SUPPORTING INFORMATION

POTENT AND SELECTIVE TYPE II INHIBITORS OF CDK8

Philippe Bergeron[†*], Michael F.T. Koehler[†], Elizabeth M. Blackwood[‡], Krista Bowman[§], Kevin Clark[ǁ], Ron Firestein, James R. Kiefer[§], Klaus Maskos[ǂ], Mark L. McCleland[#], Linda Orren, Sreemathy Ramaswamy[ǁ], Laurent Salphati[⊥], Steve Schmidt[ǁ], Ellisabeth V. Schneider[ǂ], Jiansheng Wu[◊] and Maureen Beresini[ǁ]

[†]Department of Discovery Chemistry, [‡]Department of Translational Oncology, [§]Department of Structural Biology, [ǁ]Department of Small Molecule Biochemical Pharmacology, [#]Department of Pathology, [⊥]Department of Drug Metabolism and [◊]Department of Protein Chemistry, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, United States

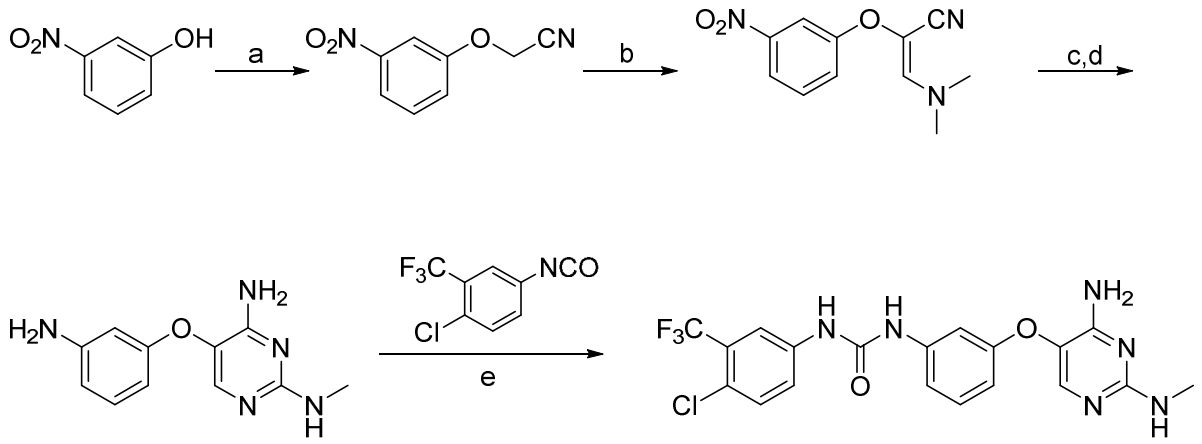
[ǂ]Proteros Biostructures GmbH, Bunsenstr. 7a, 82152 Martinsried, Germany

1. Chemistry

Experimental details:

General. Unless otherwise indicated, all reagents and solvents were purchased from commercial sources and were used without further purification Moisture or oxygen sensitive reactions were conducted under an atmosphere of argon or nitrogen gas. Unless otherwise stated, ^{1}H NMR spectra were recorded at 300 or 400 MHz using Varian or Bruker instruments operating at the indicated frequencies. Chemical shifts are expressed in ppm relative to a tetramethylsilane (ppm = 0.00) internal standard. The following abbreviations are used: br = broad signal, s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, p = pentet, m = multiplet. Purification by silica gel chromatography was carried out using Isco systems with prepacked cartridges. Chemical purities were >95% for all final compounds, as assessed by LC/MS analysis at UV 220 nm (10 min CAD gradient, 0.7 mL/min on Agilent 1200/G6140 system).

Scheme S1. Synthesis of compound 2.[a]



[a] (a) 2-iodoacetonitrile, K_2CO_3, CAN, r.t., 2h, 69%; (b) 1-*tert*-butoxy-N,N,N',N'-tetramethyl-methanediamine, DMF, 90°C, 2h, 99%; (c) 1-mehtylguanidine hydrochloride, K_2CO_3, 1-BuOH, 100°C, 2h, 84%; (d) Iron powder, AcOH, 40°C, 1h, 66%; (e) DIPEA, DCM, 1h, 82%.

2-(3-nitrophenoxy)acetonitrile.

Potassium carbonate (7.5 g, 54 mmol) was added to a solution of 3-nitrophenol (5.0 g, 36 mmol) in acetonitrile (50 ml). The mixture was stirred for 20 minutes then 2-iodoacetonitrile (3.9 mL, 54 mmol) was added. The reaction was stirred at 90°C for 2h. The mixture was then cooled down and extracted with ethyl acetate. The organic phase was dried with

$MgSO_4$, filtered and concentrated to a red solid. The residue was taken up in ether and the precipitate was removed by filtration. The filtrate was concentrated to afford 4.43 g of 2-(3-nitrophenoxy)acetonitrile as a yellow solid. RT = 1.26 min, m+H = 179.2. ^{1}H NMR (400 MHz, DMSO) δ 7.95 (d, J = 8.1 Hz, 1H), 7.91 (s, 1H), 7.68 (t, J = 8.2 Hz, 1H), 7.56 (dd, J = 8.3, 2.2 Hz, 1H), 5.35 (s, 2H).

(*E*)-3-(dimethylamino)-2-(3-nitrophenoxy)prop-2-enenitrile

1-tert-butoxy-N,N,N',N'-tetramethyl-methanediamine (3.0 mL, 15 mmol) was added to a solution of 2-(3-nitrophenoxy)acetonitrile (2.0 g, 11 mmol) in dimethylformamide (15 mL) and the mixture was stirred at 90°C for 2h. The mixture was then cooled down and water was added. The aqueous phase was extracted with ethyl acetate. The organic phase was dried with $MgSO_4$, filtered and concentrated on silica gel. Purification by flash chromatography with a gradient of 0 to 100% ethyl acetate in heptane afforded 2.6 g of (E)-3-(dimethylamino)-2-(3-nitrophenoxy)prop-2-enenitrile as a brown solid.

(*E*)-3-anilino-2-(3-nitrophenoxy)prop-2-enenitrile

Aniline hydrochloride (3.2 g, 25 mmol) was added to a solution of (E)-3-(dimethylamino)-2-(3-nitrophenoxy)prop-2-enenitrile (2.6 g, 11 mmol) in N,N-dimethylformamide (10 mL). The mixture was stirred at 110°C for 3h. The mixture was then cooled to room temperature and poured on ice water. The solid that formed was collected by filtration and washed with water to afford 2.8 g of (E)-3-anilino-2-(3-nitrophenoxy)prop-2-enenitrile as a yellow solid. RT = 1.64 min, m+H = 282.2. ^{1}H NMR (400 MHz, DMSO) δ 9.46 (d, J = 12.7 Hz, 1H), 7.99 (q, J = 4.9 Hz, 2H), 7.87 (s, 1H), 7.73 (t, J = 8.2 Hz, 1H), 7.66 – 7.57 (m, 1H), 7.34 – 7.20 (m, 4H), 6.97 (t, J = 6.8 Hz, 1H).

N2-methyl-5-(3-nitrophenoxy)pyrimidine-2,4-diamine

A vial was charged with (E)-3-anilino-2-(3-nitrophenoxy)prop-2-enenitrile (2.3 g, 8.2 mmol), methylguanidine hydrochloride (1.8 g, 16 mmol) and potassium carbonate-1.5-hydrate (3.4 g, 25 mmol) in N,N-dimethylformamide (10 mL) and 1-butanol (30 mL). The mixture was stirred at 120°C for 7h. The mixture was then cooled down and dichloromethane was added. The precipitate was removed by filtration and the filtrate was concentrated on silica gel. Purification by flash chromatography with a gradient of 0 to 100% ethyl acetate in heptane afforded 1.0 g of N2-methyl-5-(3-nitrophenoxy)pyrimidine-2,4-diamine as a white solid. ^{1}H NMR (400 MHz, DMSO) δ 7.90 (d, J = 9.8 Hz, 1H), 7.73 (s, 1H), 7.63 – 7.57 (m, 2H), 7.39 (d, J = 8.2 Hz, 1H), 6.51 (s, 2H), 6.44 (s, 1H), 2.75 (d, J = 4.9 Hz, 3H).

