Organic and Biomolecular Chemistry Full Paper

Electronic Supplementary Information (ESI)

Inhibitors of the Kinase IspE: Structure–Activity Relationships and Co-Crystal Structure Analysis

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Fig. 1ESI. Active site of *E. coli* IspE from the ternary complex with CDP-ME and the non-hydrolysable ATP analogue 5'-adenyl-β,γ-amidotriphosphate (AppNp) (PDB code: 1OJ4).[19] Colour code: protein skeleton: C: grey; inhibitor skeleton: C: green; O: red; N: blue; P: orange. The colour code is maintained throughout the ESI, if not otherwise stated.

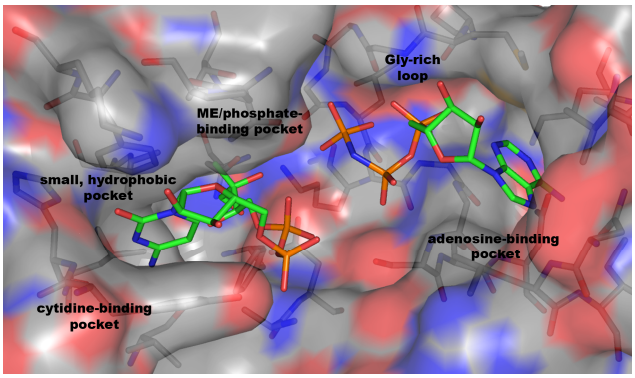


Fig. 2ESI. MOLOC-generated molecular model of inhibitor **22** in the active site of *E. coli* IspE (PDB code: 1OJ4).[19] Colour code: S: yellow. Distances are given in Å. The units for the indicated distances are maintained throughout the ESI.

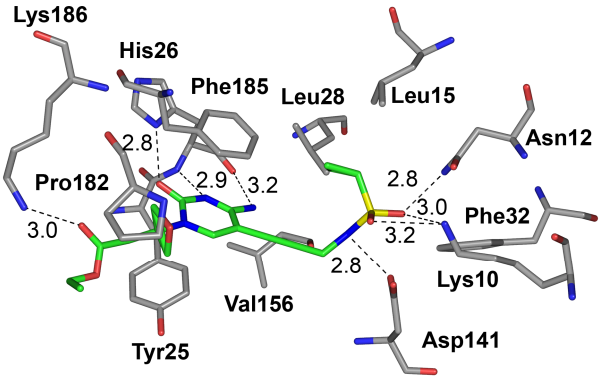
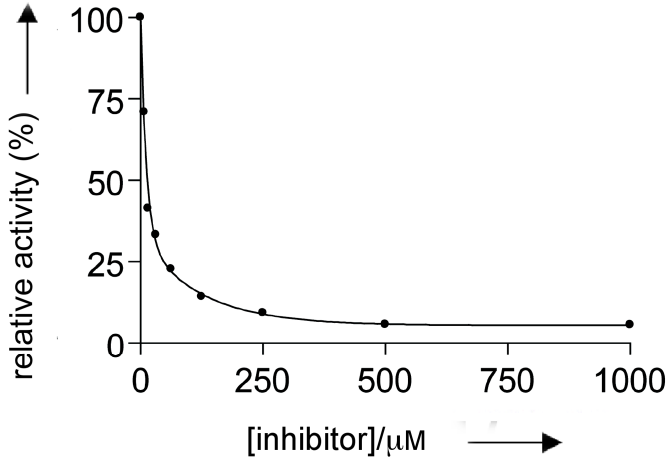


Fig. 3ESI. Exemplary IC_{50} curves for inhibition of *E. coli* IspE by inhibitors (±)-**9** (a) and **22** (b). [CDP-ME] = 1 mM; [IspE] = 2.5 μg/mm^3.

a)



b)

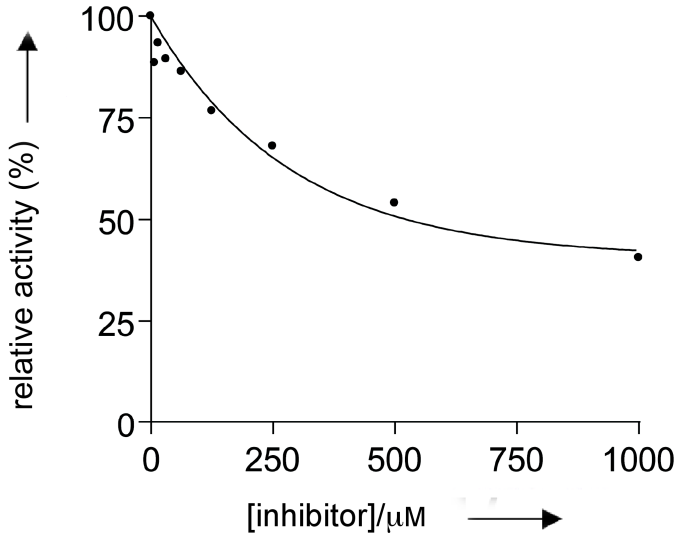
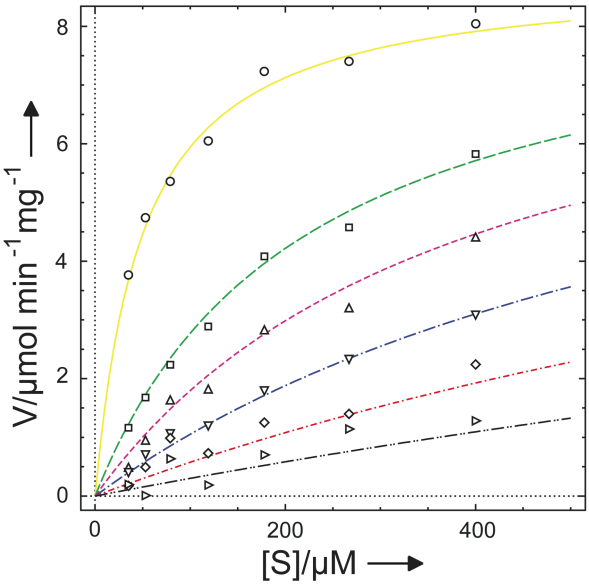


