SUPPORTING INFORMATION

Identification and Structure-Guided Development of Pyrimidinone Based USP7 Inhibitors

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1. Materials and reagents:

Common organic solvents that were used in reactions (e.g. THF, DMF, DCM, and methanol) were purchased anhydrous from Sigma-Aldrich® in Sure/Seal™ bottles and were handled appropriately under nitrogen. Water was deionised using an Elga PURELAB Option-Q. All other solvents used (i.e. for work-up procedures and purification) were generally HPLC grade and were used as supplied from various commercial sources. Unless otherwise stated, all starting materials used were purchased from commercial suppliers and used as supplied.

For assay purposes, all reagents and chemicals were purchased from Sigma-Aldrich® unless otherwise stated. All inhibitors were prepared as 10 mM DMSO stocks for cell culture experiments and stored in a controlled environment using the MultiPod system. CellTiter-Glo® was purchased from Promega (#G7571). The ubiquitin-propargylamine (Ub-PA) probe was purchased from UbiQ (#UbiQ-057). Unless otherwise stated, all other reagents were obtained from commercial sources and used without further purification.

2. Experimental Procedures and Spectroscopic Data:

Abbreviations and Acronyms

aq: aqueous; Boc: *tert*-butyloxycarbonyl; DCM: dichloromethane; br: broad; d: doublet; DIPEA: diisopropylethylamine; DMAP: 4-(dimethylamino)pyridine; DMF: *N*,*N*-dimethylformamide; DMSO: dimethylsulfoxide; dppf: 1,1′-bis(diphenylphosphino)ferrocene; EDC: *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; EtOAc: ethyl acetate; ESI: electrospray ionisation; h: hour; HATU: *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HPLC: high pressure liquid chromatography; LC: liquid chromatography; LCMS: liquid chromatography mass spectrometry; *m/z*: mass-to-charge ratio; MeCN: acetonitrile; MeOH: methanol; min: minutes; MS: mass spectrometry; m: multiplet (spectral); OAc: acetate; PE: petroleum ether (40-60 °C); R_T: retention time; rt: room temperature; s: singlet; t: triplet; TFA: trifluoroacetic acid; THF: tetrahydrofuran.

General Experimental Conditions

Microwave synthesis

Microwave experiments were carried out using a Biotage Initiator™ Eight system or a CEM Discover™/Explorer24™ system controlled by Synergy 1.5 software. Both machines give good reproducibility and control at temperature ranges from 60-250 °C and pressures of up to a maximum of 20 bar.

Flash chromatography

Purification of compounds by flash chromatography was achieved using a Biotage Isolera Four system. Unless otherwise stated, Biotage KP-Sil SNAP cartridge columns (10-340 g) were used along with the stated solvent system and an appropriate solvent gradient depending on compound polarity. In the case of more polar and basic compounds, Biotage KP-NH SNAP cartridge columns (11 g) were used.

NMR spectroscopy

^{1}H NMR spectra were recorded at ambient temperature using a Bruker Avance (300 MHz), Bruker Avance III (400 MHz) or Bruker Ascend (500 MHz) spectrometer. All chemical shifts (δ) are expressed in ppm. Residual solvent signals were used as an internal standard and the characteristic solvent peaks were corrected to the reference data outlined in *J. Org. Chem.*, 1997, 62, p7512-7515; in other cases, NMR solvents contained tetramethylsilane, which was used as an internal standard.

Liquid Chromatography Mass Spectrometry (LCMS)

Liquid Chromatography Mass Spectrometry (LCMS) experiments to determine retention times (R_T) and associated mass ions were performed using the following method:

Method A: The system consisted of an Agilent Technologies 6130 quadrupole mass spectrometer linked to an Agilent Technologies 1290 Infinity LC system with UV diode array detector and autosampler. The spectrometer consisted of an electrospray ionization source operating in positive and negative ion mode. LCMS experiments were performed on each sample submitted using the following conditions: LC Column: Agilent Eclipse Plus C18 RRHD, 1.8 µm, 50 x 2.1 mm maintained at 40 °C. Mobile phases: A) 0.1% (v/v) formic acid in water; B) 0.1% (v/v) formic acid in acetonitrile.

Gradient Time (min)	Flow (mL/min)	%A	%B
0.00	0.5	80	20
1.80	0.5	0	100
2.20	0.5	0	100
2.50	0.5	80	20
3.00	0.5	80	20

Method B: The system consisted of an Agilent Technologies 6140 single quadrupole mass spectrometer linked to an Agilent Technologies 1290 Infinity LC system with UV diode array detector and autosampler. The spectrometer consisted of a multimode ionization source (electrospray and atmospheric pressure chemical ionizations) operating in positive and negative ion mode. LCMS experiments were performed on each sample submitted using the following conditions: LC Column: Zorbax Eclipse Plus C18 RRHD, 1.8 µm, 50 x 2.1 mm maintained at 40 °C. Mobile phases: A) 0.1% (v/v) formic acid in water; B) 0.1% (v/v) formic acid in acetonitrile.

Gradient Time (min)	Flow (mL/min)	%A	%B
0.00	1.0	95	5
1.80	1.0	0	100
2.20	1.0	0	100
2.21	1.0	95	5
2.50	1.0	95	5

Preparative High Pressure Liquid Chromatography

Method A: This system consisted of an Agilent Technologies 6120 single quadrupole mass spectrometer linked to an Agilent Technologies 1200 Preparative LC system with multiple wavelength detector and autosampler. The mass spectrometer used a multimode ionization source (electrospray and atmospheric pressure chemical ionizations) operating in positive and negative ion mode. Fraction collection was mass-triggered (multimode positive and negative ion). Purifi-

cation experiments, unless otherwise stated, were performed under basic conditions at an appropriate solvent gradient that was typically determined by the retention time found using the LCMS method. In cases where the basic conditions were unsuccessful, acidic conditions were employed.

Basic conditions: LC Column: Waters XBridge™ Prep C18 5 μm OBDTM 19 x 50 mm column at rt. Mobile phase: A) 0.1% (v/v) ammonium hydroxide in water; B) 0.1% (v/v) ammonium hydroxide in 95:5, acetonitrile/water. Total experiment time was *ca*. 10 min and an example method is given:

Gradient Time (min)	Flow (mL/min)	%A	%B
0.00	20.0	50	50
3.00	20.0	12	88
5.00	20.0	12	88
7.00	20.0	0	100
8.0	20.0	0	100
8.20	20.0	50	50

Acidic conditions: LC Column: Waters XBridge™ Prep C18 5μm OBDTM 19 x 50 mm column at rt. Mobile phase: A) Water 0.1% (v/v) formic acid in water; B) 0.1% (v/v) formic acid in 95:5, acetonitrile/water. Total experiment time was *ca*. 10 min and an example method is given:

Gradient Time (min)	Flow (mL/min)	%A	%B
0.00	20.0	95	5
7.00	20.0	0	100
9.00	20.0	0	100
9.20	20.0	95	5

Method B: This system consisted of a Waters Autopurification HPLC/MS, with a Gemini NX-C18 column from Phenomenex, 5 µm, 50 mm x 30 mm i.d., running at a flow rate of 60 mL/min, 25 °C with UV diode array detection (210–400 nm) and mass-directed collection. A typical gradient was 5-50% HPLC grade acetonitrile (mobile phase B) in HPLC grade water + 0.1% (v/v) ammonia solution (mobile phase A) over 10 min, or modified as necessary. The mass spectrometer used was a Waters Micromass ZQ2000 spectrometer, operating in positive or negative ion electrospray ionisation modes, with a molecular weight scan range of 150 to 1000.

The pure fractions were combined and concentrated using a Genevac EZ-2 Elite, unless stated otherwise.

Final compound purity analysis

The purity of final compounds was assessed by LCMS and ^{1}H NMR. The HPLC purity of each compound was measured using the method stated compared to a blank.

High Resolution Mass Spec

High resolution mass spectra were acquired on a Thermo ScientificLTQ Orbitrap XL spectrometer at the EPSRC UK National Mass Spectrometry Facility (University of Swansea).

General procedures

General procedure 1: Epoxide opening with a pyrimidinone

A suspension of the pyrimidinone (1 equiv.), epoxide (1-3 equiv.) and Cs_2CO_3 (1-3 equiv.) in DMF were heated at 80 °C for 10-24 h. The reaction was allowed to cool to rt, saturated $NH_4Cl_{(aq)}$ was added and the resulting mixture was extracted with DCM (x3) using a Biotage phase separator. The combined organic phases were concentrated under reduced pressure and the residue was purified by flash chromatography (Biotage KP-Sil and KP-NH, 0-100% EtOAc in cyclohexane or PE, then 0-30% MeOH in EtOAc) to give the product.

General procedure 2: *N*-Boc deprotection

A solution of the *N*-Boc piperidine in DCM/TFA was stirred for 1-24 h at rt before being concentrated under reduced pressure. The residue was then dissolved in triethylamine and DCM before being purified by flash chromatography (Biotage KP-NH, 0-100% EtOAc in cyclohexane or PE, then 0-30% MeOH in EtOAc or 0-100% DCM in cyclohexane or PE, then 0-30% MeOH in DCM) to give the product.

General procedure 3: EDC coupling

A solution of amine (1 equiv.), carboxylic acid (1 equiv.) and EDC (3 equiv.) was stirred in (DCM) for 1-24 h at rt. The reaction was quenched by the addition of water and the resulting mixture was extracted with DCM (x3) using a Biotage phase separator. The combined organic phases were concentrated under reduced pressure and the residue was purified by flash chromatography (Biotage KP-Sil and KP-NH, 0-100% EtOAc in cyclohexane or PE, then 0-30% MeOH in EtOAc) to give the product.