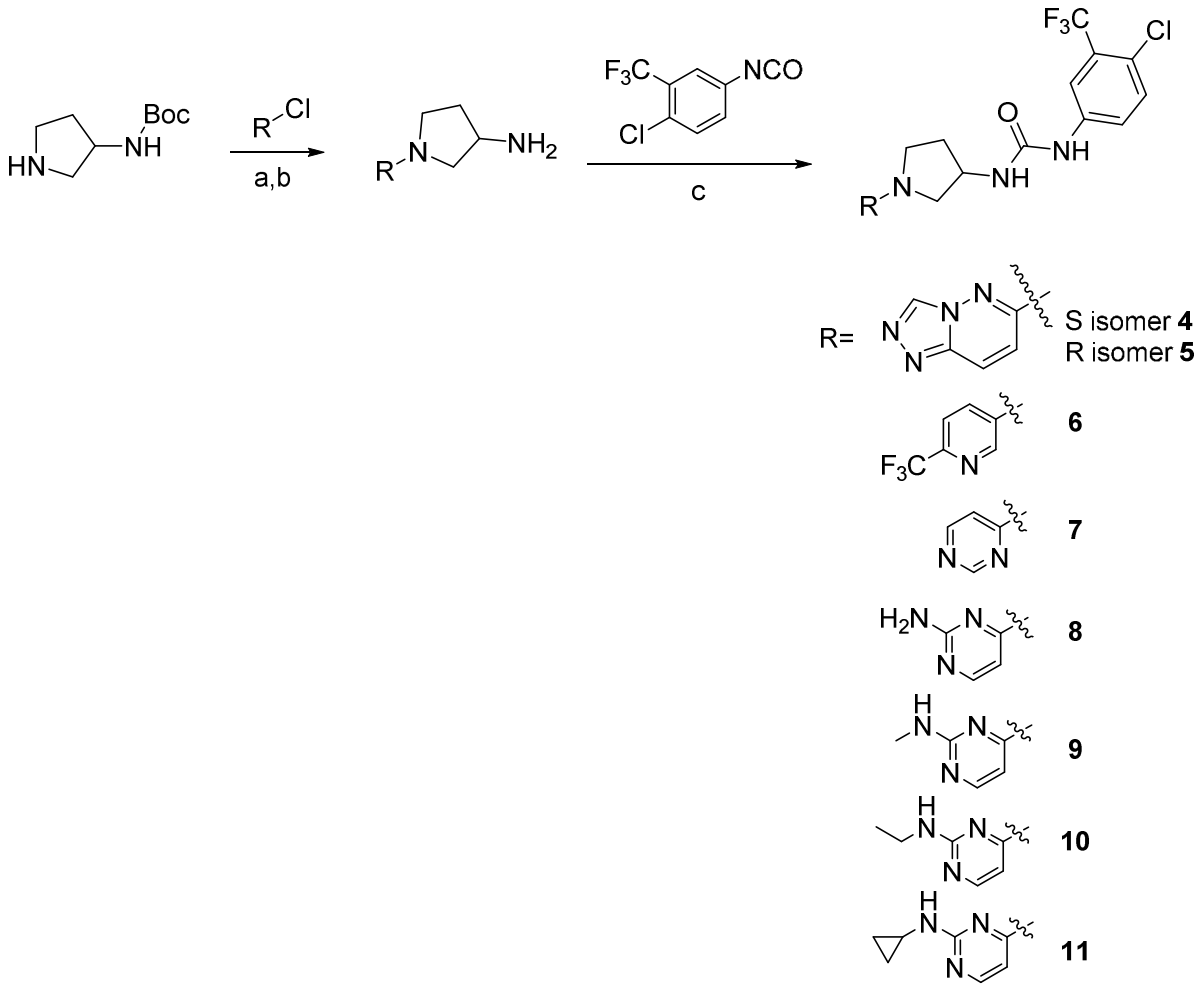
5-(3-aminophenoxy)-N2-methyl-pyrimidine-2,4-diamine

Iron powder (1.1 g, 19 mmol) was added to a solution of N-2-methyl-5-(3-nitrophenoxy)pyrimidine-2,4-diamine (1.0 g, 3.8 mmol) in glacial acetic acid (5 mL) at 40°C and stirred for 2h. The mixture was then cooled down and water was added. The aqueous phase was extracted with dichloromethane, dried with $MgSO_4$, filtered and concentrated afford 400 mg of 5-(3-aminophenoxy)-N2-methyl-pyrimidine-2,4-diamine as a white solid. ^{1}H NMR (400 MHz, DMSO) δ 7.54 (s, 1H), 6.90 (t, J = 8.3 Hz, 1H), 6.25 (d, J = 4.7 Hz, 1H), 6.19 (d, J = 8.1 Hz, 2H), 6.07 (d, J = 6.3 Hz, 2H), 5.12 (s, 2H), 2.70 (t, J = 11.0 Hz, 3H).

1-(3-((4-amino-2-(methylamino)pyrimidin-5-yl)oxy)phenyl)-3-(4-chloro-3-(trifluoromethyl)phenyl)urea (2)

4-Chloro-3-(trifluoromethyl)phenyl isocyanate (0.057mL, 0.52 mmol) dissolved in 3 ml of dichloromethane was slowly added to a solution of 5-(3-aminophenoxy)-N2-methyl-pyrimidine-2,4-diamine (100 mg, 0.43 mmol and N,N-diisopropylethylamine (0.15 mL, 0.86 mmol) in dichloromethane (10 ml). The mixture was stirred at room temperature for 30 min. The mixture was concentrated and purified by HPLC to afford 19.4 mg of 1-[3-[4-amino-2-(methylamino)pyrimidin-5-yl]oxyphenyl]-3-phenyl-urea as a white solid. RT = 3.48 min, m+H = 351.1. ^{1}H NMR (400 MHz, DMSO) δ 8.76 (s, 1H), 8.56 (s, 1H), 7.60 (s, 1H), 7.42 (d, J = 8.0 Hz, 2H), 7.26 (t, J = 7.8 Hz, 2H), 7.18 (t, J = 8.1 Hz, 1H), 7.13 – 7.01 (m, 2H), 6.96 (t, J = 7.4 Hz, 1H), 6.52 (dd, J = 8.1, 2.3 Hz, 1H), 6.30 (s, 3H), 2.73 (d, J = 4.8 Hz, 3H).

Scheme S2. Synthesis of compounds 4-11[a]



[a] (a) DIPEA EtOH, 70°C, 3h, 71%; (b) 4N HCl/dioxane, r.t., 30 min, 99%; (c) DIPEA, DCM, 1h.

***Tert*-butyl N-[(3S)-1-([1,2,4]triazolo[4,3-b]pyridazin-6-yl)pyrrolidin-3-yl]carbamate**

A mixture of 6-chloro-[1,2,4]triazolo[4,3-b]pyridazine (200 mg, 1.2940 mmol), tert-butyl N-[(3S)-pyrrolidin-3-yl]carbamate (265 mg., 1.4234 mmol) and N,N-diisopropylethylamine (0.29 mL, 1.6822 mmol) in ethanol (6 mL, 98.6 mmol) was stirred at 70°C for 6h. The mixture was then cooled down and concentrated. The residue was triturated in water and the solid obtained was collected by filtration, washed with water and dried under vacuum to afford 240 mg of tert-butyl N-[(3S)-1-([1,2,4]triazolo[4,3-b]pyridazin-6-yl)pyrrolidin-3-yl]carbamate as a white solid. LCMS (Method Waters, ESI): RT = 0.79 min, m+H = 305.2. ^{1}H NMR (400 MHz, DMSO) δ 9.18 (s, 1H), 8.03 (d, J = 10.0 Hz, 1H), 7.26 (d, J = 6.6 Hz, 1H), 7.03 (d, J = 10.1 Hz, 1H), 4.14 (dd, J = 11.7, 6.0 Hz, 1H), 3.75 – 3.43 (m, 4H), 2.14 (dq, J = 13.4, 7.0 Hz, 1H), 1.91 (dt, J = 12.5, 6.5 Hz, 1H), 1.39 (s, 9H).