Fig. 4ESI. Exemplary kinetics for the inhibition of *E. coli* IspE by inhibitors (±)-**9** (a) and **22** (b). Inhibitor concentrations were 0, 2, 4, 8, 16 and 32 µM (a) and 0, 8, 16, 31, 62 and 125 µM (b).

a)



b)

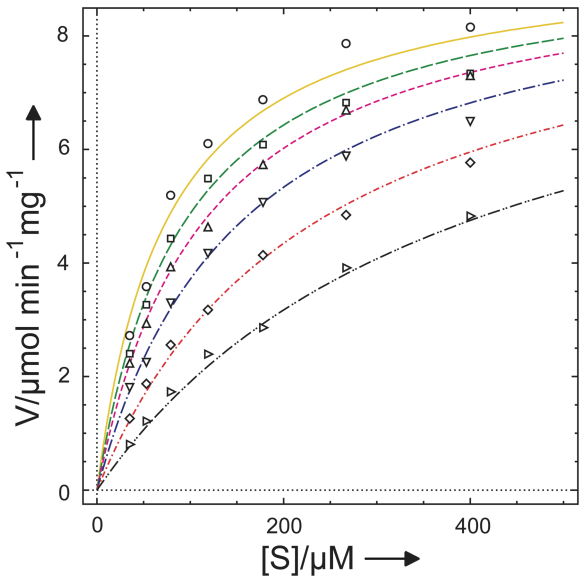


Fig. 5ESI. MOLOC-generated molecular model of (±)-**3**,[12] showing hydrophobic contacts in the small, hydrophobic pocket of *E. coli* IspE (PDB code: 1OJ4).[19]

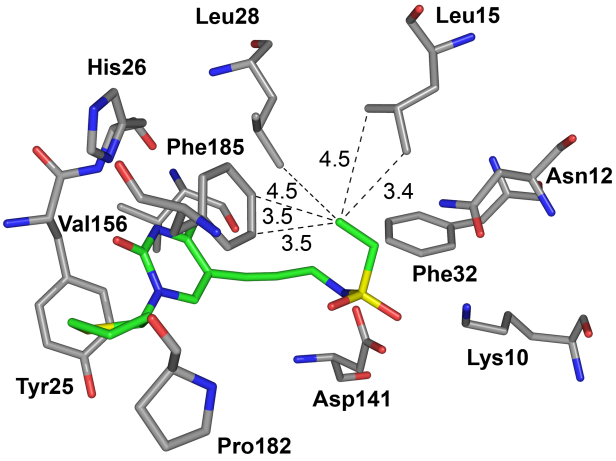


Fig 6ESI. MOLOC-generated molecular model of inhibitors featuring *n*-alkyl chains bound within the active site of *E. coli* IspE (PDB code: 1OJ4).[19] Colour code: C-skeleton of (±)-**2**[12]: green, C-skeleton of (±)-**3**[12]: cyan, C-skeleton of (±)-**4**[12]: magenta, C-skeleton of (±)-**6**: light pink.

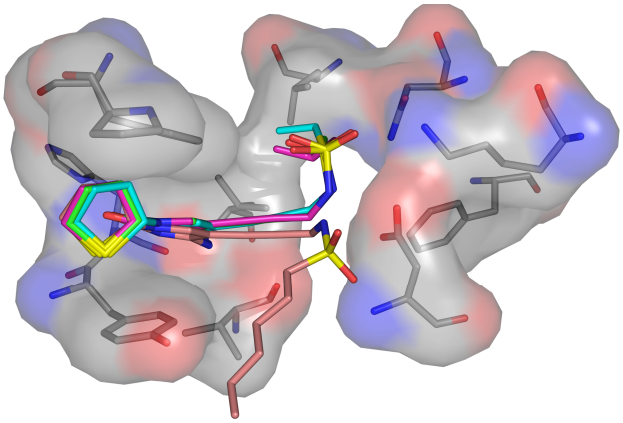


Fig 7ESI. MOLOC-generated molecular model of inhibitor (±)-**16** in the active site of *E. coli* IspE (PDB code:1OJ4).[19]