General procedure 4: HATU coupling in DCM

To a suspension of the amine (1 equiv.), carboxylic acid (1-1.5 equiv.) and HATU (1-1.5 equiv.) in DCM was added DIPEA (1-4 equiv.). The reaction was stirred for 1-24 h at rt before being quenched by the addition of saturated $NaHCO_{3(aq)}$ and the resulting mixture was extracted with DCM (x3) using a Biotage phase separator. The combined organic phases were concentrated under reduced pressure and the residue was purified by flash chromatography (Biotage KP-Sil and KP-NH, 0-100% EtOAc in cyclohexane or PE, then 0-30% MeOH in EtOAc) to give the product.

General procedure 5: Suzuki-Miyaura coupling

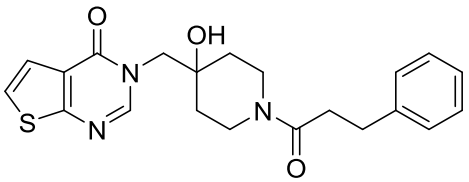
A reaction vial was charged with a mixture of the bromide (1 equiv.), the organoboron reagent (1-3 equiv.), a Pd catalyst (0.05-0.1 equiv.) and an inorganic base (2-5 equiv.) in 1,4-dioxane/water and the O_2 was removed by evacuating and refilling with N_2 three times or by bubbling N_2 through the mixture before the reaction tube was sealed. The reaction was heated under the indicated conditions for the indicated time before being cooled to rt and saturated $NH_4Cl_{(aq)}$ added. The resulting mixture was extracted with DCM (x3) using a Biotage phase separator. The combined organic phases were concentrated under reduced pressure and the residue was purified by flash chromatography (Biotage KP-Sil and/or KP-NH, 0-100% EtOAc in cyclohexane or PE, then 0-30% MeOH in EtOAc) to give the product.

General procedure 6: Sonagashira coupling

A reaction tube was charged with the bromide (1 equiv.), a Cu catalyst (0.2-0.4 equiv.) and a Pd catalyst (0.1-0.2 equiv.) and then evacuated and refilled with N_2 three times. To this was added toluene, triethylamine (20-40 equiv.) and the alkyne (1-4 equiv.) before the mixture was again evacuated and refilled with N_2 three times. The reaction tube was sealed and the reaction was heated under the indicated conditions for the indicated time. The reaction was cooled to rt and quenched by the addition of saturated $NH_4Cl_{(aq)}$. The mixture was extracted with DCM (x 3) using a Biotage phase separator, the combined organic phases were concentrated under reduced pressure and the residue was purified by flash chromatography (Biotage KP-Sil and KP-NH, 0-100% EtOAc in cyclohexane or PE, then 0-30% MeOH in EtOAc) to give the product.

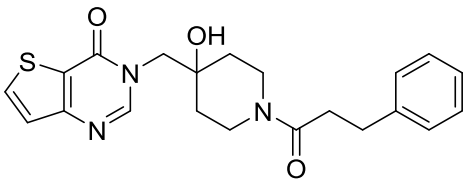
Compounds 11, **16**, ***ent*-16**, **17**, **34**, ***ent*-34** and **47** were prepared as described previously.[1]

Compound 2: 3-((4-Hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one



General procedure 1 using 3-phenyl-1-(1-oxa-6-azaspiro[2.5]octan-6-yl)propan-1-one[1] (63 mg, 0.257 mmol), thieno[2,3-*d*]pyrimidin-4(3*H*)-one (36 mg, 0.237 mmol), Cs_2CO_3 (91 mg, 0.279 mmol) and DMF (1.5 ml) gave title compound (48 mg, 51%) as a colourless solid. LCMS (Method A, ES^+): R_T = 1.08 min (purity >97% at 254 nm), *m/z* Calcd for $C_{21}H_{24}N_3O_3S$ $[M+H]^+$ 398, found 398. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07 (s, 1H), 7.45 (d, 1H), 7.34-7.23 (m, 3H), 7.22-7.11 (m, 3H), 4.32 (d, 1H), 4.09 (d, 1H), 3.98 (d, 1H), 3.58 (d, 1H), 3.42-3.21 (m, 1H), 3.10-2.98 (m, 1H), 2.96-2.84 (m, 2H), 2.69-2.49 (m, 2H), 1.68-1.29 (m, 4H).

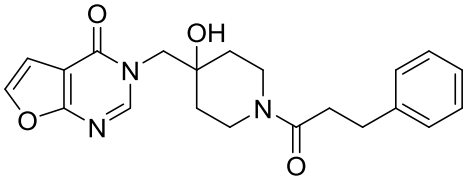
Compound 3: 3-((4-Hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl)thieno[3,2-*d*]pyrimidin-4(3*H*)-one



Step 1: *tert*-Butyl 4-hydroxy-4-((4-oxothieno[3,2-*d*]pyrimidin-3(4*H*)-yl)methyl)piperidine-1-carboxylate: General procedure 1 using *tert*-butyl 1-oxa-6-azaspiro[2.5]octane-6-carboxylate[1] (640 mg, 3.00 mmol), thieno[3,2-*d*]pyrimidin-4(3*H*)-one (502 mg, 3.30 mmol), Cs_2CO_3 (977 mg, 3.00 mmol) and DMF (6 mL) gave the title compound (889 mg, 81%) as a pale yellow foam. LCMS (Method A, ES^+): R_T = 1.07 min, *m/z* Calcd for $C_{17}H_{24}N_3O_4S$ $[M+H]^+$ 366, found 366. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.18 (s, 1H), 7.70 (d, 1H), 7.18 (d, 1H), 4.25-3.87 (m, 2H), 3.76 (br s, 2H), 3.08 (t, 2H), 1.67-1.39 (m, 4H), 1.33 (s, 9H).

Step 2: 3-((4-Hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl)thieno[3,2-*d*]pyrimidin-4(3*H*)-one: A solution of *tert*-butyl 4-hydroxy-4-((4-oxothieno[3,2-*d*]pyrimidin-3(4*H*)-yl)methyl)piperidine-1-carboxylate (15 mg, 41.0 µmol) was stirred in TFA (1 mL) for 16 h at rt. The resulting mixture was concentrated under reduced pressure and to the residue were successively added DCM (0.33 mL), DIPEA (13.8 μL), 3-phenylpropanoic acid (4.75 mg, 0.032 mmol), EDC (10.1 mg, 0.053 mmol), and HOBt (8 mg, 0.053 mmol). The reaction mixture was stirred for 30 min at rt before being diluted with water (10 mL) and the resulting mixture was extracted with DCM (3 x 10 mL) using a Biotage phase separator. The combined organic phases were concentrated under reduced pressure and the residue was purified by flash chromatography (Biotage KP-Sil 10 g cartridge, 0-100% EtOAc in PE, then 0-30% MeOH in EtOAc) to give the title compound (4 mg, 24%) as a colourless solid. LCMS (Method A, ES^+): R_T = 1.00 min (purity >95% at 254 nm), *m/z* Calcd for $C_{21}H_{24}N_3O_3S$ $[M+H]^+$ 398, found 398. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.05 (s, 1H), 7.84 (d, 1H), 7.35 (d, 1H), 7.32-7.19 (m, 5H), 4.41 (d, 1H), 4.13-4.00 (m, 2H), 3.62 (s, 1H), 3.60 (d, 1H), 3.33 (t, 1H), 3.08-2.94 (m, 3H), 2.66-2.59 (m, 2H), 1.69-1.25 (m, 4H).

Compound 4: 3-((4-Hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl)furo[2,3-*d*]pyrimidin-4(3*H*)-one



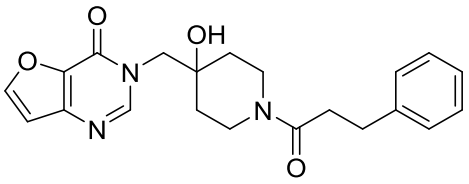
Step 1: *tert*-Butyl 4-hydroxy-4-((2-iodofuran-3-carboxamido)methyl)piperidine-1-carboxylate: General procedure 3 using *tert*-butyl 4-(aminomethyl)-4-hydroxypiperidine-1-carboxylate (100 mg, 0.434 mmol), 2-iodofuran-3-carboxylic acid (103 mg, 0.434 mmol), EDC (166 mg, 0.868 mmol) and DCM (5.4 mL) gave the title compound (123 mg, 62%) as a yellow solid. LCMS (Method A, ES^+): R_T = 1.22 min, *m/z* Calcd for $C_{16}H_{23}IN_2NaO_5$ $[M+Na]^+$ 473, found 473.

Step 2: *tert*-Butyl 4-hydroxy-4-((4-oxofuro[2,3-*d*]pyrimidin-3(4*H*)-yl)methyl)piperidine-1-carboxylate: A suspension of *tert*-butyl 4-hydroxy-4-((2-iodofuran-3-carboxamido)methyl)piperidine-1-carboxylate (400 mg, 0.888 mmol), formamidine hydrochloride (358 mg, 4.44 mmol), CuI (17 mg, 89.2 µmol) and K_2CO_3 (368 mg, 2.67 mmol) in DMF (6 mL) was heated in a microwave at 150 °C for 8 h. The reaction mixture was partitioned between 1:1 brine/water (40 mL) and EtOAc (10 mL) and the mixture was filtered through a plug of Celite®. The aqueous layer was separated and extracted into EtOAc (3 x 10 mL). The combined organic layers were washed with 1:1 brine/water (40 mL), dried over Na_2SO_4, concentrated under reduced pressure and the residue was purified by flash chromatography (Biotage KP-Sil 50 g cartridge, 0-100% EtOAc in PE) to give the title compound (34 mg, 10%) as a pale yellow solid. LCMS (Method A, ES^+): R_T = 1.17 min, *m/z* Calcd for $C_{17}H_{23}N_3NaO_5$ $[M+Na]^+$ 372, found 372.