(3*S*)-1-([1,2,4]triazolo[4,3-b]pyridazin-6-yl)pyrrolidin-3-amine hydrochloride

Tert-butyl N-[(3S)-1-([1,2,4]triazolo[4,3-b]pyridazin-6-yl)pyrrolidin-3-yl]carbamate (240 mg, 0.7884 mmol) was stirred in hydrogen chloride (4 mol/L) in 1,4-dioxane (9.8 mL., 39.42 mmol) at room temperature for 30 minutes. The mixture was then concentrated and dried under vacuum to afford 190 mg of (3S)-1-([1,2,4]triazolo[4,3-b]pyridazin-6-yl)pyrrolidin-3-amine hydrochloride as a yellow solid that was used for the next step without purification. RT = 1.17 min, m+H = 205.2

1-[(3*S*)-1-([1,2,4]triazolo[4,3-b]pyridazin-6-yl)pyrrolidin-3-yl]-3-[3-(trifluoromethyl)phenyl]urea (4)

1-isocyanato-3-(trifluoromethyl)benzene (28 mg, 0.150 mmol) was added to a solution of (3*S*)-1-([1,2,4]triazolo[4,3-b]pyridazin-6-yl)pyrrolidin-3-amine hydrochloride (30 mg, 0.124 mmol) and N-ethyldiisopropylamine (0.065 mL, 0.372 mmol) in methylene chloride (1 mL). The mixture was stirred at room temperature for 1h. The mixture was then concentrated and purified by HPLC (conditions) to afford 21.6 mg of 1-[(3*S*)-1-([1,2,4]triazolo[4,3-b]pyridazin-6-yl)pyrrolidin-3-yl]-3-[3-(trifluoromethyl)phenyl]urea as a white solid. RT = 4.34 min, m+H = 392.2. ^{1}H NMR (400 MHz, DMSO) δ 9.24 – 9.17 (s, 1H), 8.83 – 8.69 (s, 1H), 8.14 – 8.02 (d, J = 10.0 Hz, 1H), 8.02 – 7.94 (s, 1H), 7.53 – 7.38 (m, 2H), 7.29 – 7.18 (d, J = 7.0 Hz, 1H), 7.15 – 7.05 (d, J = 10.0 Hz, 1H), 6.80 – 6.67 (d, J = 6.6 Hz, 1H), 4.42 – 4.26 (q, J = 5.7 Hz, 1H), 3.82 – 3.68 (dd, J = 10.9, 6.0 Hz, 1H), 3.66 – 3.51 (m, 2H), 3.48 – 3.37 (dd, J = 10.9, 4.1 Hz, 1H), 2.30 – 2.16 (dq, J = 13.6, 7.0 Hz, 1H), 2.05 – 1.90 (dq, J = 12.4, 6.2 Hz, 1H).

1-[(3*R*)-1-([1,2,4]triazolo[4,3-b]pyridazin-6-yl)pyrrolidin-3-yl]-3-[3-(trifluoromethyl)phenyl]urea (5)

RT = 4.33 min, m+H = 392.2. ^{1}H NMR (400 MHz, DMSO) δ 9.26 – 9.18 (s, 1H), 8.78 – 8.65 (s, 1H), 8.12 – 8.02 (d, J = 10.0 Hz, 1H), 8.03 – 7.93 (s, 1H), 7.53 – 7.38 (m, 2H), 7.31 – 7.19 (d, J = 7.0 Hz, 1H), 7.14 – 7.01 (d, J = 10.0 Hz, 1H), 6.80 – 6.65 (d, J = 6.8 Hz, 1H), 4.45 – 4.27 (q, J = 5.7 Hz, 1H), 3.80 – 3.68 (dd, J = 10.9, 6.1 Hz, 1H), 3.65 – 3.52 (m, 2H), 3.49 – 3.37 (dd, J = 10.6, 4.0 Hz, 1H), 2.30 – 2.16 (dd, J = 12.9, 6.5 Hz, 1H), 2.05 – 1.92 (dd, J = 12.6, 6.4 Hz, 1H).

1-[4-chloro-3-(trifluoromethyl)phenyl]-3-[(3S)-1-[5-(trifluoromethyl)-2-pyridyl]pyrrolidin-3-yl]urea (6)

RT = 5.21 min, m+H = 453.1. ^{1}H NMR (400 MHz, DMSO) δ 8.85 (s, 1H), 8.45 – 8.32 (m, 1H), 8.07 (s, 1H), 7.76 (dd, J = 9.0, 2.5 Hz, 1H), 7.54 (d, J = 1.5 Hz, 2H), 6.77 (d, J = 6.7 Hz, 1H), 6.60 (d, J = 9.0 Hz, 1H), 4.34 (q, J = 5.5 Hz, 1H), 3.70 (dd, J = 11.0, 6.0 Hz, 1H), 3.55 (dd, J = 13.4, 6.6 Hz, 2H), 3.39 (d, J = 10.6 Hz, 1H), 2.23 (td, J = 13.6, 7.2 Hz, 1H), 1.98 (td, J = 12.4, 6.0 Hz, 1H).

1-[4-chloro-3-(trifluoromethyl)phenyl]-3-[(3S)-1-pyrimidin-4-ylpyrrolidin-3-yl]urea (7)

RT = 4.49 min, m+H = 386.1. ^{1}H NMR (400 MHz, DMSO) δ 8.84 (s, 1H), 8.47 (s, 1H), 8.14 (d, J = 6.0 Hz, 1H), 8.07 (d, J = 1.7 Hz, 1H), 7.55 (d, J = 1.3 Hz, 2H), 6.76 (d, J = 6.7 Hz, 1H), 6.51 (dd, J = 6.2, 1.2 Hz, 1H), 4.33 (s, 1H), 3.57 (d, J = 60.6 Hz, 4H), 2.21 (s, 1H), 1.96 (s, 1H).

1-[(3S)-1-(2-aminopyrimidin-4-yl)pyrrolidin-3-yl]-3-[4-chloro-3-(trifluoromethyl)phenyl]urea (8)

RT = 4.55 min, m+H = 401.1. ^{1}H NMR (400 MHz, DMSO) δ 8.83 (s, 1H), 8.12 – 8.00 (m, 1H), 7.73 (d, J = 5.8 Hz, 1H), 7.54 (d, J = 1.4 Hz, 2H), 6.72 (d, J = 6.6 Hz, 1H), 5.90 (s, 2H), 5.74 (d, J = 5.8 Hz, 1H), 4.26 (s, 1H), 3.58 (t, J = 8.1 Hz, 1H), 3.42 (s, 2H), 2.15 (dt, J = 13.8, 6.9 Hz, 1H), 1.91 (dd, J = 11.2, 6.0 Hz, 1H).

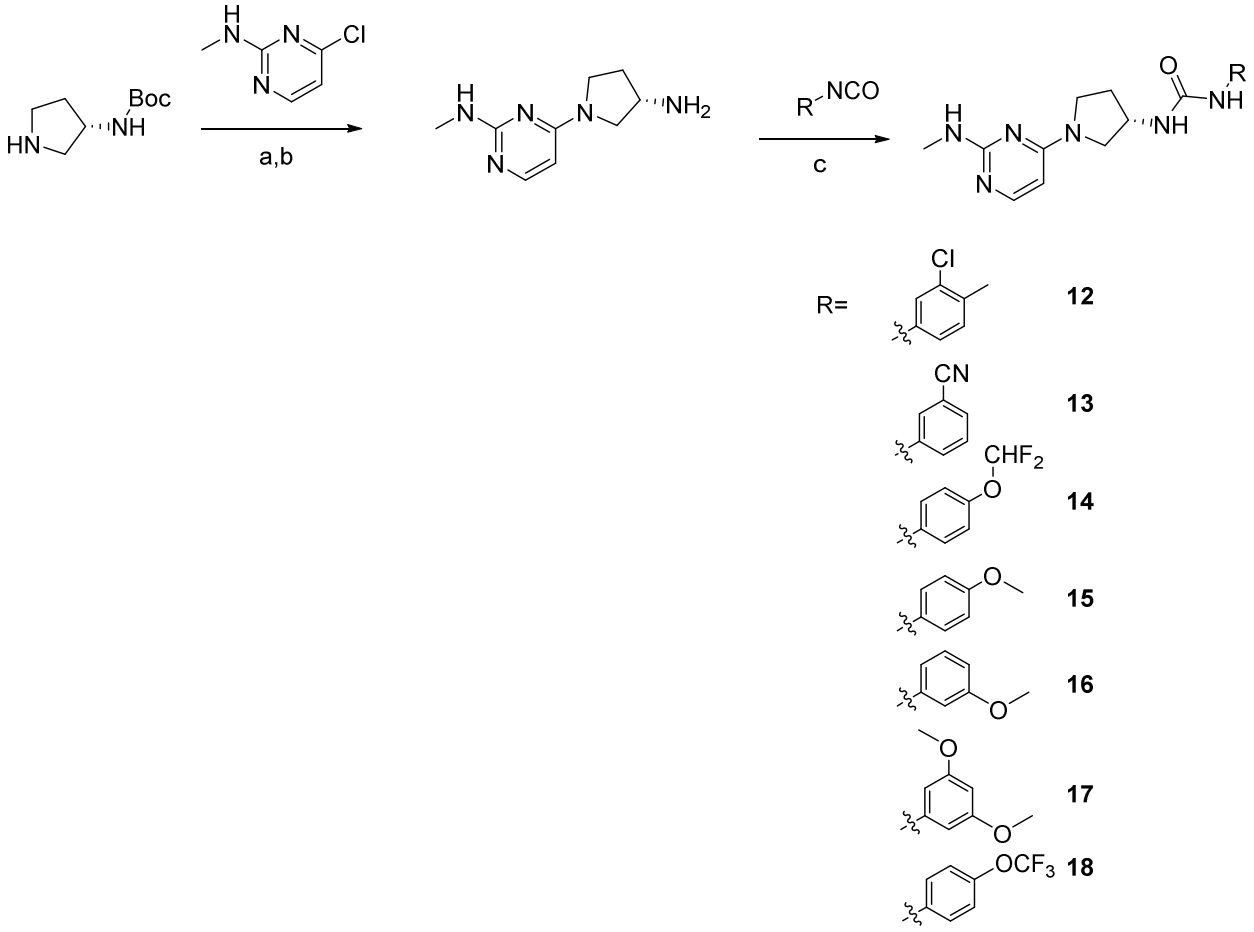
1-[4-chloro-3-(trifluoromethyl)phenyl]-3-[(3S)-1-[2-(ethylamino)pyrimidin-4-yl]pyrrolidin-3-yl]urea (10)