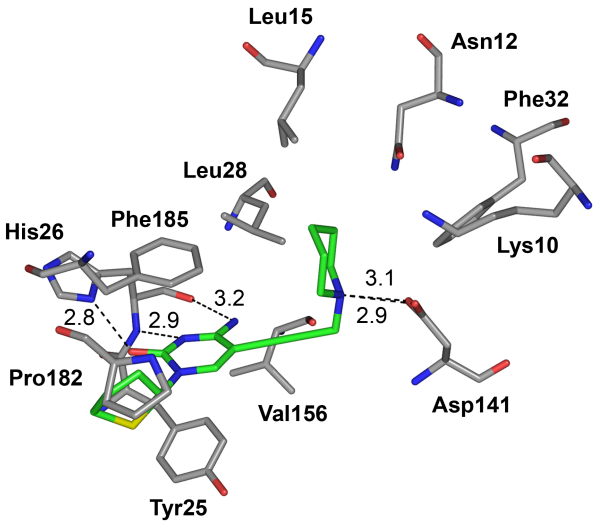
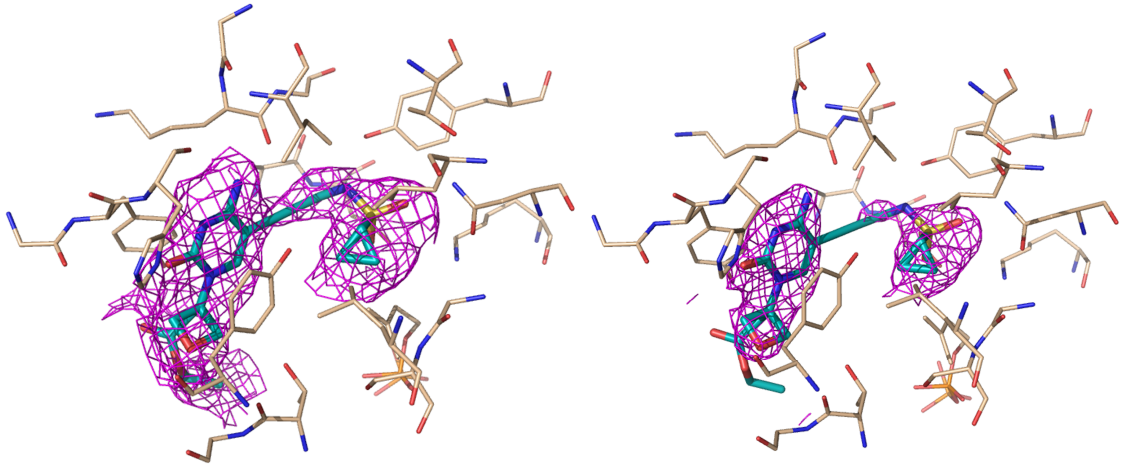
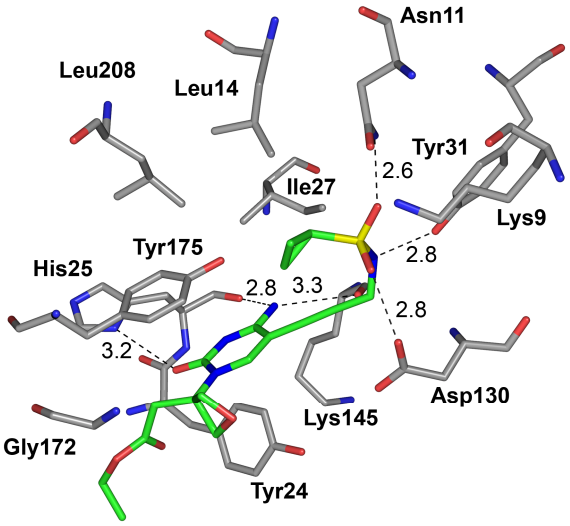


Fig. 8ESI. a) Omit difference density maps for **22** in the active site of *A. aeolicus* IspE. The purple chicken wire represents the F_o–F_c, acalc calculation, where F_o and F_c are the observed and calculated structure factors, respectively and acalc the model phases calculated from all atomic positions except for the ligand itself; contoured at the 1 σ (left) and 2.5 σ level (right). Colour code: protein skeleton: C: light pink; inhibitor skeleton: C: cyan. b) X-ray crystal structure of *A. aeolicus* IspE co-crystallised with **22** and diphosphate (PDB code: 2VF3). Shown is active site A. c) Superposition of **22**, as observed in active site A of *A. aeolicus* IspE, onto **22,** as observed in active site B of *A. aeolicus* IspE (PDB code: 2VF3). Colour code: C-skeleton of **22** as observed in active site A: light pink.

a)



b)



c)

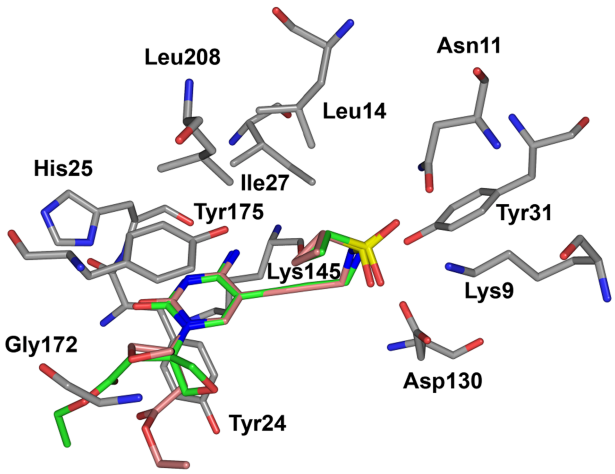
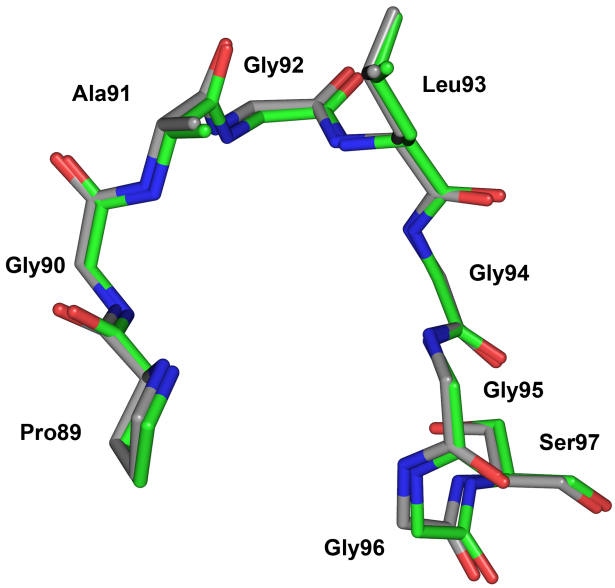
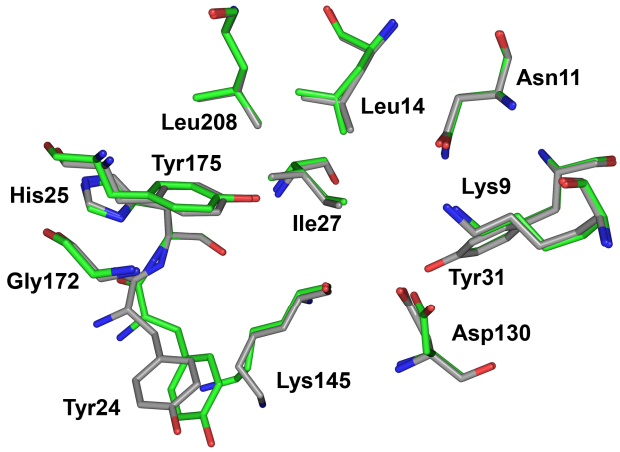


Fig. 9ESI. Superposition of the active sites from the X-ray crystal structures of *A. aeolicus* IspE with a cytidine-based ligand and **22** (PDB codes: 2V2V and 2VF3, respectively).[13] a) Gly-rich loop; b) Cytidine-binding pocket. Colour code: protein skeleton of 2VF3: C: green.

