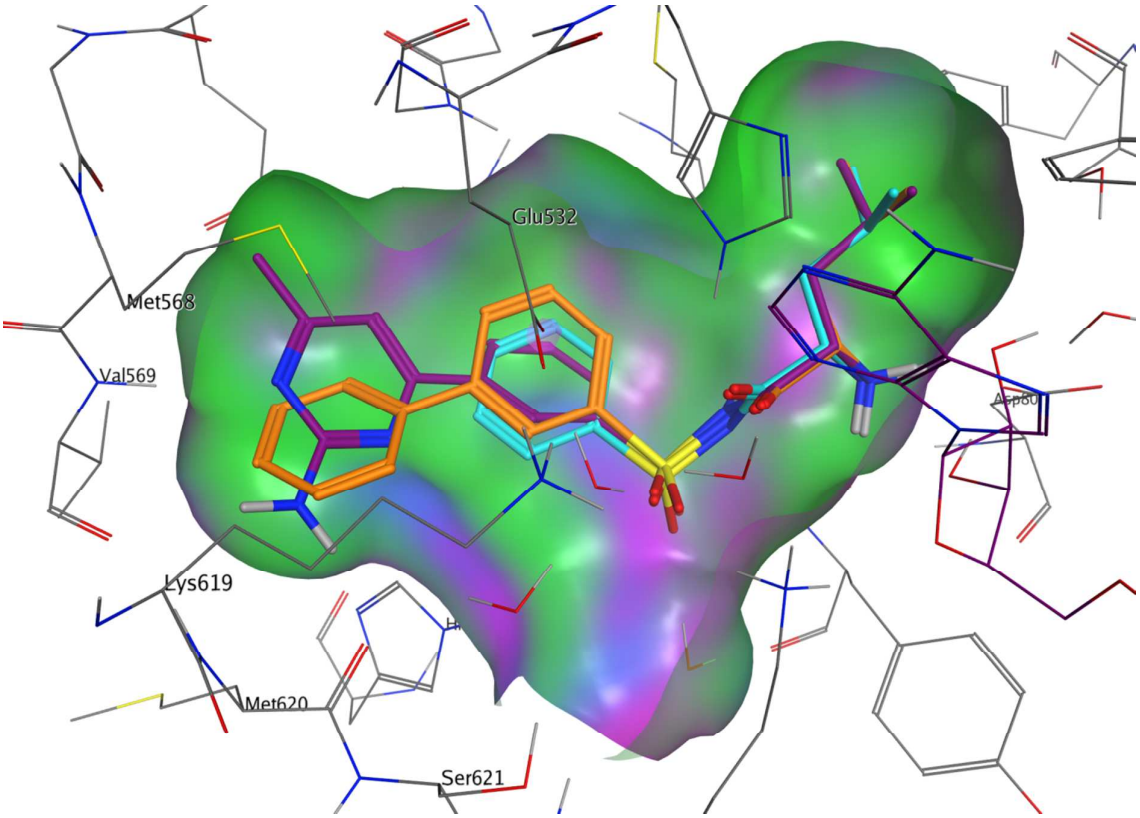


Figure M6. Docked poses of **7c** (purple), **7h** (orange) and **7j** (cyan)

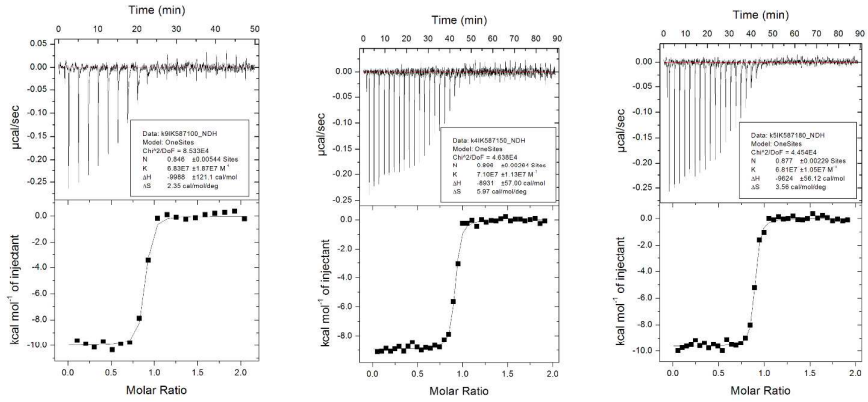
The biphenyl group of **7h** does not have appropriate functionality to mimic the binding of the 3zgz and 4aq7 ligands. The illustrated binding mode might be expected to have poor affinity as the hydrophobic phenyl is buried in a polar region of the pocket. However, the IC_{50} and ITC data show little difference between the two. This might indicate further (as yet unobserved) conformational changes in the LeuRS structure apo ligand binding. **7j** is shorter than **7h** and leaves the adenine-binding portion of the cavity empty. The rest of the predicted binding is as expected, mimicking **7h** closely. Predicted binding modes of all three ligands in the amino acid pocket is as expected.