RT = 4.93 min, m+H = 429.2. ^{1}H NMR (400 MHz, DMSO) δ 8.95 (s, 1H), 8.14 – 7.96 (m, 1H), 7.75 (d, J = 5.8 Hz, 1H), 7.54 (d, J = 1.4 Hz, 2H), 6.83 (d, J = 6.6 Hz, 1H), 6.35 (t, J = 6.0 Hz, 1H), 5.72 (d, J = 5.8 Hz, 1H), 4.27 (s, 1H), 3.59 (s, 1H), 3.44 (s, 2H), 3.23 (dt, J = 12.8, 7.1 Hz, 2H), 2.16 (s, 1H), 1.91 (s, 1H), 1.08 (t, J = 7.1 Hz, 3H).

1-[4-chloro-3-(trifluoromethyl)phenyl]-3-[(3S)-1-[2-(cyclopropylamino)pyrimidin-4-yl]pyrrolidin-3-yl]urea (11)

RT = 4.89 min, m+H = 441.2. ^{1}H NMR (400 MHz, DMSO) δ 8.83 (s, 1H), 8.06 (d, J = 1.8 Hz, 1H), 7.78 (d, J = 5.7 Hz, 1H), 7.54 (d, J = 1.4 Hz, 2H), 6.73 (d, J = 6.6 Hz, 1H), 6.58 (d, J = 3.6 Hz, 1H), 5.77 (d, J = 5.8 Hz, 1H), 4.27 (s, 1H), 3.60 (s, 1H), 3.45 (s, 2H), 2.76 – 2.59 (m, 1H), 2.17 (s, 1H), 1.91 (s, 1H), 0.58 (td, J = 6.8, 4.5 Hz, 2H), 0.47 – 0.33 (m, 2H).

Scheme S3. Synthesis of compounds 12-18.[a]



[a] (a) DIPEA EtOH, 70°C, 3h, 71%; (b) 4N HCl/dioxane, r.t., 30 min, 99%; (c) DIPEA, DCM, 1h.

1-(3-cyanophenyl)-3-[(3*S*)-1-[2-(methylamino)pyrimidin-4-yl]pyrrolidin-3-yl]urea (13)

RT = 3.80 min, m+H = 338.2. ^{1}H NMR (400 MHz, DMSO) δ 8.69 (s, 1H), 7.92 (t, J = 1.9 Hz, 2H), 7.76 (d, J = 5.9 Hz, 1H), 7.57 (dd, J = 8.4, 2.0 Hz, 1H), 7.43 (t, J = 7.9 Hz, 1H), 7.34 (d, J = 7.6 Hz, 1H), 6.72 (d, J = 6.8 Hz, 1H), 6.32 (d, J = 5.3 Hz, 1H), 5.73 (d, J = 5.8 Hz, 1H), 4.34 – 4.17 (m, 1H), 3.60 (s, 2H), 2.73 (d, J = 4.7 Hz, 3H), 2.16 (dt, J = 13.7, 6.5 Hz, 1H), 1.91 (s, 1H).

1-[4-(difluoromethoxy)phenyl]-3-[(3*S*)-1-[2-(methylamino)pyrimidin-4-yl]pyrrolidin-3-yl]urea (14)

RT = 4.09 min, m+H = 379.2. ^{1}H NMR (400 MHz, DMSO) δ 8.39 (s, 1H), 7.76 (d, J = 5.8 Hz, 1H), 7.47 – 7.33 (m, 2H), 7.11 – 6.99 (m, 2H), 6.50 (d, J = 6.8 Hz, 1H), 6.32 (d, J = 5.3 Hz, 1H), 5.73 (d, J = 5.8 Hz, 1H), 4.26 (s, 1H), 3.57 (s, 4H), 2.73 (d, J = 4.7 Hz, 3H), 2.23 – 2.05 (m, 1H), 1.89 (s, 1H).

1-(4-methoxyphenyl)-3-[(3*S*)-1-[2-(methylamino)pyrimidin-4-yl]pyrrolidin-3-yl]urea (15)

RT = 3.68 min, m+H = 343.2. ^{1}H NMR (400 MHz, DMSO) δ 8.14 (d, J = 15.3 Hz, 2H), 7.76 (d, J = 5.9 Hz, 1H), 7.27 (d, J = 8.7 Hz, 2H), 6.85 – 6.73 (m, 2H), 6.40 (d, J = 7.5 Hz, 2H), 5.74 (d, J = 6.0 Hz, 1H), 4.25 (s, 1H), 3.69 (s, 3H), 2.73 (d, J = 4.7 Hz, 3H), 2.21 – 2.04 (m, 1H), 1.87 (d, J = 11.4 Hz, 1H).

1-(3-methoxyphenyl)-3-[(3*S*)-1-[2-(methylamino)pyrimidin-4-yl]pyrrolidin-3-yl]urea (16)

RT = 3.81 min, m+H = 343.2. ^{1}H NMR (400 MHz, DMSO) δ 8.30 (s, 1H), 7.76 (d, J = 5.8 Hz, 1H), 7.15 – 7.03 (m, 2H), 6.83 (dd, J = 7.9, 1.9 Hz, 1H), 6.53 – 6.40 (m, 2H), 6.32 (d, J = 5.5 Hz, 1H), 5.73 (d, J = 5.8 Hz, 1H), 4.25 (s, 1H), 3.70 (s, 3H), 3.58 (s, 1H), 3.44 (s, 2H), 2.73 (d, J = 4.7 Hz, 3H), 2.15 (dd, J = 13.7, 7.2 Hz, 1H), 1.89 (d, J = 11.1 Hz, 1H).

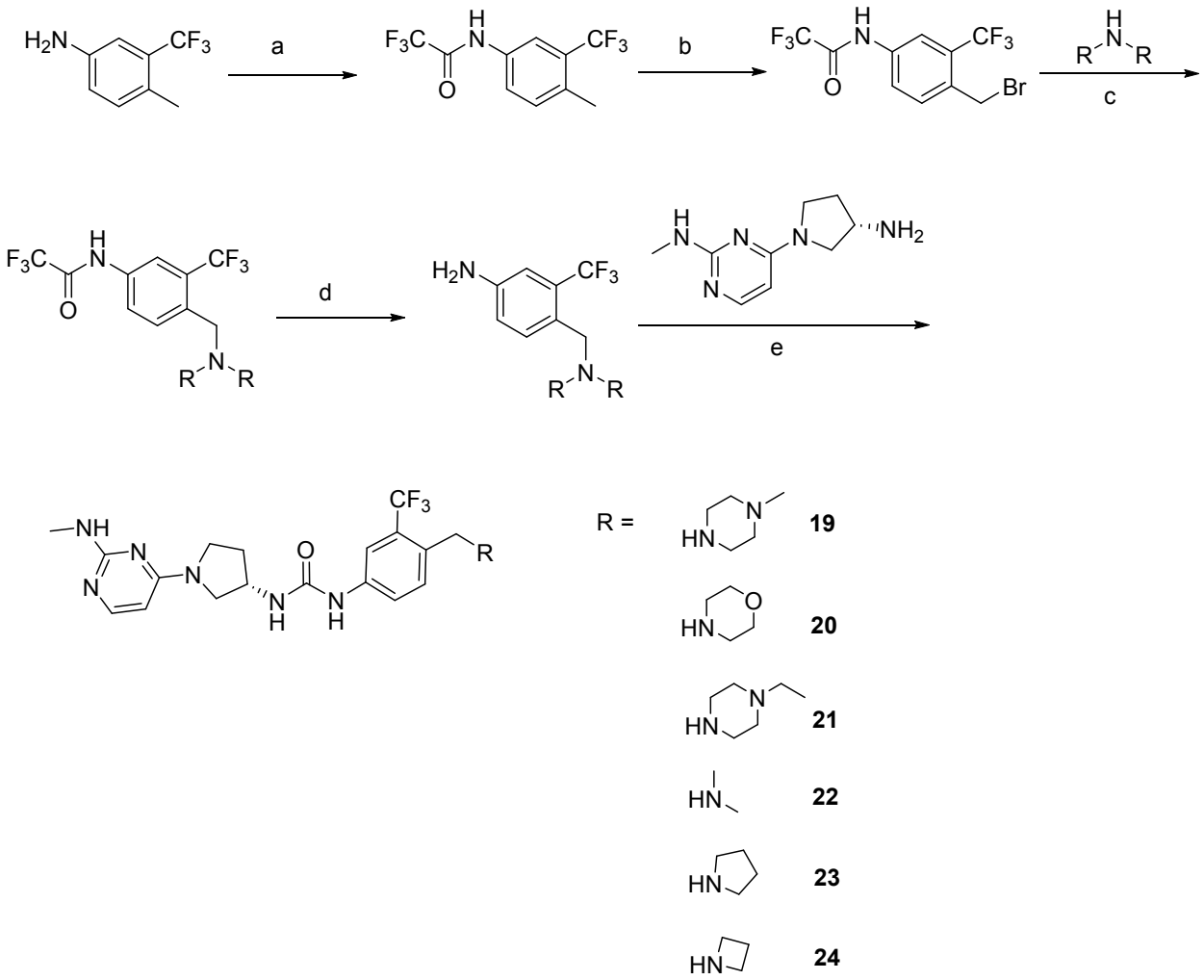
1-(3,5-dimethoxyphenyl)-3-[(3*S*)-1-[2-(methylamino)pyrimidin-4-yl]pyrrolidin-3-yl]urea (17)