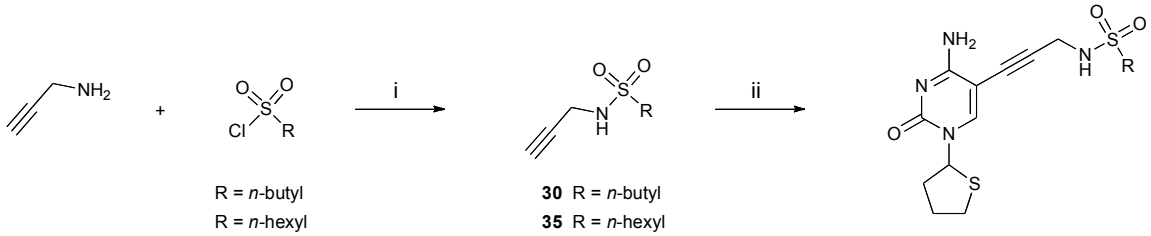
a)



b)



Scheme 1ESI. Synthesis of inhibitors (±)-**5** – (±)-**8** and (±)-**11**. (i) Et_3N, CH_2Cl_2, 0 °C → 25 °C, 15–30 min, **30**,[33] **35** (quantitative), (±)-**36** (35%), **37** (31%), **40** (78%); (ii) (±)-**29**,[12] Et_3N, $[PdCl_2(PPh_3)_2]$, CuI, DMF, 25 °C, 2.5–3 h, (±)-**5** (72%), (±)-**6** (41%), (±)-**7** (72%), (±)-**8** (92%), (±)-**11** (66%).

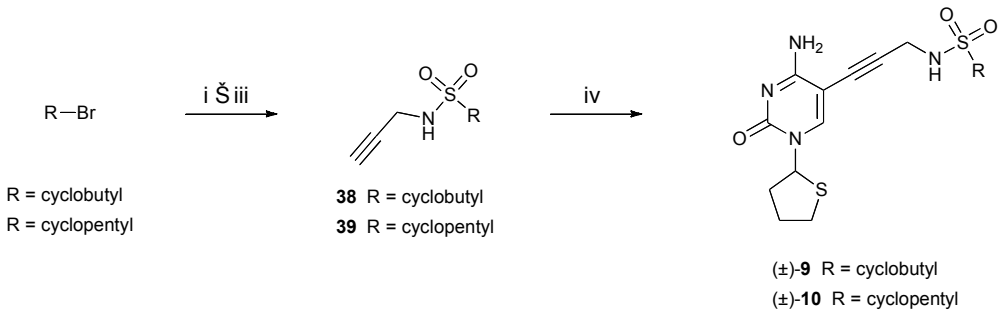


R = *n*-butyl
R = *n*-hexyl
R = *sec*-butyl
R = isopropyl
R = cyclohexyl

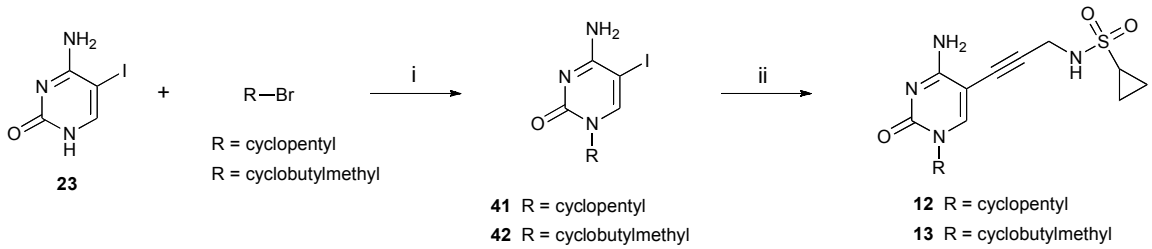
30 R = *n*-butyl
35 R = *n*-hexyl
(±)-**36** R = *sec*-butyl
37 R = isopropyl
40 R = cyclohexyl

(±)-**5** R = *n*-butyl
(±)-**6** R = *n*-hexyl
(±)-**7** R = *sec*-butyl
(±)-**8** R = isopropyl
(±)-**11** R = cyclohexyl

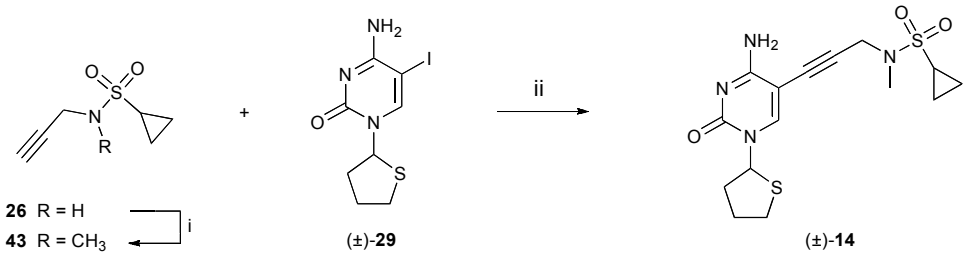
Scheme 2ESI. Synthesis of inhibitors (±)-**9** and (±)-**10**. (i) Mg, Et_2O, reflux, 30 min; (ii) SO_2Cl_2, CH_2Cl_2, 0 °C → 25 °C; (iii) propargyl amine, CH_2Cl_2, 0 °C → 25 °C, 15–30 min, **38** (31%), **39** (30%); (iv) (±)-**29**,[12] Et_3N, $[PdCl_2(PPh_3)_2]$, CuI, DMF, 25 °C, 2.5 h, (±)-**9** (54%), (±)-**10** (75%).