Step 3: 3-((4-Hydroxypiperidin-4-yl)methyl)furo[2,3-*d*]pyrimidin-4(3*H*)-one: General procedure 2 using *tert*-butyl 4-hydroxy-4-((4-oxofuro[2,3-*d*]pyrimidin-3(4*H*)-yl)methyl)piperidine-1-carboxylate (34 mg, 97.3 µmol), TFA (0.6 mL) and DCM (0.6 mL) gave the title compound (23 mg, 94%) as a pale yellow solid. LCMS (Method A, ES^+): R_T = 0.23 min, *m/z* Calcd for $C_{12}H_{16}N_3O_3$ $[M+H]^+$ 250, found 250.

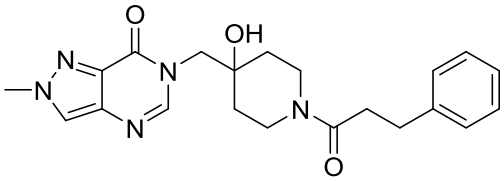
Step 4: 3-((4-Hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl)furo[2,3-*d*]pyrimidin-4(3*H*)-one: General procedure 3 using 3-((4-hydroxypiperidin-4-yl)methyl)furo[2,3-*d*]pyrimidin-4(3*H*)-one (23 mg, 92.3 µmol), 3-phenylpropanoic acid (14 mg, 92.3 µmol), EDC (53 mg, 0.277 mmol) and DCM (0.9 mL) gave the title compound (13 mg, 36%) as a colourless solid. LCMS (Method A, ES^+): R_T = 0.91 min (purity >95% at 254 nm), *m/z* Calcd for $C_{21}H_{24}N_3O_4$ $[M+H]^+$ 382, found 382. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.99 (s, 1H), 7.49 (d, 1H), 7.33-7.21 (m, 5H), 6.92 (d, 1H), 4.41 (d, 1H), 4.16-4.00 (m, 2H), 3.56 (d, 1H), 3.32 (t, 1H), 3.08-2.94 (m, 4H), 2.66-2.60 (m, 2H), 1.65-1.27 (m, 4H).

Compound 5: 3-((4-Hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl)furo[3,2-*d*]pyrimidin-4(3*H*)-one



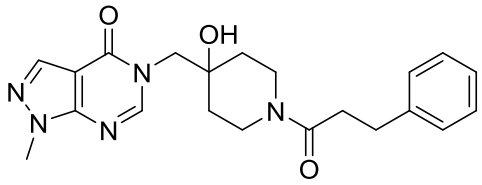
General procedure 1 using 3-phenyl-1-(1-oxa-6-azaspiro[2.5]octan-6-yl)propan-1-one[1] (81 mg, 0.331 mmol), furo[3,2-*d*]pyrimidin-4(3*H*)-one (45 mg, 0.331 mmol), Cs_2CO_3 (323 mg, 0.992 mmol) and DMF (0.6 mL) gave the title compound (100 mg, 79%) as a colourless solid. LCMS (Method A, ES^+): R_T = 0.94 min (purity >98% at 254 nm), *m/z* Calcd for $C_{21}H_{24}N_3O_4$ $[M+H]^+$ 382, found 382. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.81 (d, 1H), 7.32-7.19 (m, 5H), 6.80 (d, 1H), 4.35 (d, 1H), 4.17-4.00 (m, 2H), 3.92 (s, 1H), 3.60 (d, 1H), 3.33 (t, 1H), 3.09-2.92 (m, 3H), 2.65-2.58 (m, 2H), 1.61-1.26 (m, 4H).

Compound 6: 6-((4-Hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one



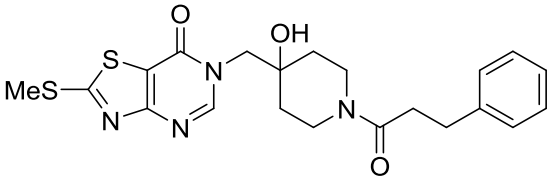
General procedure 1 using 3-phenyl-1-(1-oxa-6-azaspiro[2.5]octan-6-yl)propan-1-one[1] (65 mg, 0.266 mmol), 2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (Oslob, J. D.; Yu, C. H. WO 2007/013964 A1; Feb 01, 2007) (40 mg, 0.266 mmol) and Cs_2CO_3 (260 mg, 0.799 mmol) gave the title compound (54 mg, 51%) as a colourless solid. LCMS (Method A, ES^+): R_T = 0.85 min (purity >96% at 254 nm), *m/z* Calcd for $C_{21}H_{26}N_5O_3$ $[M+H]^+$ 396, found 396. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.91 (s, 1H), 7.78 (s, 1H), 7.31-7.18 (m, 5H), 4.32 (d, 1H), 4.21-3.95 (m, 5H), 3.60 (d, 1H), 3.38-3.30 (m, 1H), 3.10-3.01 (m, 1H), 2.92 (t, 2H), 2.66-2.55 (m, 2H), 1.62-1.37 (m, 4H).

Compound 7: 5-((4-Hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl)-1-methyl-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one



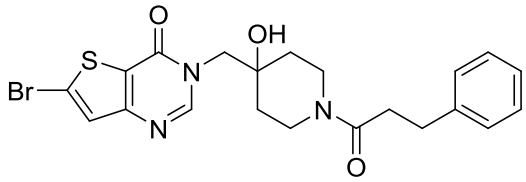
General procedure 1 using 3-phenyl-1-(1-oxa-6-azaspiro[2.5]octan-6-yl)propan-1-one[1] (48 mg, 0.196 mmol), 1-methyl-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (27 mg, 0.180 mmol), Cs_2CO_3 (70 mg, 0.213 mmol) and DMF (1.5 mL) gave the title compound (43 mg, 60%) as a colourless solid. LCMS (Method A, ES^+): R_T = 0.93 min (purity >98% at 254 nm), *m/z* Calcd for $C_{21}H_{26}N_5O_3$ $[M+H]^+$ 396, found 396. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.05 (s, 1H), 8.03 (s, 1H), 7.36-7.24 (m, 2H), 7.24-7.10 (m, 3H), 4.31 (d, 1H), 4.13-4.07 (m, 1H), 3.97 (s, 3H), 3.96-3.88 (m, 1H), 3.58 (d, 1H), 3.41-3.21 (m, 1H), 3.12-2.97 (m, 1H), 2.97-2.84 (m, 2H), 2.71-2.48 (m, 2H), 1.65-1.29 (m, 4H).

Compound 8: 6-((4-Hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl)-2-(methylthio)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one



General procedure 1 using 3-phenyl-1-(1-oxa-6-azaspiro[2.5]octan-6-yl)propan-1-one[1] (55 mg, 0.224 mmol), 2-(methylthio)thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (Prepared according to *Liebigs Annalen der Chemie*, 1989, p409-411) (41 mg, 0.206 mmol), Cs_2CO_3 (80 mg, 0.246 mmol) and DMF (1.5 mL) gave the title compound (36 mg, 39%) as a colourless solid. LCMS (Method A, ES^+): R_T = 1.12 min (purity >99% at 254 nm), *m/z* Calcd for $C_{21}H_{25}N_4O_3S_2$ $[M+H]^+$ 445, found 445. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.20 (s, 1H), 7.47-7.06 (m, 5H), 4.69-4.28 (m, 2H), 4.17-3.90 (m, 2H), 3.62 (d, 1H), 3.49-3.26 (m, 1H), 3.19-2.87 (m, 3H), 2.74 (s, 3H), 2.69-2.50 (m, 2H), 1.87-1.32 (m, 4H).

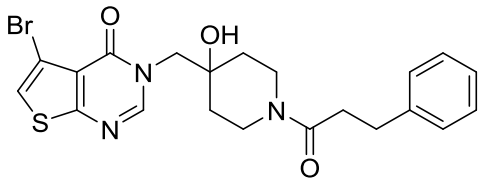
Compound 9: 6-Bromo-3-((4-hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl)thieno[3,2-*d*]pyrimidin-4(3*H*)-one



Step 1: 6-Bromothieno[3,2-*d*]pyrimidin-4(3*H*)-one: To a solution of 6-bromo-4-chlorothieno[3,2-*d*]pyrimidine (600 mg, 2.40 mmol) in THF (5 mL) was added 1 M $NaOH_{(aq)}$ (3.61 mL, 3.61 mmol). The reaction was stirred at rt for 17 h before the reaction was heated to 50 °C for 3 h. The reaction was cooled to rt and 1 M $HCl_{(aq)}$ was added until a neutral pH was achieved. The product was collected by filtration and subsequently three additional batches of the product were collected from the mother liquor after allowing it to stand for ~1 h. All the batches were dried in vacuo to give the title compound (471 mg, 84%) as a yellow solid. LCMS (Method A, ES^+): R_T = 0.75 min, *m/z* Calcd for $C_6H_4BrN_2OS$ $[M+H]^+$ 231, 233, found 231, 233. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.15 (s, 1H), 7.61 (s, 1H).