RT = 3.91 min, m+H = 373.2. ^{1}H NMR (400 MHz, DMSO) δ 8.30 (s, 1H), 7.76 (d, J = 5.8 Hz, 1H), 6.67 – 6.55 (m, 2H), 6.46 (d, J = 6.8 Hz, 1H), 6.32 (d, J = 5.2 Hz, 1H), 6.07 (q, J = 1.9 Hz, 1H), 5.73 (d, J = 5.8 Hz, 1H), 4.24 (s, 1H), 3.68 (s, 6H), 3.58 (s, 2H), 2.73 (d, J = 4.7 Hz, 3H), 2.15 (dd, J = 13.5, 6.0 Hz, 1H), 1.88 (s, 1H).

1-[(3*S*)-1-[2-(methylamino)pyrimidin-4-yl]pyrrolidin-3-yl]-3-[4-(trifluoromethoxy)phenyl]urea (18)

RT = 4.42 min, m+H = 397.2. ^{1}H NMR (400 MHz, DMSO) δ 8.53 (s, 1H), 7.76 (d, J = 5.8 Hz, 1H), 7.54 – 7.39 (m, 2H), 7.22 (d, J = 8.5 Hz, 2H), 6.56 (d, J = 6.8 Hz, 1H), 6.32 (d, J = 5.3 Hz, 1H), 5.73 (d, J = 5.8 Hz, 1H), 4.27 (s, 1H), 3.59 (s, 1H), 3.44 (s, 2H), 2.73 (d, J = 4.7 Hz, 3H), 2.16 (dd, J = 13.0, 6.8 Hz, 1H), 1.89 (s, 1H).

Scheme S3. Synthesis of compounds 19-24.[a]



[a] (a) Pyridine, TFA, 0°C to r.t., 1h, 93%; (b) NBS, BPO, CCl_4, 100°C, 36h, 48%; (c) ACN, 0°C, 1.5h; (d) K_2CO_3, MeOH, Water, 80°C, 1h; (e) CDI, DIPEA, DMF, r.t., 16h.

N-(3-trifluoromethyl-4-methylphenyl)-2,2,2-trifluoroacetamide

To a solution of 3-trifluoromethy-4-methylaniline (14.2 g, 100 mmol) dissolved in dichloromethane (250 mL) was added pyridine (75 mL, 0.80 mmol) and trifluoroacetic anhydride (16.5 mL, 112.5 mmol) at 0°C. The mixture was stirred at room temperature for 1 hour. The reaction mixture was washed with 10% aqueous HCl, then with water. The organic phase was dried over sodium sulfate, filtered and concentrated to give 22 g (92.8%) of the title compound, which was used without further purification.

N-(-4-Bromomethyl)-3-trifluoromethylphenyl)-2,2,2-trifluoroacetamide

To a solution of N-(3-trifluoromethyl-4-methylphenyl)-2,2,2-trifluoroacetamide (22 g, 92.2 mmol) in CCl_4 was added BPO (6.7 g, 27.7 mmol) and NBS (19.7 g, 110.6 mmol). The mixture was warmed to 100°C and illuminated for 1 h by a high voltage lamp. The reaction was stirred at 100°C for 36h. After cooling down to 0°C, the reaction mixture was filtered and the filtrate was washed with brine, then concentrated. The crude product was washed with petroleum ether and filtered to give 14 g (48.3 %) of the title compound, which was used without further purification.

N-(3-trifluoromethyl-4-((dimethylamino)methyl)phenyl)-2,2,2-trifluoroacetamide

To a solution of dimethylamine hydrochloride (4.6g, 56.4 mmol) in MeCN (150 ml) was added dropwise N-(4-(bromomethyl)-3-trifluoromethylphenyl)-2,2,2-trifluoroacetamide (3.0 g, 9.4 mmol) dissolved in MeCN (150 ml) over 1

hour at 0°C, and it was stirred at 0°C for 30 minutes. Subsequently, 100 mL of saturated $NaHCO_3$ was added, and the mixture was partitioned between DCM and water. The organic layer was washed with water (15 mL), dried over Na_2SO_4, concentrated and purified by prep-TLC (petroleum ether:EtOAc = 2:1) to give the title compound(2.2g, 83%).

3-trifluoromethyl-4-((dimethylamino)methyl)aniline

To a solution of N-(3-trifluoromethyl-4-((dimethylamino) methyl) phenyl)-2,2,2-trifluoroacetamide (2.2 g, crude) in MeOH (30 mL) and H_2O (30ml) was added K_2CO_3 (3.25g, 23.6mmol) at room temperature . The mixture was then stirred at 80°C for 1 hour. The reaction mixture was concentrated to give the title compound (1.3g, 90.1%).

(*S*)-1-(4-((dimethylamino)methyl)-3-(trifluoromethyl)phenyl)-3-(1-(2-(methylamino)pyrimidin-4-yl)pyrrolidin-3-yl)urea (22).

To a solution of 3-trifluoromehtyl-4-((dimethylamino) methyl) aniline (200 mg, 1.08mmol) in dry DMF (5 ml) was added CDI (224mg, 1.4 mmol), and DIPEA (413.2mg, 3.26mmol)at 250C. The mixture was stirred at 25°C for 2 hours. (*S*)-4-(3-aminopyrrolidin-1-yl)-N-methylpyrimidin-2-amine (208mg, 1.08 mmol) was then added, and the mixture was stirred at 25°C for 16 hours. The mixture was purified by prep-HPLC to give title compound (22.0 mg, 5.0%).

^{1}H NMR (400 MHz, CDCl3) δ 8.22 (br s, 1 H), 7.72-7.51 (m, 4 H), 7.59-7.45 (m, 2 H), 6.83 (br s, 1 H), 5.61-5.57 (m, 1 H), 4.99 (brs, 1 H), 4.53 (brs, 1 H), 3.82-3.25 (m, 6 H), 2.93 (d, J = 4.4 Hz, 3 H), 2.36-2.05 (m, 8 H). LCMS (ESI): m/z 438.2 [M+H+].

(*S*)-1-(1-(2-(methylamino)pyrimidin-4-yl)pyrrolidin-3-yl)-3-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)urea (19).