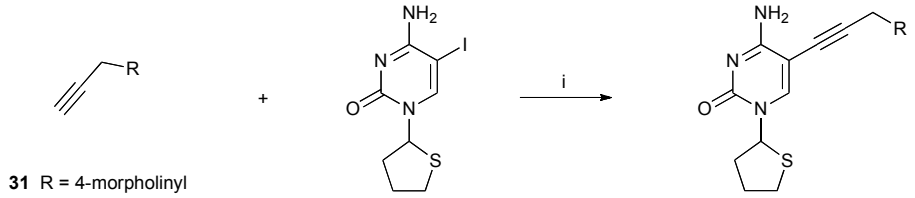
Scheme 3ESI. Synthesis of inhibitors **12** and **13**. (i) Cs_2CO_3 or NaH, DMF, 50 °C, 16 h or 8 h, **41** (37%), **42** (54%); (ii) **26**,[12] Et_3N, $[PdCl_2(PPh_3)_2]$, CuI, DMF, 25 °C, 2.5 h, **12** (90%), **13** (74%).



Scheme 4ESI. Synthesis of inhibitor (±)-**14**. (i) NaH, MeI, DMF, 25 °C, 1 h, 91%; (ii) Et_3N, [$Pd(PPh_3)_4$], CuI, DMF, 50 °C, 26 h, 31%.



Scheme 5ESI. Synthesis of inhibitors (±)-**15** – (±)-**17**. (i) Et_3N, $[PdCl_2(PPh_3)_2]$, CuI, DMF, 25 °C, 2 h, (±)-**15** (95%), (±)-**16** (43%), (±)-**17** (53%).



31 R = 4-morpholinyl
32 R = 1-piperidinyl
33 R = 1-pyrrolidinyl

(±)-**29**

(±)-**15** R = 4-morpholinyl
(±)-**16** R = 1-piperidinyl
(±)-**17** R = 1-pyrrolidinyl

Scheme 6ESI. Synthesis of inhibitors **19** – **21**. (i) NaH, DMF, 25 °C, 21 h or 15 h, **44** (89%), **45** (67%); (ii) **26** or **34**,[12] Et_3N, $[Pd(PPh_3)_4]$ or $[PdCl_2(PPh_3)_2]$, CuI, DMF, 50 °C or 25 °C, 3.5 h or 20 h, **20** (75%), **21** (73%); (iii) Et_3N, H_2O, reflux, 1 h, 90%.

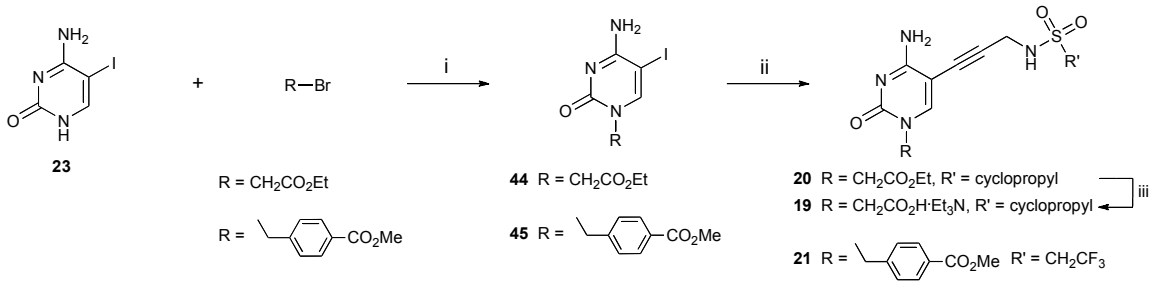


Table 1ESI. X-ray co-crystal structure: statistics for data collection and refinement.

Space Group	$P2_13$
Unit cell length (Å)	137.3
Resolution range (Å)	97.1–2.2
Observed / unique reflections	271,731 / 43,998
Wilson B (Å^2)	48.6
Completeness (%)	99.9 (100.0)
Multiplicity/R_{merge} (%)	6.2 (6.1) / 6.0 (69.0)
$\langle I/\sigma(I)\rangle$	19.4 (2.5)
R_{work} / R_{free} (%)	22.7 (29.8) / 27.6 (38.0)
r.m.s.d from ideal values, bond lengths (Å)	0.013
r.m.s.d from ideal values, bond angles (°)	1.585
B-factors	
Overall / main chain / side chain /	46.1 / 45.6 / 46.5 /
water molecules / **22** / diphosphate / Cl^- / Br^-	43.5 / 56.1 / 50.4 / 46.5 / 32.4
Residues in most favourable regions (%)	91.8
Residues in additionally allowed regions (%)	7.5
Cruickshanks DPI[a] (Å) based on R_{free}	0.23

Numbers in parenthesis represent the highest resolution bin of width approx. 0.12 Å.

[a] Diffraction-component Precision Index.[43]

Synthesis ESI

General procedures

General Procedure B for the preparation of a sulfonamide from a sulfonyl chloride

To a solution of propargyl amine (1.0 eq) in dry CH_2Cl_2, Et_3N (1.1 eq) and the sulfonyl chloride (1.0 eq) were slowly added at 0 °C. After completion of the addition, the mixture was left to stir at 25 °C for 15–30 min and concentrated *in vacuo*. The residue was purified by CC (SiO_2; EtOAc–cyclohexane 1:2).