Step 2: 6-Bromo-3-((4-hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl)thieno[3,2-*d*]pyrimidin-4(3*H*)-one: General procedure 1 using 3-phenyl-1-(1-oxa-6-azaspiro[2.5]octan-6-yl)propan-1-one[1] (45 mg, 0.183 mmol), 6-bromothieno[3,2-*d*]pyrimidin-4(3*H*)-one (39 mg, 0.169 mmol), Cs_2CO_3 (65 mg, 0.199 mmol) and DMF (1.5 mL) gave, after purification by preparative HPLC, the title compound (4.2 mg, 5%) as a colourless solid. LCMS (Method A, ES^+): R_T = 1.29 min (purity >98% at 254 nm), *m/z* Calcd for $C_{21}H_{23}BrN_3O_3S$ $[M+H]^+$ 476, 478, found 476, 478. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.03 (s, 1H), 7.45-7.15 (m, 6H), 4.40 (d, 1H), 4.04 (dd, 2H), 3.61 (d, 1H), 3.44-3.19 (m, 1H), 3.14-2.90 (m, 4H), 2.78-2.51 (m, 2H), 1.65-1.39 (m, 2H), 1.39-1.14 (m, 2H).

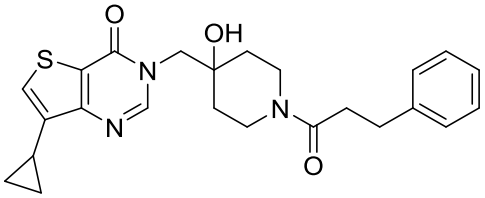
Compound 10: 5-Bromo-3-((4-hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one



Step 1: 5-Bromothieno[2,3-*d*]pyrimidin-4(3*H*)-one: To a stirred suspension of thieno[2,3-*d*]pyrimidin-4(3*H*)-one (250 mg, 1.64 mmol) in acetic acid (1.6 mL) in a reaction tube was dropwise added Br_2 (0.254 mL, 4.93 mmol). The reaction tube was sealed and the mixture was heated at 95 °C for 16 h before the reaction was allowed to cool to rt. To this mixture was added MeOH (7 mL) and Et_2O (7 mL). The resulting precipitate was collected by filtration and washed with Et_2O (50 mL) before being dried under high vacuum to give the title compound (352 mg, 93%) as a beige solid. LCMS (Method A, ES^+): R_T = 0.84 min, *m/z* Calcd for $C_6H_4BrN_2OS$ $[M+H]^+$ 231, 233, found 231, 233. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.67 (br s, 1H), 8.15 (s, 1H), 7.55 (s, 1H).

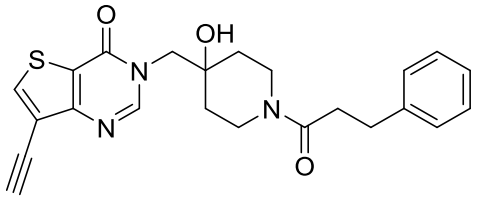
Step 2: 5-Bromo-3-((4-hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one: General procedure 1 using 3-phenyl-1-(1-oxa-6-azaspiro[2.5]octan-6-yl)propan-1-one[1] (49 mg, 0.200 mmol), 5-bromothieno[2,3-*d*]pyrimidin-4(3*H*)-one (42 mg, 0.182 mmol), Cs_2CO_3 (71 mg, 0.218 mmol) in DMF (1.5 ml) gave the title compound (26 mg, 30%) as a colourless solid. LCMS (Method A, ES^+): R_T = 1.34 min (purity >96% at 254 nm), *m/z* Calcd for $C_{21}H_{23}BrN_3O_3S$ $[M+H]^+$ 476, 478, found 476, 478. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.00 (s, 1H), 7.48 (s, 1H), 7.42-7.14 (m, 5H), 4.36 (d, 1H), 4.11 (d, 1H), 3.97 (d, 1H), 3.73-3.54 (m, 2H), 3.46-3.21 (m, 1H), 3.18-2.87 (m, 3H), 2.79-2.49 (m, 2H), 1.69-1.30 (m, 4H).

Compound 12: 7-Cyclopropyl-3-((4-hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl)thieno[3,2-*d*]pyrimidin-4(3*H*)-one



General procedure 5 using 7-bromo-3-((4-hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl)thieno[3,2-*d*]pyrimidin-4(3*H*)-one[1] (23 mg, 48.3 µmol), cyclopropylboronic acid (12 mg, 0.145 mmol), $PdCl_2$(dppf) (3.5 mg, 4.83 µmol) and K_3PO_4 (46 mg, 0.217 mmol) in 1,4-dioxane (0.5 mL) and water (0.1 mL) at 110 °C for 16 h gave the title compound (9.5 mg, 44%) as a colourless solid. LCMS (Method A, ES^+): R_T = 1.29 min (purity >98% at 254 nm), *m/z* Calcd for $C_{24}H_{28}N_3O_3S$ $[M+H]^+$ 438, found 438. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.07 (s, 1H), 7.40-7.14 (m, 6H), 4.43 (d, 1H), 4.18-4.06 (m, 2H), 3.75-3.48 (m, 1H), 3.44-3.24 (m, 1H), 3.14-2.89 (m, 3H), 2.72-2.53 (m, 2H), 2.33-2.14 (m, 1H), 1.70-1.30 (m, 5H), 1.12-0.92 (m, 2H), 0.92-0.70 (m, 2H).

Compound 13: 7-Ethynyl-3-((4-hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl)thieno[3,2-*d*]pyrimidin-4(3*H*)-one

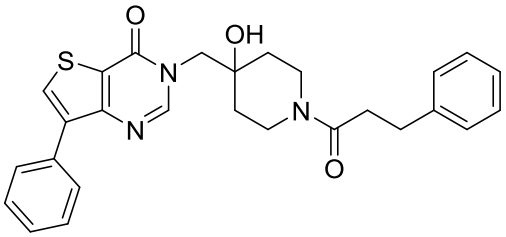


Step 1: *tert*-Butyl 4-hydroxy-4-((4-oxo-7-((trimethylsilyl)ethynyl)thieno[3,2-*d*]pyrimidin-3(4*H*)-yl)methyl)piperidine-1-carboxylate: General procedure 6 using *tert*-butyl 4-((7-bromo-4-oxothieno[3,2-*d*]pyrimidin-3(4*H*)-yl)methyl)-4-hydroxypiperidine-1-carboxylate[1] (102 mg, 0.230 mmol), CuI (8.7 mg, 45.7 µmol), $Pd(PPh_3)_2Cl_2$ (16 mg, 22.8 µmol), triethylamine (0.640 mL, 4.59 mmol), ethynyltrimethylsilane (0.127 mL, 0.917 mmol) and toluene (2 mL) at 110 °C for 16 h gave the title compound (100 mg, 94%) as a yellow oil. LCMS (Method A, ES^+): R_T = 1.76 min, *m/z* Calcd for $C_{22}H_{32}N_3O_4SSi$ $[M+H]^+$ 462, found 462. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.21 (s, 1H), 7.96 (s, 1H), 4.27-4.03 (m, 2H), 3.97-3.75 (m, 2H), 3.15 (t, 2H), 3.06 (s, 1H), 1.78-1.49 (m, 4H), 1.45 (s, 9H), 0.29 (s, 9H).

Step 2: 7-Ethynyl-3-((4-hydroxypiperidin-4-yl)methyl)thieno[3,2-*d*]pyrimidin-4(3*H*)-one: General procedure 2 using *tert*-butyl 4-hydroxy-4-((4-oxo-7-((trimethylsilyl)ethynyl)thieno[3,2-*d*]pyrimidin-3(4*H*)-yl)methyl)piperidine-1-carboxylate (112 mg, 0.243 mmol), TFA (2 mL) and DCM (2 mL) gave a mixture of the TMS and desilated alkyne. This material was treated with K_2CO_3 (101 mg, 0.731 mmol) in MeOH for 45 min before the reaction mixture was purified directly by flash chromatography (Biotage KP-NH 11 g cartridge, 0-100% DCM in PE, then 0-40% MeOH in DCM) to give the title compound (51 mg, 72%) as a colourless solid. LCMS (Method A, ES^+): R_T = 0.23 min, *m/z* Calcd for $C_{14}H_{16}N_3O_2S$ $[M+H]^+$ 290, found 290. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.27 (s, 1H), 7.98 (s, 1H), 4.14 (s, 2H), 3.41 (s, 1H), 3.01-2.84 (m, 4H), 1.91-1.44 (m, 6H).

Step 3: 7-Ethynyl-3-((4-hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl)thieno[3,2-*d*]pyrimidin-4(3*H*)-one: General procedure 4 using 7-ethynyl-3-((4-hydroxypiperidin-4-yl)methyl)thieno[3,2-*d*]pyrimidin-4(3*H*)-one (18 mg, 62.2 µmol), 3-phenylpropanoic acid (14 mg, 93.3 µmol), HATU (35 mg, 93.3 µmol), DIPEA (42 μL, 0.243 mmol) and DCM (0.5 mL) gave the title compound (24 mg, 91%) as a colourless solid. LCMS (Method A, ES^+): R_T = 1.15 min (purity >95% at 254 nm), *m/z* Calcd for $C_{23}H_{24}N_3O_3S$ $[M+H]^+$ 422, found 422. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19 (s, 1H), 7.99 (s, 1H), 7.33-7.25 (m, 2H), 7.24-7.16 (m, 3H), 4.34 (d, 1H), 4.12 (d, 1H), 4.02 (d, 1H), 3.59 (d, 1H), 3.52 (s, 1H), 3.41-3.25 (m, 2H), 3.11-2.99 (m, 1H), 2.99-2.89 (m, 2H), 2.70-2.52 (m, 2H), 1.67-1.28 (m, 4H).