^{1}H NMR (400 MHz, DMSO-d6) δ 8.83 (s, 1 H), 8.16 (s, 2 H), 7.90 (d, J = 2.0 Hz, 1 H), 7.74 (d, J = 6.0 Hz, 1 H), 7.52 (d, J = 8.4 Hz, 1 H), 7.46-7.44 (m, 1 H), 6.79 (d, J = 7.2 Hz, 1 H), 6.48 (brs, 1 H), 5.73 (d, J = 6.0 Hz, 1 H), 4.24 (s, 2 H), 3.58-3.55 (m, 2 H), 3.43 (s, 3 H), 2.71 (d, J = 4.8 Hz, 3 H), 2.48-2.37 (m, 8 H), 2.20 (s, 3 H), 2.15-2.13 (m, 1 H), 1.89-1.86 (m, 1 H). ^{13}C NMR (101 MHz, DMSO) δ 164.04, 160.83, 155.61, 155.32, 139.96, 131.74, 129.53, 121.42, 55.00, 54.99, 52.80, 45.84, 44.48, 41.38, 40.86, 40.64, 40.42, 40.21, 40.00, 39.79, 39.57, 39.36, 39.11. LCMS (ESI): m/z 493.1 [M+H+]. HRMS (ESI): m/z calcd: $C_{23}H_{32}ON_8F_3$ = 493.2646; found: 493.2659.

(*S*)-1-(1-(2-(methylamino)pyrimidin-4-yl)pyrrolidin-3-yl)-3-(4-(morpholinomethyl)-3-(trifluoromethyl)phenyl)urea (20).

^{1}H NMR (400 MHz, DMSO-d6) δ 8.80 (s, 1 H), 7.93 (s, 1 H), 7.77 (d, J = 6.0 Hz, 1 H), 7.58 (d, J = 8.4 Hz, 1 H), 7.49-7.47 (m, 1 H), 6.76 (d, J = 6.4 Hz, 1 H), 5.81 (d, J = 6.0 Hz, 1 H), 4.27 (s, 2 H), 3.65-3.62 (m, 1 H), 3.58-3.56 (m, 5 H), 3.51 (s, 3 H), 2.75 (d, J = 8.8 Hz, 3 H), 2.35 (d, J = 4.4 Hz, 4 H), 2.18-2.17 (m, 1 H), 1.92-1.91 (m, 1 H). ^{13}C NMR (101 MHz, DMSO) δ 163.71, 160.80, 155.26, 139.94, 131.87, 121.42, 66.69, 53.72, 44.52, 41.30, 41.29, 40.93, 40.69, 40.64, 40.48, 40.42, 40.21, 39.79, 39.57, 39.36, 39.22, 39.21. LCMS (ESI): m/z 480.2 [M+H+]. HRMS (ESI): m/z calcd: $C_{22}H_{29}ON_7F_3$ = 480.2329; found: 480.2340.

(*S*)-1-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(1-(2-(methylamino)pyrimidin-4-yl)pyrrolidin-3-yl)urea (21).

^{1}H NMR (400 MHz, Methanol-d4)) δ 7.81 (s, 1 H), 7.74 (d, J = 6.0 Hz, 1 H), 7.65 (d, J = 8.8 Hz, 1 H), 7.53 (d, J = 8.0 Hz, 1 H), 5.84 (d, J = 6.4 Hz, 1 H), 4.62-4.51 (m, 2 H), 4.49-4.32 (m, 1 H), 3.61-3.37 (m, 5 H), 2.88 (s, 3 H), 2.65-1.95 (m, 11 H), 1.12 (t, J = 7.2 Hz, 3 H). ^{13}C NMR (101 MHz, DMSO) δ 162.87, 160.87, 156.20, 155.24, 139.79, 131.70, 129.77, 121.45, 114.88, 53.28, 52.87, 52.05, 43.94, 40.83, 40.00, 39.79, 39.57, 39.36, 39.21, 39.07, 39.01, 38.87, 38.86, 12.49. LCMS (ESI): m/z 507.2 [M+H+]. HRMS (ESI): m/z calcd: $C_{24}H_{34}ON_8F_3$ = 507.2802; found: 507.2814.

(*S*)-1-(1-(2-(methylamino)pyrimidin-4-yl)pyrrolidin-3-yl)-3-(4-(pyrrolidin-1-ylmethyl)-3-(trifluoromethyl)phenyl)urea (23)

^{1}H NMR (400 MHz, CDCl3) δ 8.12 (brs, 1 H), 7.72-7.52 (m, 4 H), 7.59-7.45 (m, 2 H), 6.89 (brs, 1 H), 5.64-5.62 (m, 1 H), 5.39 (brs, 1 H), 4.59 (brs, 1 H), 3.76 (s, 3 H), 3.72-3.41 (m, 3 H), 2.92 (d, J = 4.0 Hz, 3 H), 2.58 (s, 4 H), 2.36-1.85 (m, 6 H). LCMS (ESI): m/z 464.2 [M+H+].

1-(4-Azetidin-1-ylmethyl-3-trifluoromethyl-phenyl)-3-[(S)-1-(2-methylamino-pyrimidin-4-yl)-pyrrolidin-3-yl]-urea (24).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (brs, 1 H), 7.73-7.61 (m, 2 H), 7.59-7.45 (m, 2 H), 6.82 (brs, 1 H), 5.62-5.51 (m, 1 H), 4.84 (brs, 1 H), 4.51 (brs, 1 H), 3.71 (s, 2 H), 3.72-3.05 (m, 8 H), 2.93 (d, J = 4.8 Hz, 3 H), 2.32-2.05 (m, 4 H). LCMS (ESI): m/z 450.2 [M+H+].

2. LanthaScreen binding assay:

Compound potencies were determined in a competitive ATP site binding assay. In a polystyrene or cyclo-olefin copolymer plate, a complex of full-length His-tagged CDK8 and CyclinC (Genentech; 5 nM, final), europium-labeled anti-His (Life Technologies, Carlsbad, CA; 4 nM, final), Tracer236 (Life Technologies; 10 nM, final), and test compound were incubated together for 1 hour at room temperature. Test compounds that competed directly or indirectly with Tracer236, an Alexafluor647-labeled staurosporine analog, for binding to CDK8/CyclinC resulted in a loss of TR-FRET signaling. TR-FRET donor and acceptor fluorescence emission were measured on a Viewlux imager (PerkinElmer, Waltham, MA) at 615 nm and 665 nm, respectively, after excitation at 340 nm. Normalized inhibition of binding, by varying concentrations of test compounds (10 point titration, with N = 2), was calculated relative to untreated controls. IC50 values were calculated using a 4-parameter logistic nonlinear regression dose-response model, from plots of normalized inhibition as a function of compound concentration. The average IC50 values, standard deviations, standard errors of the means, and the numbers of individual experiments per average are summarized in the table below. Although this assay uses the CDK8/cyclin C complex, this complex exists as part of the larger mediator complex in cells. Association with with MED12, a part of this complex, is known to significantly increase the kinase activity of CDK8; however, there have been no reports that its presence impacts the binding of compounds to CDK8.

Compound	Average	SD	SEM	N
1	32.5	12.3	4.4	8
2	14.6	5.6	2.5	5
3	18.7	6.9	4.0	3
4	4.2	0.6	0.3	5
5	166	43.5	25.1	3
6	117	11.8	5.3	5
7	60.3	17.5	7.8	5
8	42.3	10.9	4.9	5
9	10.1	5.0	2.2	5
10	8.9	2.0	0.9	5
11	20.3	4.6	2.1	5
12	18.4	8.9	5.1	3
13	3478	557	321	3
14	143	12.4	5.5	5
15	7157	759	438	3
16	3619	644	372	3
17	5012	917	649	2
18	44.1	9.7	4.3	5
19	20.5	16.1	6.6	6
20	17.4	7.1	3.2	5
21	14.7	5.0	2.2	5
22	40.1	7.4	4.3	3
23	157	72.0	36.0	4
24	99.9	28.4	14.2	4

3. Cellular Stat1 (S727) Phosphorylation Assay

Cellular activity of CDK8 inhibitors was evaluated by stimulating $2.5x10^4$ HCT116 cells with 10 ng/ml of IFN-gamma in the presence of inhibitor for 24 h in media containing 10% FBS. Following treatment, cells were fixed with 4% paraformaldehyde diluted in PBS for 30min. Following fixation, cells were washed four times with permeabilization buffer (0.1% Triton X-100 in PBS) and blocked with Odyssey block buffer (LI-COR) for 1 h. Subsequently, cells were incubated at room temperature for 2 h with p-Stat1 (S727) antibody (CST) diluted 1:300 and beta Actin antibody (CST) diluted 1:1000 in block buffer. Following the primary antibody incubation, cells were washed four times with TBST wash buffer (Sigma) and incubated for 1 h with IRdye-700 conjugated goat anti-rabbit secondary antibody (1:600, Invitrogen) and IRdye-800CW conjugated goat anti-mouse secondary antibody (1:2000, LI-COR). Finally, cells were washed four times with TBST wash buffer, and the plate was scanned on a LI-COR Odyssey Infrared scanner. Staining was quantified using the scanner software. Normalized p-Stat1 intensity was calculated by dividing the integrated intensity for p-Stat1 staining on the 700 nm channel by the integrated intensity of Actin staining on the 800 nm channel. Normalized values were plotted as a function of the concentration of compound to determine IC_{50} values.