General procedure C for the base-mediated alkylation of 23[24]

A solution of **23**[24] (1.0 eq) and NaH (1.1 eq) in dry DMF was left to stir at 25 °C for 1 h. The alkyl bromide (1.1 eq) in dry DMF was slowly added, and the mixture was left to stir at 25–50 °C for 8–21 h and concentrated *in vacuo*. NaH was used as suspension of NaH in mineral oil (55–65%).

General procedure D for the preparation of sulfonamides from bromides

To a suspension of Mg turnings (1.7 eq) in dry Et_2O, a solution of the alkyl bromide (1.0 eq) in dry Et_2O was slowly added in small portions under strong stirring. After the initial exothermic reaction had ceased, the mixture was further heated to reflux for 30 min. The suspension was cooled to 25 °C and slowly added to a solution of sulfuryl chloride (3.0 eq) in dry CH_2Cl_2 at 0 °C. The mixture was warmed up to 25 °C and concentrated in *vacuo*. The residue was extracted with *n*-hexane and concentrated in *vacuo*. The remaining oil was used without further purification and slowly added to a solution of propargyl amine (1.0 eq) and Et_3N (1.1 eq) in dry CH_2Cl_2 at 0 °C. After completion of the addition, the mixture was left to stir at 25 °C for 15–30 min and then concentrated *in vacuo*. The residue was purified by CC (SiO_2; EtOAc–cyclohexane 1:2) to afford the corresponding sulfonamide.

Preparation of the precursors

***N*-Prop-2-yn-1-ylhexane-1-sulfonamide (35):**

General procedure B, starting from propargyl amine (0.13 cm^3, 2.0 mmol), Et_3N (0.30 cm^3, 2.2 mmol) and hexanesulfonyl chloride (0.30 cm^3, 2.0 mmol) in dry CH_2Cl_2 (15 cm^3). Purification by CC afforded **35** (387 mg, quantitative) as a yellow oil (Found C 52.9, H 8.4, N 6.9. Calcd for $C_9H_{17}NO_2S$: C 53.2, H 8.4, N 6.9%); ν_{max}(neat)/cm^{-1} 3274, 2930, 2860, 1431, 1325, 1251, 1143, 1078, 993, 920, 835, 663; δ_H(300 MHz, $CDCl_3$) 0.90 (t, J = 6.9, 3 H), 1.28–1.48 (m, 6 H), 1.79–1.89 (m, 2 H), 2.35 (t, J = 2.7, 1 H), 3.11–3.16 (m, 2 H), 3.96 (dd, J = 2.7, 6.3, 2 H), 4.44 (br s, 1 H); δ_C(75 MHz, $CDCl_3$) 13.9, 22.3, 23.5, 27.9, 31.2, 32.6, 53.5, 72.9, 109.8; EI-HR-MS: calcd for $C_9H_{16}NO_2S^+$ ($[M-H]^+$): 202.0897; found: 202.0894.

(±)-***N*-Prop-2-yn-1-ylbutane-2-sulfonamide ((±)-36):**

General procedure B, starting from propargyl amine (0.13 cm^3, 2.0 mmol), Et_3N (0.30 cm^3, 2.2 mmol), and *sec*-butanesulfonyl chloride (0.13 cm^3, 2.0 mmol) in dry CH_2Cl_2 (15 cm^3). Purification by CC afforded (±)-**36** (120 mg, 35%) as a yellow oil; ν_{max}(neat)/cm^{-1} 3275, 2974, 2935, 2889, 1458, 1315, 1140, 1078, 985, 918, 853, 719, 645; δ_H(300 MHz, $CDCl_3$) 1.02 (t, J = 7.5, 3 H), 1.38 (d, J = 6.9, 3 H), 1.49–1.64 (m, 1 H), 2.00–2.13 (m, 1 H), 2.32 (t, J = 2.6, 1 H), 2.98-3.10 (m, 1 H), 3.93 (dd, J = 2.6, 6.2, 2 H), 4.81 (br. s, 1 H); δ_C(75 MHz, $CDCl_3$) 11.2, 13.3, 23.4, 32.7, 59.7, 72.6, 79.2; EI-HR-MS: calcd for $C_7H_{13}NNaO_2S^+$ ($[M+Na]^+$): 198.0565; found: 198.0560.

***N*-Prop-2-yn-1-ylpropane-2-sulfonamide (37):**

General procedure B, starting from propargyl amine (0.13 cm^3, 2.0 mmol), Et_3N (0.30 cm^3, 2.2 mmol) and isopropanesulfonyl chloride (0.23 cm^3, 2.0 mmol) in dry CH_2Cl_2 (15 cm^3). Purification by CC afforded **37** (100 mg, 31%) as a red oil; ν_{max}(neat)/cm^{-1} 3274, 2956, 2930, 2863, 1458, 1324, 1248, 1143, 1076, 990, 925, 838,

668; δ_H(300 MHz, $CDCl_3$) 1.40 (d, J = 6.9, 6 H), 2.33 (t, J = 2.4, 1 H), 3.29 (sept, J = 6.9, 1 H), 3.94 (dd, J = 2.4, 6.0, 2 H), 4.75 (br s, 1 H); δ_C(75 MHz, $CDCl_3$) 16.5 (2 C), 32.8, 53.9, 72.7, 79.2; EI-HR-MS: calcd for $C_6H_{11}NO_2S^+$ ($[M]^+$): 161.0510; found: 161.0497.