Compound 14: 3-((4-Hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl)-7-phenylthieno[3,2-*d*]pyrimidin-4(3*H*)-one



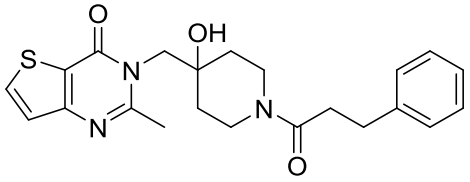
Step 1: *tert*-Butyl 4-hydroxy-4-((4-oxo-7-phenylthieno[3,2-*d*]pyrimidin-3(4*H*)-yl)methyl)piperidine-1-carboxylate: General procedure 5 using *tert*-butyl 4-((7-bromo-4-oxothieno[3,2-*d*]pyrimidin-3(4*H*)-yl)methyl)-4-hydroxypiperidine-1-carboxylate[1] (50 mg, 0.113 mmol), phenylboronic acid (41 mg, 0.338 mmol), $PdCl_2$(dppf) (8.2 mg, 11.2 µmol) and K_3PO_4 (108 mg, 0.506 mmol) in 1,4-dioxane (1 mL) and water (0.2 mL) at 110 °C for 16 h gave the title compound (48 mg, 96%) as a beige solid. LCMS (Method A, ES^+): R_T = 1.59 min, *m/z* Calcd for $C_{23}H_{28}N_3O_4S$

$[M+H]^+$ 442, found 442. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.17 (s, 1H), 7.87 (s, 1H), 7.82-7.72 (m, 2H), 7.54-7.34 (m, 3H), 4.28-3.99 (m, 2H), 3.89 (br s, 2H), 3.41 (s, 1H), 3.15 (t, 2H), 1.72-1.48 (m, 4H), 1.45 (s, 9H).

Step 2: 3-((4-Hydroxypiperidin-4-yl)methyl)-7-phenylthieno[3,2-*d*]pyrimidin-4(3*H*)-one: General procedure 2 using *tert*-butyl 4-hydroxy-4-((4-oxo-7-phenylthieno[3,2-*d*]pyrimidin-3(4*H*)-yl)methyl)piperidine-1-carboxylate (48 mg, 0.109 mmol), TFA (1 mL) and DCM (1 mL) gave the title compound (30 mg, 80%) as a colourless solid. LCMS (Method A, ES^+): R_T = 0.73 min, *m/z* Calcd for $C_{18}H_{20}N_3O_2S$ $[M+H]^+$ 342, found 342. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.21 (s, 1H), 7.85 (s, 1H), 7.83-7.73 (m, 2H), 7.65-7.32 (m, 3H), 4.12 (s, 2H), 3.12-2.74 (m, 4H), 2.19 (br s, 2H), 1.83-1.47 (m, 4H).

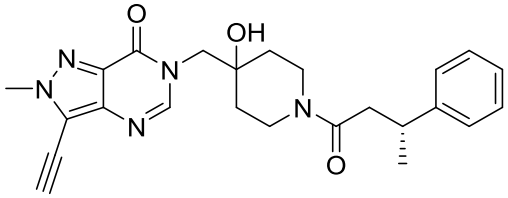
Step 3: 3-((4-Hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl)-7-phenylthieno[3,2-*d*]pyrimidin-4(3*H*)-one: General procedure 4 using 3-((4-hydroxypiperidin-4-yl)methyl)-7-phenylthieno[3,2-*d*]pyrimidin-4(3*H*)-one (30 mg, 87.9 µmol), 3-phenylpropanoic acid (20 mg, 0.132 mmol), HATU (50 mg, 0.132 mmol), DIPEA (61 μL, 0.351 mmol) and DCM (1 mL) gave the title compound (22 mg, 52%) as a colourless solid. LCMS (Method A, ES^+): R_T = 1.47 min (purity >97% at 254 nm), *m/z* Calcd for $C_{27}H_{28}N_3O_3S$ $[M+H]^+$ 474, found 474. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.09 (s, 1H), 7.91 (s, 1H), 7.87-7.76 (m, 2H), 7.59-7.45 (m, 2H), 7.44-7.37 (m, 1H), 7.35-7.16 (m, 5H), 4.44 (d, 1H), 4.14 (d, 1H), 4.05 (d, 1H), 3.63 (d, 1H), 3.45 (br s, 1H), 3.42-3.24 (m, 1H), 3.18-2.88 (m, 3H), 2.76-2.55 (m, 2H), 1.77-1.34 (m, 4H).

Compound 15: 3-((4-Hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl)-2-methylthieno[3,2-*d*]pyrimidin-4(3*H*)-one



General procedure 1 using 3-phenyl-1-(1-oxa-6-azaspiro[2.5]octan-6-yl)propan-1-one[1] (70 mg, 0.285 mmol), 2-methylthieno[3,2-*d*]pyrimidin-4(3*H*)-one (43 mg, 0.259 mmol), Cs_2CO_3 (101 mg, 0.311 mmol) in DMF (1.5 ml) gave the title compound (29 mg, 27%) as a pale yellow solid. LCMS (Method A, ES^+): R_T = 1.08 min (purity >98% at 254 nm), *m/z* Calcd for $C_{22}H_{26}N_3O_3S$ $[M+H]^+$ 412, found 412. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.82-7.66 (m, 1H), 7.29-7.08 (m, 6H), 4.45-4.34 (m, 1H), 4.26 (br s, 1H), 4.19 (d, 1H), 4.07 (d, 1H), 3.59-3.47 (m, 1H), 3.34-3.18 (m, 1H), 2.95-2.80 (m, 3H), 2.60 (s, 3H), 2.58-2.44 (m, 2H), 1.61-1.21 (m, 4H).

Compound 18: (*R*)-3-Ethynyl-6-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one



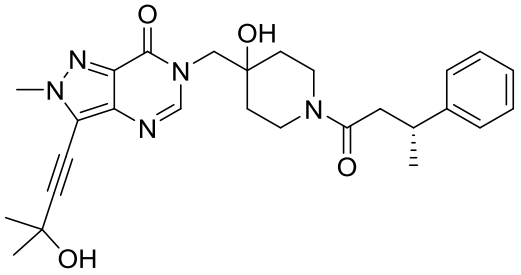
Step 1: *tert*-Butyl 4-hydroxy-4-((2-methyl-7-oxo-3-((trimethylsilyl)ethynyl)-2,7-dihydro-6*H*-pyrazolo[4,3-*d*]pyrimidin-6-yl)methyl)piperidine-1-carboxylate: General procedure 6 using *tert*-butyl 4-((3-bromo-2-methyl-7-oxo-2,7-dihydro-6*H*-pyrazolo[4,3-*d*]pyrimidin-6-yl)methyl)-4-hydroxypiperidine-1-carboxylate[1] (60 mg, 0.136 mmol), CuI (5.2 mg, 27.3 µmol), $Pd(PPh_3)_2Cl_2$ (9.5 mg, 13.5 µmol), triethylamine (0.378 mL, 2.71 mmol), ethynyltrimethylsilane (75 μL, 0.918 mmol) and toluene (1.4 mL) at 110 °C for 16 h gave the title compound (41 mg, 65%) as a yellow oil. LCMS (Method A, ES^+): R_T = 1.60 min, *m/z* Calcd for $C_{22}H_{34}N_5O_4Si$ $[M+H]^+$ 460, found 460. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.94 (s, 1H), 4.27-4.00 (m, 5H), 4.00-3.77 (m, 2H), 3.27-3.06 (m, 3H), 1.75-1.39 (m, 13H), 0.31 (s, 9H).

Step 2: 3-Ethynyl-6-((4-hydroxypiperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one: General procedure 2 using *tert*-butyl 4-hydroxy-4-((2-methyl-7-oxo-3-((trimethylsilyl)ethynyl)-2,7-dihydro-6*H*-pyrazolo[4,3-*d*]pyrimidin-6-yl)methyl)piperidine-1-carboxylate (32 mg, 69.6 µmol), TFA (1 mL) and DCM (1 mL) gave a mixture of the TMS and desilated alkyne. This material was treated with K_2CO_3 (29 mg, 0.209 mmol) in MeOH (1 mL) for 45 min before the reaction mixture was purified directly by flash chromatography (Biotage KP-NH 11 g

cartridge, 0-100% DCM in PE then 0-40% MeOH in DCM) affording the title compound (16 mg, 79%) as a colourless solid. LCMS (Method A, ES^+): R_T = 0.33 min, *m/z* Calcd for $C_{14}H_{18}N_5O_2$ $[M+H]^+$ 288, found 288.

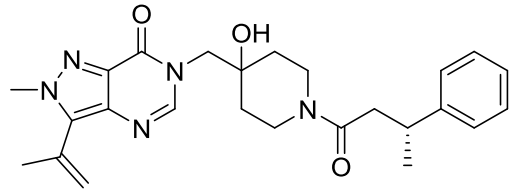
Step 3: (*R*)-3-Ethynyl-6-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one: General procedure 3 using 3-ethynyl-6-((4-hydroxypiperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (12 mg, 41.8 µmol), (*R*)-3-phenylbutanoic acid (7 mg, 41.8 µmol), EDC (24 mg, 0.125 mmol) and DCM (0.4 mL) gave the title compound (9 mg, 49%) as a colourless solid. LCMS (Method A, ES^+): R_T = 1.06 min (purity >99% at 254 nm), *m/z* Calcd for $C_{24}H_{28}N_5O_3$ $[M+H]^+$ 434, found 434. ^{1}H NMR (300 MHz, $CDCl_3$, this molecule appears conformers in a 2:3 ratio): δ 7.94 (s, 0.4H), 7.82 (s, 0.6H), 7.39-7.16 (m, 5H), 4.42-4.32 (m, 1H), 4.17 (s, 3H), 4.01-3.88 (m, 2H), 3.61-2.87 (m, 5H), 2.71-2.41 (m, 2H), 1.67-1.23 (m, 7.4H), 0.89-0.75 (m, 0.6).