4. Kinase selectivity data

Compounds were evaluated for selectivity of kinase inhibition in biochemical activity or binding assays with a panel of recombinant kinases at Life Technologies (Madison, WI). Compounds were assayed at a single concentration (0.1 µM for compounds 1 and 2 or 1 µM for compounds 12 and 20) with N = 2. For activity assays, ATP concentrations were set at Km values. Data are expressed as percent inhibition of the kinase activity or binding interaction.

Compound	1	2	12	20
Concentration (µM)	0.1	0.1	1.0	1.0
CDK8/cyclinC	51.4	58.5	101.4	79.6
Abl	8.9	5	2.3	4.2
ACVR1B	-9	-0.1	-2.5	-2.3
ACVR2B	3.5	-0.6	-2.4	3.6
AKT1	2	3.2	0.2	0.1
AKT2	4	3.6	0.2	-2.7
ALK2	-3.5	1.9	3.3	0
ARK5	-2	2.3	-0.4	3.1
ASK1	5.5	-0.7	-5.3	6
Aurora_A	4.1	2.5	-0.1	-1.6
Aurora_B	5	8.3	-0.4	0.7
Axl	-2	-2	-3.5	-1.3
Blk	1	5	-8.4	-18
BMPR1A	-2	4.3	-1.5	-1.9
Bmx	2	7.5	5.6	3.9
B-Raf	22	5.9	1.4	6.3
Brk	-10.5	1.2	-9.8	-7.6
BrSK1	4	5.7	-4.1	-3.7
BTK	1	-0.9	-1.9	-0.4
CaMKI	-9	1.4	10.9	11.3
CaMKI_delta	-4	8.3	-1.7	0.3
CamKII_alpha	0	5.5	-0.2	4.5
CaMKII_beta	-2	10.9	0.4	-2.9
CamKIV	-1	6.1	-1.2	-3.7
CAMKK1	-4	5.7	6.8	12.4

CAMKK2	4.5	-0.8	2.5	14.2
CDK1/cyclinB	4.6	8.1	1.7	-0.9
CDK2/cyclinA	3.8	0.8	-6.4	-4.9
CDK5/p25	5.8	1.9	-4.9	-10.3
CDK7/cyclinH	4.3	4.5	1.3	1.7
CDK9/cyclinT1	-9.8	1.5	3.5	-4
CHK1	3	3	3.7	6.2
CHK2	-1	-1.1	-3.1	-4.7
CK1_alpha1	-0.5	3	4	2
CK1_delta	7.5	2.9	6.3	7.9
CK1_epsilon1	2	2.3	3.1	6.3
CK1_gamma1	-5	0.6	-1.9	3.3
CK1_gamma2	0	0.6	3.1	1.9
CK2_alpha1	6.9	5.7	6.3	3
CLK1	-0.5	-3.1	0	-7.4
CLK2	3.5	8	2.4	-1.4
CLK3	1	7.3	-0.2	0.9
CLK4	4.5	-0.8	6	9.7
Cot	48.8	13.7	3.3	-1
CSF1R	51	34.9	7	19.1
CSK	-3	-1.3	-2.3	-1.3
DAPK1	6.5	18.4	6.2	4.8
DCAMKL2	0	-0.1	-2	-1.6
DDR1	67.9	7.2	-5.5	4.7
DMPK	-6	1.6	-5.9	-10.4
DNA-PK	-5.5	4	6.2	7
DRAK1	6	4.2	-1.6	-6.3
DYRK1A	-4.2	9.7	7	5.8
DYRK3	4	3.6	0.9	4.6
DYRK4	5.5	5.2	1.2	-0.4
eEF-2K	-1	8.3	0.3	2
EGFR	-5	-0.3	6.4	4.7
EGFR(T790M,L858R)	-1.5	-9	-7	-8.5
EphA1	5	8.4	-1	-0.7
EphA3	3.5	4.1	-2.7	-2.7
EphA7	14.5	2.1	0.9	-4.4
EphA8	3	1	1.6	1.9
EphB1	1.5	2.7	1.3	1.3
EphB3	0.5	1.2	0.6	0.4
ErbB2	6	-9.1	-3.2	-1.8
ErbB4	-3.5	1.8	-1.3	-0.6
ERK2	2.3	4.4	2.3	2.6
FAK	2	9.1	7.8	7.5
Fes	2.5	2.6	2.9	0.1
FGFR1	0.3	0.9	5.2	5.3
FGFR3	1	1.8	1.7	-7.8
FGFR4	2.5	1.3	-1.6	-3.9
Fgr	3	7.6	4	0.4
Flt1	22.5	1	-2.6	1

Flt3	70.7	56.4	7.8	73.6
Flt4	35.5	10.6	8.3	7.8
Frk	4	10.6	3	1.9
GRK2	-1.5	9.3	4.5	3.5
GRK3	2	-3.7	-2.4	-0.9
GRK5	-3.5	-3.3	0.8	1.3
GRK6	5.5	-2.8	4	3.5
GSK3_alpha	10.9	3.5	4.4	2.5
GSK3_beta	-1	3.8	4.3	1.7
HIPK1	-2	1.5	-2.1	2.5
HIPK2	4	9.2	0	1.4
HIPK4	63.8	69.9	0.6	-3.6
Hyl	-3	4	2	1.1
IGF1R	3	8.6	6.8	10.3
IKK_alpha	0.5	1	12	6.1
IKK_beta	0	0.8	2	0.7
IKK_epsilon	-3.5	3.4	3.8	2
InsR	2	4.1	2.2	2.4
IRAK1	-9.5	1.3	9.3	8.9
IRAK4	0	-3.6	1.1	-1.2
IRR	6.5	1.8	2.9	1
ITK	-16.5	4.1	5.3	9.1
JAK1	-5.5	-4.8	5.3	3.1
JAK2	2.5	-0.2	-2.1	3.6
JAK3	0	1.3	-4.4	-5.3
JNK1_alpha1	-7	-5.5	1.4	3.5
JNK2	7	7	1.5	7.2
JNK3	3	3	-3.8	-1.3
KDR	63.8	16.6	2.5	5.6
KHS1	1.6	35	2.6	-8.9
Kit	9.6	1.4	1.8	6.8
Lck	35.3	13.6	1.2	3.5
LIMK1	16.5	-4.1	9.3	-5.2
LRRK2	13.5	-18.4	2.7	4.2
LTK	6	2.9	3	2.4
Lyn	11.7	8.3	1.1	4.5
MAP4K4	3.4	17.7	9.3	4.5
MAPKAPK2	0.1	2.5	6.9	5.4
MAPKAPK3	4	5	-6.5	-6.3
MARK1	5.5	3.8	-1	-1.7
MARK3	5	6.1	0.9	-1.8
MEK1	1	-1	2.6	-1.8
MEK3	0.5	2	-2	13
MEKK2	-4	1	-7.9	-7.6
MELK	5	15.1	3.5	8.3
Mer	2.5	3.5	0.9	1
Met	8	0.8	-0.5	4.1
Mink1	-6.3	-7.1	0.5	-12.3
MKK6	7.5	4	-4.7	-8.4
MKNK1	10.5	-1.8	-2.4	-1.1