***N*-Prop-2-yn-1-ylcyclobutanesulfonamide (38):**

General procedure D, starting from Mg turnings (100 mg, 4.2 mmol) in dry Et_2O (4.0 cm^3) and cyclobutyl bromide (0.20 cm^3, 2.5 mmol) in dry Et_2O (4.0 cm^3); sulfuryl chloride (0.60 cm^3, 7.4 mmol) in dry CH_2Cl_2 (6.0 cm^3); propargyl amine (0.13 cm^3, 2.0 mmol) and Et_3N (0.30 cm^3, 2.2 mmol) in dry CH_2Cl_2 (15 cm^3). Purification by CC afforded **38** (110 mg, 31%) as a brown oil; ν_{max}(neat)/cm^{-1} 3274, 2952, 2873, 1434, 1318, 1282, 1243, 1143, 1076, 1011, 913, 812, 737, 623; δ_H(300 MHz, $CDCl_3$) 1.96–2.07 (m, 2 H), 2.29–2.41 (m, 2 H), 2.34 (t, J = 2.7, 1 H), 2.45–2.58 (m, 2 H), 3.91–3.95 (m, 3 H), 4.57 (br s, 1 H); δ_C(75 MHz, $CDCl_3$) 16.8, 23.9, 32.7, 55.0, 72.6, 79.3; EI-HR-MS: calcd for $C_7H_{11}NO_2S^+$ ($[M]^+$): 173.0505; found: 173.0507.

***N*-Prop-2-yn-1-ylcyclopentanesulfonamide (39):**

General procedure D, starting from Mg turnings (150 mg, 6.3 mmol) in dry Et_2O (4.0 cm^3) and cyclopentyl bromide (0.36 cm^3, 3.3 mmol) in dry Et_2O (4.0 cm^3); sulfuryl chloride (0.90 cm^3, 11.1 mmol) in dry CH_2Cl_2 (6.0 cm^3); propargyl amine (0.18 cm^3, 2.7 mmol) and Et_3N (0.45 cm^3, 3.3 mmol) in dry CH_2Cl_2 (15 cm^3). Purification by CC afforded **39** (150 mg, 30%) as a brown oil that was taken directly to the next step without full characterisation; ν_{max}(neat)/cm^{-1} 3279, 2963, 1640, 1493, 1306, 1127, 1076, 972, 839, 783, 719; δ_H(300 MHz, $CDCl_3$) 1.57–1.70 (m, 2 H), 1.76–1.89 (m, 2 H), 1.95–2.14 (m, 4 H), 2.34 (t, J = 2.7, 1 H), 3.61 (quint, J = 7.5,

1 H), 3.94–3.98 (m, 2 H), 4.54 (br s, 1 H); EI-HR-MS: calcd for $C_8H_{12}NO_2S^+$ ($[M-H]^+$) 186.0584; found: 186.0585.

***N*-Prop-2-yn-1-ylcyclohexanesulfonamide (40):**

General procedure B, starting from propargyl amine (0.13 cm^3, 2.0 mmol), Et_3N (0.30 cm^3, 2.2 mmol) and cyclohexanesulfonyl chloride (0.30 cm^3, 2.0 mmol) in dry CH_2Cl_2 (15 cm^3). Purification by CC afforded **40** (320 mg, 78%) as a yellow oil; ν_{max}(neat)/cm^{-1} 3273, 2938, 2860, 1454, 1317, 1269, 1144, 1078, 985, 893, 861, 838, 668; δ_H(300 MHz, $CDCl_3$) 1.17–1.37 (m, 3 H), 1.48–1.61 (m, 2 H), 1.68–1.74 (m, 1 H), 1.89–1.94 (m, 2 H), 2.20–2.25 (m, 2 H), 2.35 (t, J = 2.5, 1 H), 3.02 (tt, J = 3.5, 12.0, 1 H), 3.95 (dd, J = 2.4, 6.0, 2 H), 4.41 (br s, 1 H); δ_C(75 MHz, $CDCl_3$) 25.1, 25.2 (2 C), 26.3 (2 C), 32.7, 61.6, 72.6, 79.2; EI-HR-MS: calcd for $C_9H_{14}NO_2S^+$ ($[M+H]^+$): 200.0740; found: 200.0742.

4-Amino-1-cyclopentyl-5-iodopyrimidin-2(1*H*)-one (41):

General procedure C, starting from **23**[24] (240 mg, 1 mmol), Cs_2CO_3 (360 mg, 1.1 mmol) and cyclopentyl bromide (0.12 cm^3, 1.1 mmol) in dry DMF (15 + 5.0 cm^3). The mixture was left to stir at 50 °C for 16 h and concentrated *in vacuo*. Purification by CC (SiO_2; CH_2Cl_2–MeOH 96:4) afforded **41** (110 mg, 37%) as a white solid; mp > 210 °C (decomposition); ν_{max}(neat)/cm^{-1} 3441, 2953, 2867, 2661, 2567, 2235, 2040, 2025, 1984, 1967, 1946, 1897, 1822, 1767, 1610, 1514, 1481, 1400, 1309, 1283, 1243, 1189, 1099, 1067, 1026, 913, 850, 777, 725, 643; δ_H(300 MHz, ($CDCl_3$–CD_3OD 7:1) 1.43–1.72 (m, 6 H), 1.97–2.03 (m, 2 H), 4.79 (quint, J = 7.9, 1 H), 7.50 (s, 1 H); δ_C(75 MHz, $CDCl_3$–CD_3OD 7:1) 23.7 (2 C), 31.6 (2 C), 56.1, 58.1, 147.5, 155.9, 163.0; MALDI-HR-MS: calcd for $C_9H_{13}IN_3O^+$ ($[M+H]^+$): 306.0098; found: 306.0103.