Compound 19: (*R*)-6-((4-Hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-3-(3-hydroxy-3-methylbut-1-yn-1-yl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one



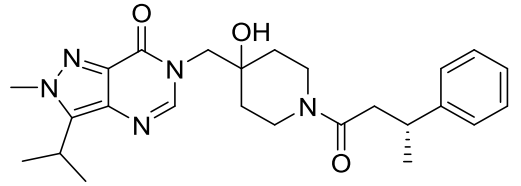
General procedure 6 using (*R*)-3-bromo-6-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one[1] (40 mg, 81.9 µmol), $CuBr{\cdot}SMe_2$ (0.7 mg, 3.28 μmol), $Pd(PPh_3)_4$ (1.9 mg, 1.64 μmol), triethylamine (0.46 mL, 3.28 mmol) and 2-methylbut-3-yn-2-ol (8 mg, 98.3 µmol) at 70 °C for 1 h gave the title compound (27 mg, 67%) as a colourless solid. LCMS (Method A, ES^+): R_T = 1.06 min (purity >99% at 254 nm), *m/z* Calcd for $C_{27}H_{34}N_5O_4$ $[M+H]^+$ 492, found 492. ^{1}H NMR (300 MHz, $CDCl_3$, this molecule appears as conformers in a 2:3 ratio): δ 7.94 (s, 0.4H), 7.86 (s, 0.6H), 7.37-7.15 (m, 5H), 4.45-3.83 (m, 6H), 3.63-3.46 (m, 1H), 3.39-3.19 (m, 2H), 3.12-2.90 (m, 1H), 2.71-2.41 (m, 2H), 2.08 (br s, 2H), 1.76-1.21 (m, 12.4H), 1.01-0.82 (m, 0.6H).

Compound 20: (*R*)-6-((4-Hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-3-(prop-1-en-2-yl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one



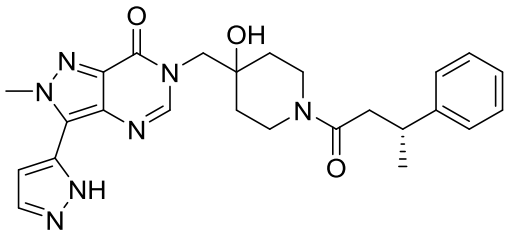
General procedure 5 using (*R*)-3-bromo-6-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one[1] (75 mg, 0.154 mmol), potassium isopropenyltrifluoroborate (68 mg, 0.461 mmol), K_3PO_4 (98 mg, 0.461 mmol), $Pd(PPh_3)_4$ (18 mg, 15.4 µmol), 1,4-dioxane (1.2 mL) and water (0.3 mL) at 130 °C under microwave irradiation for 45 min gave the title compound (56 mg, 81%) as a colourless solid. LCMS (Method A, ES^+): R_T = 1.12 min (purity >96% at 254 nm), *m/z* Calcd for $C_{25}H_{32}N_5O_3$ $[M+H]^+$ 450, found 450. ^{1}H NMR (300 MHz, $CDCl_3$, this molecule appears as conformers in a 2:3 ratio): δ 7.85 (s, 0.4H), 7.74 (s, 0.6H), 7.37-7.13 (m, 5H), 5.66-5.55 (m, 1H), 5.32-5.25 (m, 1H), 4.44-3.88 (m, 6H), 3.77-3.46 (m, 2H), 3.40-3.14 (m, 2H), 3.11-2.85 (m, 1H), 2.71-2.57 (m, 1H), 2.57-2.41 (m, 1H), 2.27 (d, 3H), 1.66-1.21 (m, 6.4H), 0.93-0.74 (m, 0.6H).

Compound 21: (*R*)-6-((4-Hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-3-isopropyl-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one



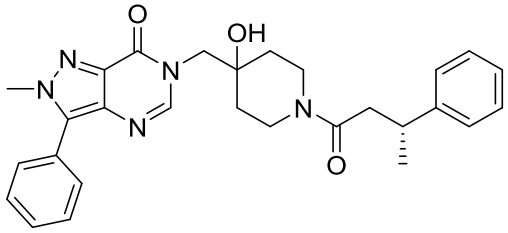
A solution of (*R*)-6-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-3-(prop-1-en-2-yl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (50 mg, 0.111 mmol) in MeOH (10 mL) was hydrogenated in an H-Cube® (10% Pd/C CatCart®, 1 mLmin^{-1}, 50 °C, 60 bar H_2). The resulting solution was concentrated under reduced pressure and the residue was purified by flash chromatography (Biotage KP-Sil 10 g cartridge, 0-100% EtOAc in PE, then 0-30% MeOH in EtOAc) to give the title compound (20 mg, 39%) as a colourless solid. LCMS (Method A, ES$^+$): R_T = 1.02 min (purity >98% at 254 nm), *m/z* Calcd for $C_{25}H_{34}N_5O_3$ $[M+H]^+$ 452, found 452. ^{1}H NMR (300 MHz, $CDCl_3$, this molecule appears as conformers in a 2:3 ratio): δ 7.70 (s, 0.4H), 7.59 (s, 0.6H), 7.38-7.15 (m, 5H), 4.48-4.26 (m, 1H), 4.19-3.83 (m, 5H), 3.65-2.84 (m, 6H), 2.72-2.58 (m, 1H), 2.58-2.42 (m, 1H), 1.64-1.21 (m, 12.4H), 0.79-0.63 (m, 0.6H).

Compound 22: (*R*)-6-((4-Hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-3-(1*H*-pyrazol-5-yl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one



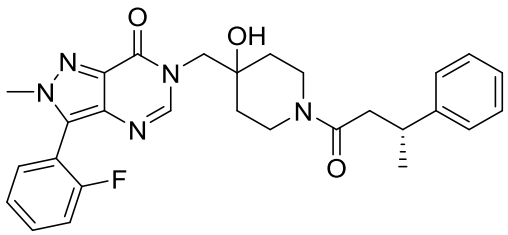
General procedure 5 using (*R*)-3-bromo-6-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one[1] (40 mg, 81.9 µmol), (1*H*-pyrazol-5-yl)boronic acid (27 mg, 0.246 mmol), K_3PO_4 (52 mg, 0.246 mmol), $Pd(PPh_3)_4$ (9 mg, 8.19 µmol), 1,4-dioxane (0.65 mL) and water (0.16 mL) at 150 °C under microwave irradiation for 10 min gave the title compound (26 mg, 66%) as a colourless solid. LCMS (Method A, ES$^+$): R_T = 0.97 min (purity >95% at 254 nm), *m/z* Calcd for $C_{25}H_{30}N_7O_3$ $[M+H]^+$ 476, found 476. ^{1}H NMR (300 MHz, $CDCl_3$, this molecule appears as conformers in a 2:3 ratio): δ 7.87 (s, 0.4H), 7.81-7.71 (m, 1.6H), 7.39-7.15 (m, 6H), 6.95 (br s, 1H), 4.46-3.90 (m, 6H), 3.77-3.47 (m, 2H), 3.44-2.88 (m, 3H), 2.72-2.42 (m, 2H), 1.79-1.24 (m, 6.4H), 0.97-0.74 (m, 0.6H).

Compound 23: (*R*)-6-((4-Hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-3-phenyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one



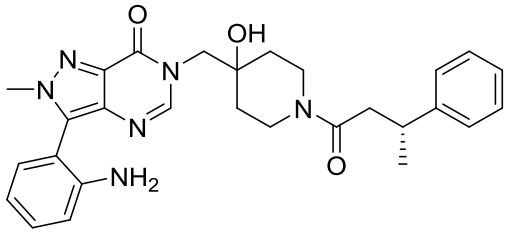
General procedure 5 using (*R*)-3-bromo-6-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one[1] (40 mg, 81.9 µmol), phenylboronic acid (30 mg, 0.246 mmol), K_3PO_4 (52 mg, 0.246 mmol), $Pd(PPh_3)_4$ (9.5 mg, 8.19 µmol), 1,4-dioxane (0.7 mL) and water (0.15 mL) at 150 °C under microwave irradiation for 10 min gave the title compound (31 mg, 77%) as a colourless solid. LCMS (Method A, ES$^+$): R_T = 1.23 min (purity >98% at 254 nm), *m/z* Calcd for $C_{28}H_{32}N_5O_3$ $[M+H]^+$ 486, found 486. ^{1}H NMR (300 MHz, $CDCl_3$, this molecule appears as conformers in a 2:3 ratio): δ 7.84 (s, 0.4H), 7.73 (s, 0.6H), 7.65-7.44 (m, 5H), 7.39-7.14 (m, 5H), 4.43-3.88 (m, 6H), 3.68-3.44 (m, 2H), 3.40-2.85 (m, 3H), 2.71-2.41 (m, 2H), 1.66-1.23 (m, 6.4H), 0.93-0.73 (m, 0.6H).

Compound 24: (*R*)-3-(2-Fluorophenyl)-6-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one



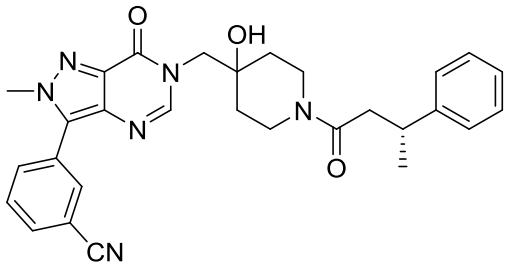
General procedure 5 using (*R*)-3-bromo-6-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one[1] (33 mg, 67.6 µmol), (2-fluorophenyl)boronic acid (28 mg, 0.203 mmol), K_3PO_4 (43 mg, 0.203 mmol), $Pd(PPh_3)_4$ (7.8 mg, 6.76 µmol), 1,4-dioxane (0.5 mL) and water (0.13 mL) at 150 °C under microwave irradiation for 10 min gave the title compound (24 mg, 70%) as a colourless solid. LCMS (Method A, ES^+): R_T = 1.23 min (purity >97% at 254 nm), *m/z* Calcd for $C_{28}H_{31}FN_5O_3$ $[M+H]^+$ 504, found 504. ^1H NMR (300 MHz, $CDCl_3$, this molecule appears as conformers in a 2:3 ratio): δ 7.86 (s, 0.4H), 7.74 (s, 0.6H), 7.61-7.44 (m, 2H), 7.41-7.15 (m, 7H), 4.44-3.89 (m, 6H), 3.62-2.86 (m, 5H), 2.70-2.41 (m, 2H), 1.63-1.23 (m, 6.4H), 0.98-0.71 (m, 0.6H).