3. Isothermal titration calorimetry

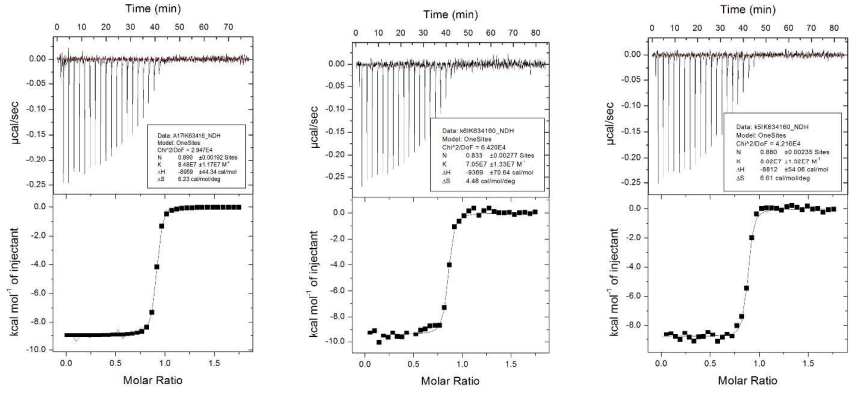
ITC experiments were carried out using a Microcal ITC200 instrument (GE Healthcare). Protein concentration was determined by measuring the absorbance at 280 nm using a theoretical molar extinction coefficient of 169,140 M-1cm-1. Ligand stock solutions were prepared in DMSO at 62.5 mM concentration. The titrations were performed at 25 oC with 10-30 μM E.Coli LeuRS in 50 mM HEPES, 150 mM NaCl, pH 7.5 buffer containing 1% DMSO (v/v). Compound IK-698 affinity was determined using competition ITC assay by

titrating 500 µM IK-698 into a solution with 40-50 µM protein and 60 µM competitive ligand with known binding parameters. The protein solution in the 200 µL sample cell was titrated with the inhibitor solution (diluted to 100-300 µM in the same buffer as the protein) using 1-2 µL injections every 140 s. All titrations were repeated at least three times. To correct for heats of dilution and mixing, the final baseline consisting of small peaks of identical size at the end of each experiment was subtracted. The experimental data were fitted to a theoretical titration curve (one site model) using MicroCal Origin 7 software. The arithmetic mean ± standard deviation (SD) of KD, ΔH, -TΔS values from at least three experiments are shown in Table 1.

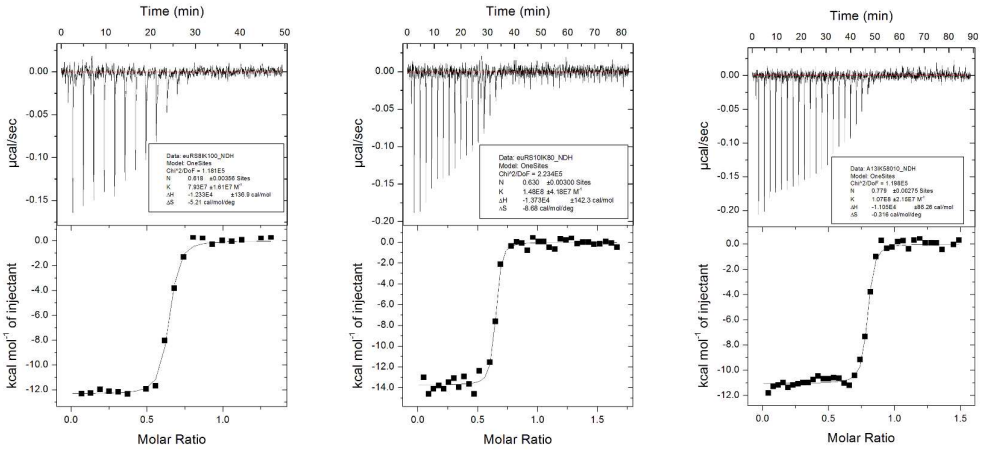
7a (IK-587) ITC curves:



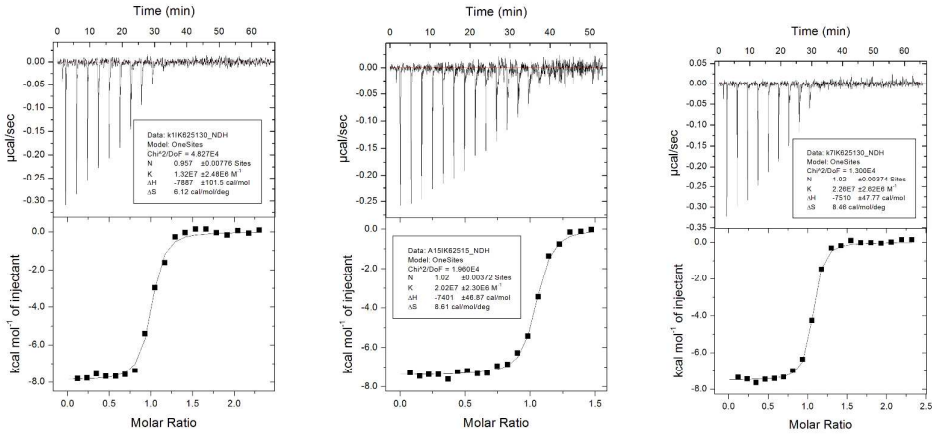
7b (IK-634) ITC curves:



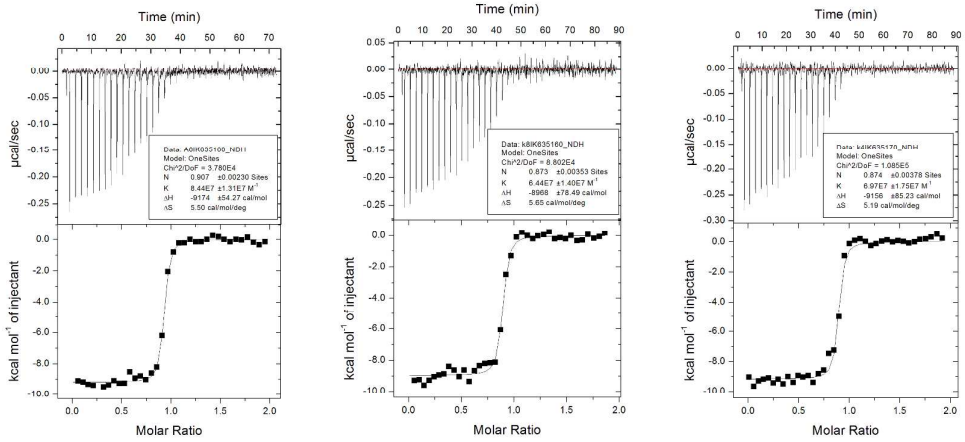
7c (IK-580) ITC curves:



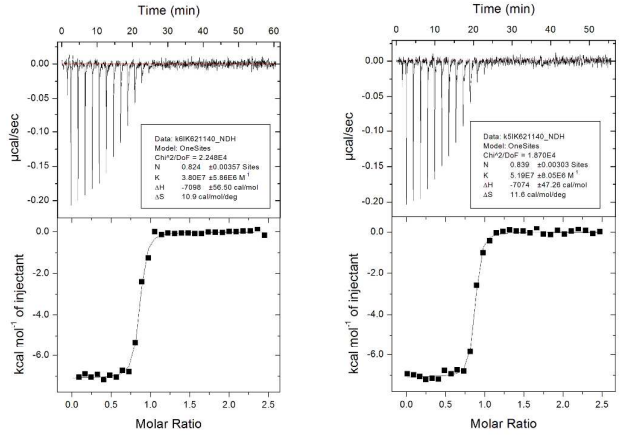
7d (IK-625) ITC curves:



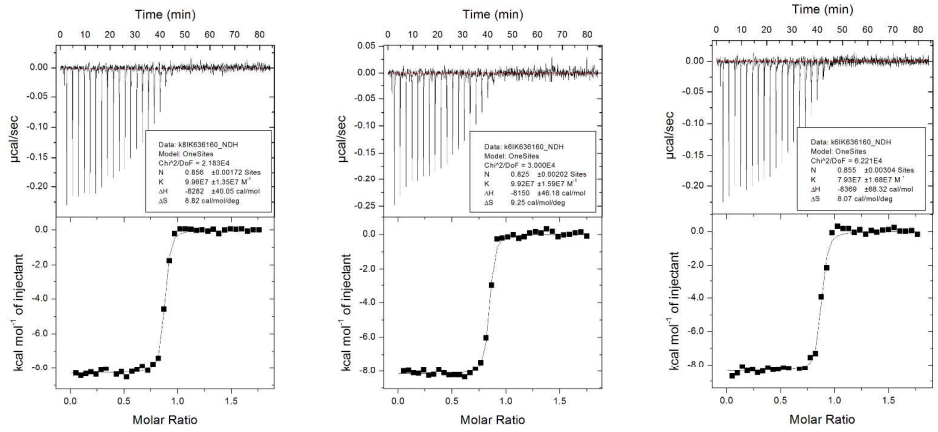
7e (IK-635) ITC curves:



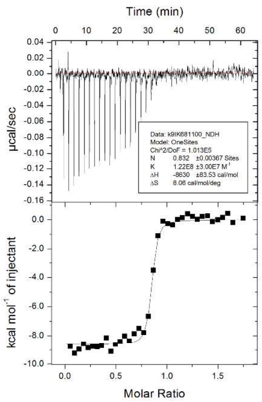
7f (IK-621) ITC curves:

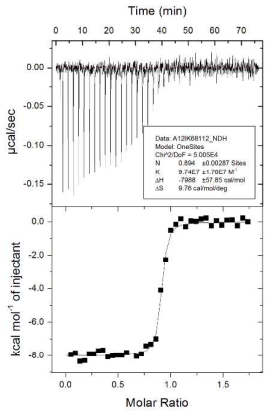


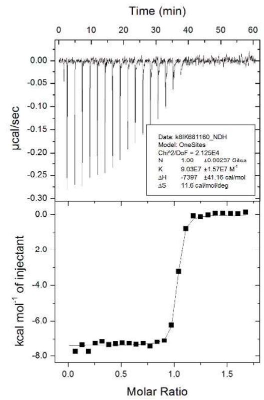
7g (IK-636) ITC curves:

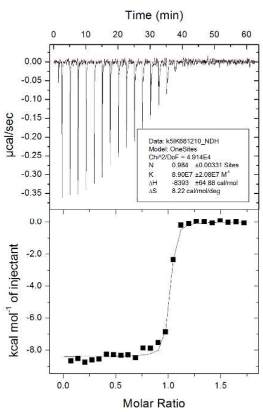


7h (IK-681) ITC curves:

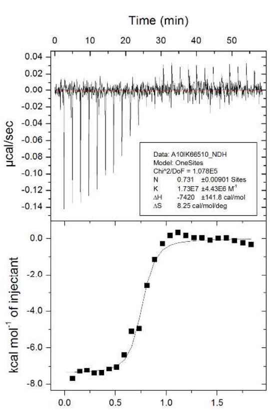


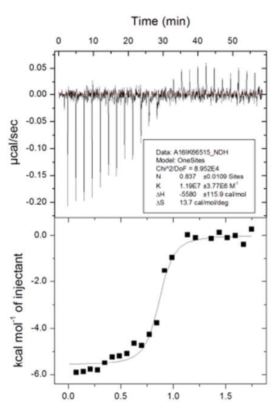


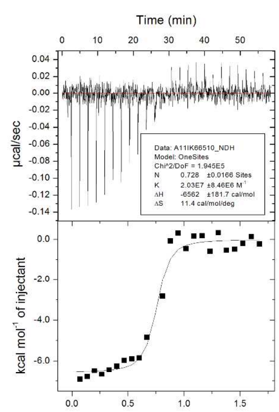


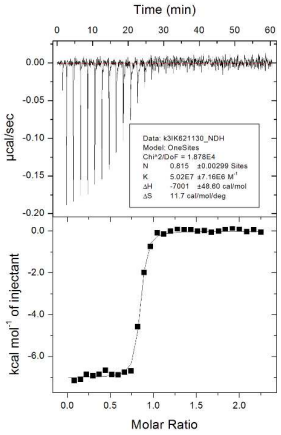


7i (IK-665) ITC curves:

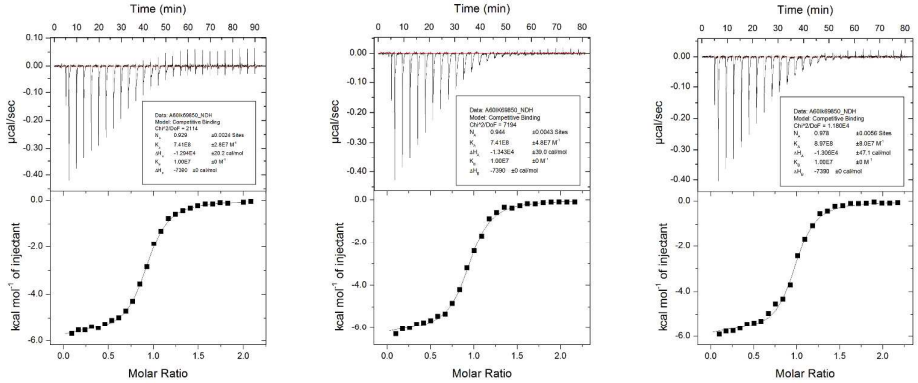








7j (IK-698) ITC curves:



4. In vitro assay for determination of LeuRS inhibitory activity

The compounds have been tested for antibacterial activity *in vitro* as aminoacyl-tRNA synthetases (aaRS) inhibitors following the following process.

Targeted aaRSs

Leucyl-tRNA synthetase (LRS) from *Escherichia coli* (Eco) and *Staphylococcus aureus* (Sau).

Protein expression and purification

Escherichia coli M15 cells transformed with plasmid pQE-60, or pQE-70, containing the open-reading frame sequence of one targeted aaRS were induced with 1 mM IPTG (Isopropyl β-D-1-thiogalactopyranoside) for 3 h at 37^{o}C. Bacterial cells were harvested and lysed with

20 mM NaH_2PO_4 (pH 8.0), 200 mM NaCl, 10 mM imidazole and protease inhibitor cocktail (Roche). Pathogenic aaRS was purified by nickel affinity standard chromatography. Protein concentration was determined by spectrophotometry.

In vitro tRNA transcription

$tRNA^{Leu}$ were transcribed *in vitro* for 4 h at 37°C using T7 RNA polymerase. Transcription reaction contained 40 mM Tris-HCl (pH 8.0), 22 mM $MgCl_2$, 1 mM spermidine, 5 mM DTT, 0.01% Triton X-100, 4 mM GTP, 4mM ATP, 4 mM UTP, 4 mM CTP, 16 mM GMP, 250µM T7 RNA polymerase and 150µg BstNI digested plasmid. Once finished, the reaction was applied on a 6% polyacrylamide-8 M urea denaturing gel to purify the transcribed tRNA and discard any impurities. Purified tRNA was quantified with Nanodrop 2000 (Thermo Scientific).

Determination of IC_{50}

The aminoacylation reaction catalyzed by aminoacyl-tRNA synthetases (aaRS) takes place in two steps. In the first step, aaRS activates its cognate amino acid with ATP; and in the second step the activated amino acid is loaded to its corresponding tRNA. This reaction can be summarized as follows:

[1] ARS + aa + ATP = ARS-aa-AMP + PPi

[2] ARS-aa-AMP + tRNA = aa-tRNA + AMP + ARS

(ARS, Aminoacyl-tRNA synthetase; aa, amino acid; ARS-aa-AMP, enzyme-bound to aminoacyl-adenylate; aa-tRNA, aminoacyl-tRNA).

The activity of the aaRSs was monitored by measuring the ATP consumption rate, since this consumption is directly proportional to the activity of the aaRS. If the tested compound, at a single point concentration of 50µM, is inhibiting the aminoacylation reaction, there is a decrease in the ATP consumption, compared to the control reaction without compound, allowing the calculation of an inhibition ratio.

When the inhibition ratio for a given compound was above 80%, IC_{50} determination was performed with the same enzymatic assay (using the commercial kit Kinase RR from BioThema AB, Sweden) in the presence of serial dilutions of inhibitor. Known inhibitors of LRS were used as a positive control of the assay. The IC_{50} was calculated based on nonlinear regression analysis.

5. Antibacterial susceptibility determinations

Minimum inhibitory concentrations (MICs) were determined by broth micro-dilution in Mueller-Hinton broth II (CLSI, 2012).[4] Briefly, bacterial suspensions (~5 X 10^5 cfu/ml) of *E. coli* were added to a two-fold dilution series of the compounds in 96-well micro-titre plates. MICs were defined as the lowest concentration of compound that inhibited visible bacterial growth after incubation at 37°C with aeration for 18 hours.

[4] *Clinical and Laboratory Standards Institute (CLSI).* Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard: ninth edition, M07-A9, Wayne, PA. **2012.**