4-Amino-1-(cyclobutylmethyl)-5-iodopyrimidin-2(1*H*)-one (42):

General procedure C, starting from: **23**[24] (240 mg, 1 mmol), NaH (44 mg, 1.1 mmol) and cyclobutylmethyl bromide (0.12 cm^3, 1.1 mmol) in dry DMF (15 + 5.0 cm^3). The mixture was left to stir at 50 °C for 8 h. Purification by CC (SiO_2; CH_2Cl_2–MeOH 96:4) afforded **42** (164 mg, 54%) as a white solid (Found C 35.4, H 4.0, N 13.7. Calcd for $C_9H_{12}IN_3O$: C 35.4, H 4.0, N 13.8%); mp 154–156 °C; ν_{max}(neat)/cm^{-1} 2967, 2933, 2858, 2569, 2497, 2427, 1808, 1603, 1467, 1429, 1358, 1312, 1280, 1241, 1191, 1156, 1128, 1015, 951, 906, 867, 773, 736, 676, 632; δ_H(300 MHz, CD_3OD) 1.75–2.06 (m, 6 H), 2.72 (quint, J = 7.6, 1 H), 3.79 (d, J = 7.6, 2 H), 8.02 (s, 1 H); δ_C(75 MHz, CD_3OD) 19.0, 26.5 (2 C), 36.1, 55.6, 56.1, 153.4, 158.0, 165.8; MALDI-HR-MS: calcd for $C_9H_{13}IN_3O^+$ ($[M+H]^+$): 306.0098; found: 306.0098.

***N*-Methyl-*N*-prop-2-yn-1-ylcyclopropanesulfonamide (43):**

A supension of **26**[12] (87 mg, 0.55 mmol) and NaH (26 mg, 1.1 mmol) in dry DMF (7.0 cm^3) was left to stir at 25 °C for 1 h. Methyl iodide (68 mm^3, 1.1 mmol) was added, and the mixture was left to stir at 25 °C for 1 h. The resulting mixture was quenched with water (10 cm^3) and extracted with CH_2Cl_2 (3 x 15 cm^3). The combined org. phases were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Filtration through a plug of silica gel (EtOAc–cyclohexane 1:2**)** afforded **43** (87 mg, 91%) as a yellow oil (Found C 48.35, H 6.4, N 7.9. Calcd for $C_7H_{11}NO_2S$: C 48.5, H 6.4, N 8.1%); ν_{max}(neat)/cm^{-1} 3273, 2969, 1635, 1456, 1328, 1306, 1200, 1148, 1067, 1041, 995, 927, 907, 888, 827, 783, 760, 742, 691; δ_H(300 MHz, $CDCl_3$) 0.96–1.03 (m, 2 H), 1.16–1.21 (m, 2 H), 2.36 (t, J = 2.5, 1 H), 2.39–2.46 (m, 1 H), 2.96 (s, 3 H), 4.06 (d, J = 2.5, 2 H); δ_C(75 MHz, $CDCl_3$) 5.1 (2 C), 27.3, 34.7, 39.8, 74.1, 77.0; HR-ESI-MS: calcd for $C_7H_{11}NNaO_2S^+$ ($[M+Na]^+$): 196.0403; found: 196.0403.

Ethyl (4-Amino-5-iodo-2-oxopyrimidin-1(2*H*)-yl)acetate (44):

General procedure C, starting from: **23**[24] (950 mg, 4.0 mmol), NaH (110 mg, 4.4 mmol) and ethyl bromoacetate (740 mg, 4.4 mmol) in dry DMF (60 + 20 cm^3). The mixture was left to stir at 25 °C for 21 h. Purification by CC (SiO_2; CH_2Cl_2–MeOH 95:5) afforded **44** (1.2 g, 89%) as a white solid (Found C 29.8, H 3.1, N 13.1. Calcd for $C_8H_{10}IN_3O_3$: C 29.7, H 3.2, N 13.0%); mp 207–209 °C; ν_{max}(neat)/cm^{-1} 3460, 2977, 2250, 1723, 1633, 1478, 1412, 1397, 1368, 1356, 1330, 1282, 1230, 1207, 1093, 1012, 958, 920, 876, 819, 776, 731, 710, 645, 625; δ_H(300 MHz, $CDCl_3$–CD_3OD 7:1) 1.30 (t, J = 7.2, 3 H), 4.25 (q, J = 7.2, 2 H), 4.51 (s, 2 H), 7.57 (s, 1 H); δ_C(75 MHz, $(CD_3)_2SO$) 14.0, 49.6, 55.6, 60.9, 152.2, 154.5, 164.3, 168.4; MALDI-HR-MS: calcd for $C_8H_{11}IN_3O_3^+$ ($[M+H]^+$): 323.9840; found: 323.9846.

Methyl 4-[(4-Amino-5-iodo-2-oxopyrimidin-1(2*H*)-yl)methyl]benzoate (45):

General procedure C, starting from: **23**[24] (470 mg, 2.0 mmol), NaH (53 mg, 2.2 mmol) and methyl 4-(bromomethyl)benzoate (500 mg, 2.2 mmol) in dry DMF (30 + 10 cm^3). The mixture was left to stir at 25 °C for 15 h. Purification by CC (SiO_2; CH_2Cl_2–MeOH 97:3) afforded **45** (520 mg, 67%) as a white solid (Found C 40.6, H 3.2, N 10.7. Calcd for $C_{13}H_{13}IN_3O_3$: C 40.5, H 3.1, N 10.9%); mp 228–230 °C; ν_{max}(neat)/cm^{-1} 3441, 3037, 2945, 1707, 1622, 1488, 1471, 1428, 1413, 1367, 1347, 1320, 1275, 1216, 1189, 1104, 1017, 965, 943, 923, 872, 793, 772, 757, 747, 706, 688, 645, 614; δ_H(300 MHz, $CDCl_3$–CD_3OD 7:1) 3.81 (s, 3 H), 4.91 (s, 2 H), 7.26 (d, J = 8.3, 2 H), 7.57 (s, 1 H), 7.91 (d, J = 8.3, 2 H); δ_C(75 MHz, $CDCl_3$–CD_3OD 7:1) 51.8, 52.0, 56.4, 127.4 (2 C), 129.5, 129.8 (2 C), 140.9, 150.8, 156.1, 164.0, 166.7; MALDI-HR-MS: calcd for $C_{13}H_{13}IN_3O_3^+$ ($[M+H]^+$): 385.9996; found: 385.9992.

Reference

43. D. W. J. Cruickshank, *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 1999, **55**, 583–601.