Compound 25: (*R*)-3-(2-Aminophenyl)-6-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one



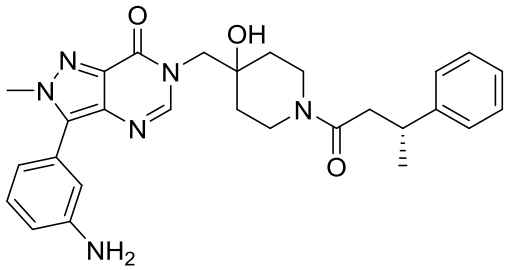
General procedure 5 using (*R*)-3-bromo-6-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one[1] (40 mg, 81.9 µmol), 2-aminophenylboronic acid hydrochloride (36 mg, 0.205 mmol), K_3PO_4 (70 mg, 0.328 mmol), $Pd(PPh_3)_4$ (9.5 mg, 8.19 µmol), 1,4-dioxane (0.7 mL) and water (0.16 mL) at 150 °C under microwave irradiation for 10 min gave the title compound (33 mg, 80%) as a colourless solid. LCMS (Method A, ES^+): R_T = 1.14 min (purity >97% at 254 nm), *m/z* Calcd for $C_{28}H_{33}N_6O_3$ $[M+H]^+$ 501, found 501. ^1H NMR (300 MHz, $CDCl_3$, this molecule appears as conformers in a 2:3): δ 7.82 (s, 0.4H), 7.72 (s, 0.6H), 7.39-7.13 (m, 5H), 7.13-7.05 (m, 1H), 6.93-6.57 (m, 3H), 4.40-3.84 (m, 6H), 3.62-2.84 (m, 5H), 2.70-2.41 (m, 2H), 1.64-1.24 (m, 6.4H), 0.92-0.77 (m, 0.6H).

Compound 26: (*R*)-3-(6-((4-Hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-7-oxo-6,7-dihydro-2*H*-pyrazolo[4,3-*d*]pyrimidin-3-yl)benzonitrile



General procedure 5 using (*R*)-3-bromo-6-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one[1] (33 mg, 67.6 µmol), (3-cyanophenyl)boronic acid (30 mg, 0.203 mmol), K_3PO_4 (43 mg, 0.203 mmol), $Pd(PPh_3)_4$ (7.8 mg, 6.76 µmol), 1,4-dioxane (0.5 mL) and water (0.13 mL) at 150 °C under microwave irradiation for 10 min gave the title compound (24 mg, 69%) as a colourless solid. LCMS (Method A, ES^+): R_T = 1.20 min (purity >95% at 254 nm), *m/z* Calcd for $C_{29}H_{31}N_6O_3$ $[M+H]^+$ 511, found 511. ^1H NMR (300 MHz, $CDCl_3$, this molecule appears as conformers in a 2:3 ratio): δ 7.92-7.67 (m, 5H), 7.33-7.18 (m, 5H), 4.43-3.90 (m, 6H), 3.65-2.86 (m, 5H), 2.72-2.41 (m, 2H), 1.66-1.23 (m, 6.4H), 0.92-0.77 (m, 0.6H).

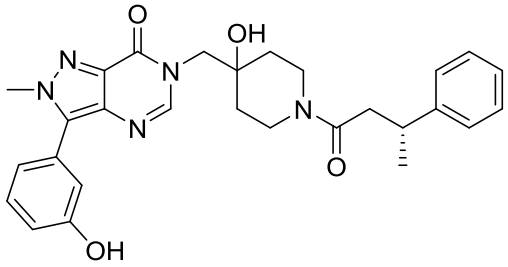
Compound 27: (*R*)-3-(3-Aminophenyl)-6-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one



General procedure 5 using (*R*)-3-bromo-6-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one[1] (40 mg, 81.9 µmol), (3-aminophenyl)boronic acid (34 mg, 0.246 mmol),

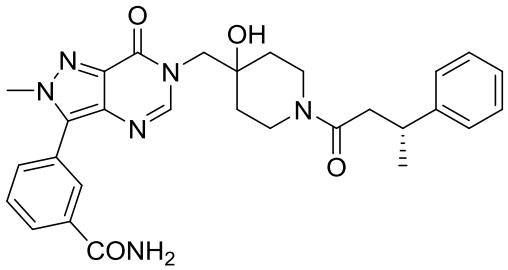
K_3PO_4 (52 mg, 0.246 mmol), $Pd(PPh_3)_4$ (9.5 mg, 8.19 µmol), 1,4-dioxane (0.65 mL) and water (0.16 mL) at 150 °C under microwave irradiation for 10 min gave the title compound (35 mg, 85%) as a colourless solid. LCMS (Method A, ES^+): R_T = 0.99 min (purity >96% at 254 nm), *m/z* Calcd for $C_{28}H_{33}N_6O_3$ $[M+H]^+$ 501, found 501. ^{1}H NMR (300 MHz, $CDCl_3$, this molecule appears as conformers in a 2:3 ratio): δ 7.83 (s, 0.4H), 7.73 (s, 0.6H), 7.38-7.15 (m, 6H), 6.87-6.71 (m, 3H), 4.42-3.71 (m, 8H), 3.59-3.43 (m, 1H), 3.42-3.11 (m, 2H), 3.09-2.82 (m, 1H), 2.71-2.40 (m, 2H), 1.93 (s, 1H), 1.63-1.23 (m, 6.4H), 0.96-0.73 (m, 0.6H).

Compound 28: (*R*)-6-((4-Hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-3-(3-hydroxyphenyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one



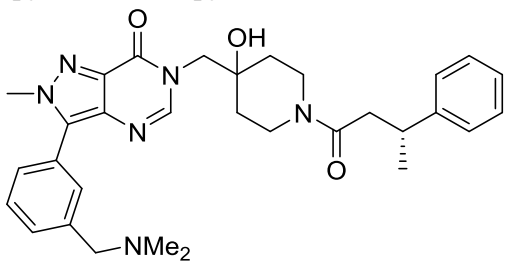
General procedure 5 using (*R*)-3-bromo-6-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one[1] (32 mg, 65.5 µmol), (3-hydroxyphenyl)boronic acid (27 mg, 0.197 mmol), K_3PO_4 (42 mg, 0.197 mmol), $Pd(PPh_3)_4$ (7.6 mg, 6.55 µmol), 1,4-dioxane (0.5 mL) and water (0.13 mL) at 150 °C under microwave irradiation for 10 min gave the title compound (21 mg, 63%) as a colourless solid. LCMS (Method A, ES^+): R_T = 1.09 min (purity >98% at 254 nm), *m/z* Calcd for $C_{28}H_{32}N_5O_4$ $[M+H]^+$ 502, found 502. ^{1}H NMR (300 MHz, methanol-d_4, this molecule appears as conformers in a 2:3 ratio): δ 7.89 (s, 0.4H), 7.83 (s, 0.6H), 7.34-7.01 (m, 6H), 7.00-6.89 (m, 2H), 6.89-6.78 (m, 1H), 4.19-3.70 (m, 6H), 3.66-3.47 (m, 1H), 3.26-2.31 (m, 5H), 1.60-1.08 (m, 6.4H), 0.85-0.69 (m, 0.6H).

Compound 29: (*R*)-3-(6-((4-Hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-7-oxo-6,7-dihydro-2*H*-pyrazolo[4,3-*d*]pyrimidin-3-yl)benzamide



General procedure 5 using (*R*)-3-bromo-6-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one[1] (40 mg, 81.9 µmol), (3-carbamoylphenyl)boronic acid (41 mg, 0.246 mmol), K_3PO_4 (52 mg, 0.246 mmol), $Pd(PPh_3)_4$ (9.5 mg, 8.19 µmol), 1,4-dioxane (0.65 mL) and water (0.16 mL) at 150 °C under microwave irradiation for 10 min gave the title compound (29 mg, 66%) as a colourless solid. LCMS (Method A, ES^+): R_T = 0.96 min (purity >96% at 254 nm), *m/z* Calcd for $C_{29}H_{33}N_6O_4$ $[M+H]^+$ 529, found 529. ^{1}H NMR (300 MHz, $CDCl_3$, this molecule appears as conformers a 2:3 ratio): δ 8.48-8.43 (m, 1H), 8.19 (s, 0.4H), 8.13-8.05 (m, 1.6H), 7.99-7.93 (m, 1H), 7.63-7.54 (m, 1H), 7.39-7.17 (m, 5H), 6.97 (s, 1H), 4.51-3.79 (m, 3H), 3.66-3.48 (m, 1H), 3.45-2.81 (m, 3H), 2.75-2.44 (m, 2H), 1.75-1.24 (m, 8.4H), 0.76-0.60 (m, 0.6H).