MKNK2	38.5	-0.2	-1.3	13
MLK1	2	-12.3	-6.9	-3.5
MLK2	0.5	-0.6	1.6	0.3
MRCK_alpha	2	0.8	2.1	1.4
MSK1	5	-3.5	1.9	0.3
MSSK1	2	7.5	0	-0.4
MST1	3	2.6	-4.8	-2.1
MST2	-11.5	2	6.1	7.3
MST3	10	4.8	0.9	-5.1
MST4	-7	20.3	1.1	-5.3
mTOR	2	-3.3	-1	-1
MuSK	33.6	46.5	3.7	23.1
MYLK(smMLCK)	-1.4	-1.2	-0.5	5.8
MYLK3(caMLCK)	0.5	-2.9	-2.7	3.5
NEK1	3.5	3	5.9	6.1
NEK4	1	4.7	4	3.5
NEK6	2.5	3.6	0.9	-2
NEK9	-5	-0.7	-7	-6.6
NLK	0.5	-1	-2.9	0.1
p38_alpha(direct)	5	13.7	2.6	3.3
p38_beta	-1	11.1	6.5	9.2
p38_delta	5	10.1	3.4	2
p38_gamma	4	9.2	3.5	4.2
p70S6K	-3.5	10.8	-2.8	-6.8
PAK1	16.5	7.6	10.1	3
PAK3	3.5	5.1	4.2	3.9
PAK4	-9	0.2	11.7	0.9
PAK6	5.5	2	6	3.7
PASK	-0.1	10.8	4.6	-3
PDGFR_alpha	57.3	12.1	0.6	4.8
PDK1(direct)	2	-1.4	2.7	0.3
PhK_gamma1	8.5	1.5	-7.2	-0.3
PhK_gamma2	-3	1.1	-3.8	-6.2
PI3K-A	7.6	7.6	5.2	9.1
PI3K-G	-2	8.9	13.1	2.3
PIM1	-2	6.3	-0.8	-2.9
PKA	-6	4.9	-1.1	-2.3
PKC_alpha	8	10.8	0.2	1.4
PKC_beta1	-3	11.9	3.6	1.6
PKC_delta	6.5	1.1	-1.8	2
PKC_epsilon	10	1.7	4.9	-5.8
PKC_eta	6	9.9	6.4	2
PKC_theta	0.5	6.9	-1.7	2.7
PKC_zeta	-0.5	-1.1	0.4	-7.5
PKD1	2	9.3	6	6.8
PKG1_alpha	3	4.2	1.4	0.6
PLK1	14	0.6	2.1	2.6
PLK2	3.5	3.7	1.8	-1.3
PLK3	-0.5	-9.6	-0.7	0.3
PRAK	-2.5	4.9	4.5	4.4

PRK1	-5.5	5.7	-9.3	-2
PRKAA1		-4.6	-1.6	-1.3
PrKX	3.5	5.3	0	3
RAF1(Y340D,Y341D)	77	52.5	-3.8	0.1
Ret	67.1	21.8	4.6	7.8
RIPK2	12	-0.7	0.2	-3.3
ROCK1	1	9.3	-4.3	-2.3
ROCK2	8	0.1	1.2	-2.9
Ron	-1.5	-0.4	3.9	1.4
Ros	2.5	7	1.3	1.2
Rse	-1	8	2.6	1.7
RSK1	5	4.2	1.3	5.9
RSK2	-4	5.5	1.5	-1
RSK3	-0.5	8.8	5.8	3.3
SGK1	2	5.3	-1	2.8
SGK2	2.5	5.6	-3	-2.1
SGK3	3	2.8	3	-0.4
SIK2	0	5.9	3.1	3.5
SLK	1.5	4	-3.6	-2.3
SPHK1	3	-5	2.5	8
Src	0	4	1.8	-0.4
Srm	0	5.2	2.4	7.9
SRPK1	0.5	4.5	0.8	-0.9
STK16	2.3	0.4	2.1	6.3
STK33	6.8	2.7	-3.4	-0.7
Syk	7	7	-0.6	-1.6
TAK1-TAB1	12.5	18	11.8	-7
TAO1	17.7	7	5.5	2
TBK1	-3.5	8.8	7.3	4.3
TEC	-2	-5.3	-0.7	-1.9
TGFBR1	-6	-0.1	12.2	6.6
Tie2	3.1	-4.2	-0.5	0.1
TNK2	-2.5	-1.4	4.5	-0.5
TrkA	11	10.1	16.4	24.1
TrkB	10	5.9	-0.9	-5.8
TSSK1	0.5	9.5	4.3	8.5
TTK	7.5	25.9	0.8	0.5
TXK	-2	0.7	0.1	-0.3
TYK2	6	4	1.2	-2.1
WEE1	-8	5	-6.3	-3.4
WNK2	1.5	0	-5.3	-6.4
Yes	7.5	9.2	4	1.8
YSK1	1	5	-8.4	-6.3
ZAK	54.9	17.1	2.8	2
ZAP-70	-8.5	7	4.5	6.9
ZIPK	-2.8	0.2	0.4	0

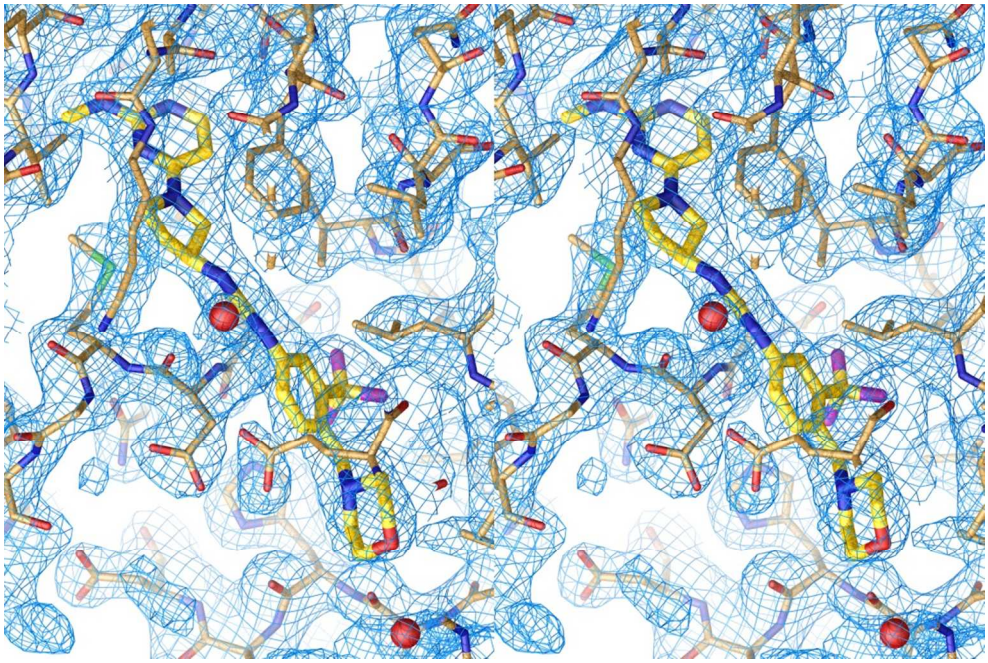
5. Crystallization and structure determination.

Proteins CDK8 and Cyclin C were both purified to greater than 95% purity and were co-crystallized with compound **20**, using previously described protocols[1]. Crystals have been flash-frozen and measured at a temperature of 100°K. The X-ray diffraction data were collected at beamline PXI/X06SA of the Swiss Light Source (SLS, Villigen, Switzerland) at a wavelength of 1.00004Å. The crystals diffracted to 2.39Å resolution and belong to space group $P2_12_12_1$. Data were processed using the programs XDS and XSCALE[2]. The structure was determined by molecular replacement using an internal structure as the search model. The model was adjusted with Coot[3] and refined in Refmac[4]. The final R_{free}=22.0%, and the final model retained good agreement with expected geometries, with the RMS deviation from ideal bond lengths = 0.008Å and only one residue in Ramachandran disallowed region (Supplementary Table S1). Contiguous electron density was observed for all atoms of the inhibitor (Supplementary Figure S1).

Supplementary Table S1. X-ray Data Collection and Refinement statistics

Data collection	
Space group	$P2_12_12_1$
Cell dimensions	
a, *b*, *c* (Å)	72.2, 71.9, 180.8
α, β, γ (°)	90, 90, 90
Resolution (Å)[a]	2.39 (2.64-2.39)
R_{merge}(%)[b]	7.4 (44.4)
$\langle I/\sigma I \rangle$	16.2 (4.4)
Completeness (%)	95.1 (97.3)
Redundancy	5.8 (6.1)
Refinement	
Resolution (Å)	90.41-2.39
No. reflections	34,934 (1,095)
R_{work} / R_{free} [c]	18.0 / 22.0
No. atoms	
Protein	5,013
Ligand / heteroatoms	39
Water	159
Ramachandran Plot	
Most favored region (%)	97.1
Allowed region (%)	2.2
Generously allowed region (%)	0
Disallowed region (%)	0.7
R.m.s. deviations	
Bond lengths (Å)	0.008
Bond angles (°)	1.07

[a] Values in parenthesis are for the highest resolution shell.
[b] $R_{merge} = \Sigma||I| - |\langle I \rangle||/ \Sigma|\langle I \rangle|$, where I is the intensity of a single observation and <I> the average intensity for symmetry equivalent observations.
[c] $R_{work} = \Sigma|F_o-F_c|/ \Sigma|F_o|$, where F_o and F_c are observed and calculated structure factor amplitudes, respectively. R_{free} is calculated as R for 5% of reflections sequestered from refinement.



Supplementary Figure S1. Electron density map of active site. Divergent-eye stereo representation of the simulated annealing composite omit $2F_O$-F_C difference electron density map contoured at 1.0σ. Inhibitor molecule (**20**) is displayed as CPK color scheme with gold carbon atoms, while the kinase active site is displayed as darker carbon atoms.

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