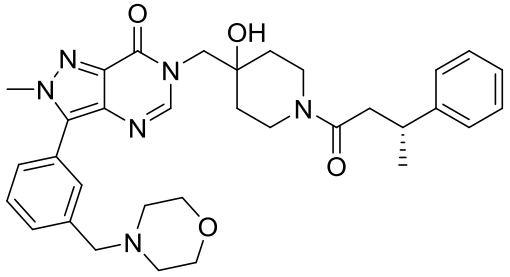
Compound 30: (*R*)-3-(3-((Dimethylamino)methyl)phenyl)-6-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one



Step 1: (*R*)-3-(3-(Aminomethyl)phenyl)-6-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one: General procedure 5 using (*R*)-3-bromo-6-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one[1] (90 mg, 0.184 mmol), (3-(aminomethyl)phenyl)boronic acid hydrochloride (86 mg, 0.461 mmol), K_3PO_4 (156 mg, 0.737 mmol), $Pd(PPh_3)_4$ (21 mg, 18.4 µmol), 1,4-dioxane (1.5 mL) and water (0.5 mL) at 150 °C under microwave irradiation for 10 min gave the title compound (30 mg, 31%) as a colourless solid. LCMS (Method B, ES^+): R_T = 0.75 min, *m/z* Calcd for $C_{29}H_{35}N_6O_3$ $[M+H]^+$ 515, found 515.

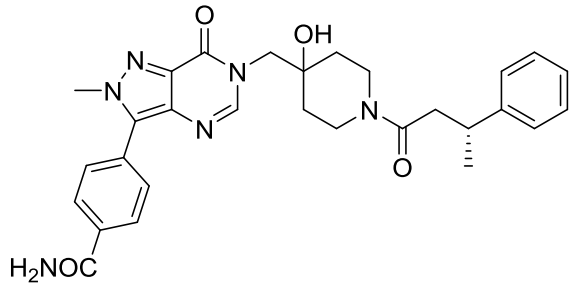
Step 2: (*R*)-3-(3-((Dimethylamino)methyl)phenyl)-6-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one: To a mixture of (*R*)-3-(3-(aminomethyl)phenyl)-6-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (30 mg, 58.3 µmol) and paraformaldehyde (8.75 mg, 0.291 mmol) in ethanol (0.3 mL) was added $NaBH_4$ (6.6 mg, 0.175 mmol) and the mixture was heated at reflux for 190 min. The reaction mixture was allowed to cool to rt before being diluted with brine (20 mL) and extracted with DCM (20 mL) using a Biotage phase separator. The organic phase was concentrated under reduced pressure and the residue was purified by flash chromatography (Biotage KP-NH 11 g cartridge, 0-100% EtOAc in PE, then 0-30% MeOH in EtOAc) to give the title compound (25 mg 79%) as a pale yellow solid. LCMS (Method B, ES^+): R_T = 0.77 min (purity >95% at 254 nm), *m/z* Calcd for $C_{31}H_{39}N_6O_3$ $[M+H]^+$ 543, found 543. ^{1}H NMR (500 MHz, DMSO-d_6, this molecule appears as conformers in a 1:1 ratio): δ 8.00 (s, 0.5H), 7.97 (s, 0.5H), 7.64-7.56 (m, 2H), 7.56-7.51 (m, 1H), 7.46-7.41 (m, 1H), 7.31-7.20 (m, 4H), 7.18-7.13 (m, 1H), 4.86 (s, 0.5H), 4.85 (s, 0.5H), 4.10 (s, 3H), 4.06-3.87 (m, 4H), 3.68-3.61 (m, 1H), 3.50 (s, 2H), 3.25-3.12 (m, 2H), 2.93-2.83 (m, 1H), 2.61-2.55 (m, 1H), 2.19 (s, 6H), 1.59-1.23 (m, 4H), 1.21 (d, 3H).

Compound 31: (*R*)-6-((4-Hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-3-(3-(morpholinomethyl)phenyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one



General procedure 5 using (*R*)-3-bromo-6-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one[1] (25 mg, 51.2 µmol), 4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)morpholine (39 mg, 0.128 mmol), K_3PO_4 (33 mg, 0.154 mmol), $Pd(PPh_3)_4$ (6 mg, 5.12 µmol), 1,4-dioxane (0.4 mL) and water (0.1 mL) at 130 °C under microwave irradiation for 15 min gave the title compound (19 mg, 63%) as a colourless solid. LCMS (Method B, ES^+): R_T = 0.76 min (purity >95% at 254 nm), *m/z* Calcd for $C_{33}H_{41}N_6O_4$ $[M+H]^+$ 585, found 585. ^{1}H NMR (500 MHz, DMSO-d_6, this molecule appears as conformers in a 1:1 ratio): δ 8.00 (s, 0.5H), 7.98 (s, 0.5H), 7.64 (s, 1H), 7.60 (d, 1H), 7.54 (t, 1H), 7.46 (d, 1H), 7.30-7.21 (m, 4H), 7.18-7.12 (m, 1H), 4.89 (s, 0.5H), 4.87 (s, 0.5H), 4.11 (s, 3H), 4.14-3.86 (m, 4H), 3.70-3.53 (m, 6H), 3.27-3.13 (m, 2H), 2.92-2.83 (m, 1H), 2.66-2.55 (m, 2H), 2.44-2.35 (m, 4H), 1.58-1.25 (m, 4H), 1.21 (d, 3H).

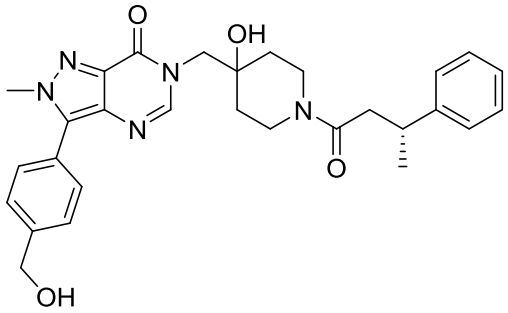
Compound 32: (*R*)-4-(6-((4-Hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-7-oxo-6,7-dihydro-2*H*-pyrazolo[4,3-*d*]pyrimidin-3-yl)benzamide



General procedure 5 using (*R*)-3-bromo-6-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one[1] (25 mg, 51.2 µmol), (4-carbamoylphenyl)boronic acid (21 mg, 0.128 mmol), K_3PO_4 (33 mg, 0.154 mmol), $Pd(PPh_3)_4$ (6 mg, 5.12 µmol), 1,4-dioxane (0.4 mL) and water (0.1 mL) at 130 °C

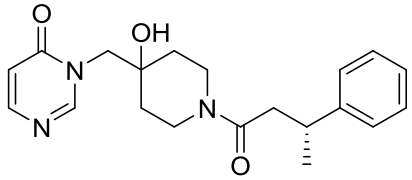
under microwave irradiation for 15 min gave the title compound (22 mg, 81%) as a colourless solid. LCMS (Method A, ES^+): R_T = 0.94 min (purity >95% at 254 nm), *m/z* Calcd for $C_{29}H_{33}N_6O_4$ $[M+H]^+$ 529, found 529. ^{1}H NMR (500 MHz, DMSO-d_6, this molecule appears as conformers in a 1:1 ratio): δ 8.11 (s, 1H), 8.06 (d, 2H), 8.03 (s, 0.5H), 8.00 (s, 0.5H), 7.82 (d, 2H), 7.49 (s, 1H), 7.30-7.21 (m, 4H), 7.19-7.13 (m, 1H), 4.88 (s, 0.5H), 4.87 (s, 0.5H), 4.15 (s, 3H), 4.05-3.89 (m, 3H), 3.69-3.61 (m, 1H), 3.28-3.12 (m, 3H), 2.90-2.84 (m, 1H), 2.62-2.56 (m, 1H), 1.55-1.29 (m, 4H), 1.21 (d, Hz, 3H).

Compound 33: (*R*)-6-((4-Hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-3-(4-(hydroxymethyl)phenyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one



General procedure 5 using (*R*)-3-bromo-6-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-2-methyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one[1] (25 mg, 51.2 µmol), (4-(hydroxymethyl)phenyl)boronic acid (19 mg, 0.128 mmol), K_3PO_4 (33 mg, 0.154 mmol), $Pd(PPh_3)_4$ (6 mg, 5.12 µmol), 1,4-dioxane (0.4 mL) and water (0.1 mL) at 130 °C under microwave irradiation for 15 min gave the title compound (19 mg, 71%) as a colourless solid. LCMS (Method A, ES^+): R_T = 1.01 min (purity >95% at 254 nm), *m/z* Calcd for $C_{29}H_{34}N_5O_4$ $[M+H]^+$ 516, found 516. ^{1}H NMR (500 MHz, DMSO-d_6, this molecule appears as conformers in a 1:1 ratio): δ 7.99 (s, 0.5H), 7.97 (s, 0.5H), 7.69-7.65 (m, 2H), 7.54-7.49 (m, 2H), 7.29-7.23 (m, 4H), 7.17-7.14 (m, 1H), 5.32 (t, 1H), 4.87 (s, 0.5H), 4.86 (s, 0.5H), 4.60 (d, 2H), 4.10 (s, 3H), 4.07-3.87 (m, 3H), 3.69-3.61 (m, 1H), 3.27-3.13 (m, 2H), 2.92-2.83 (m, 1H), 2.66-2.55 (m, 2H), 1.57-1.28 (m, 4H), 1.21 (d, 3H).

Compound 35: (*R*)-3-((4-Hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)pyrimidin-4(3*H*)-one



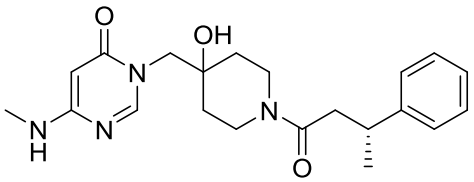
Step 1: (*R*)-1-(3-Phenylbutanoyl)piperidin-4-one*:* To freshly prepared piperidin-4-one hydrochloride (Young, J., *et al.* WO 2011/084402 A1, Jul 14, 2011) (1.70 g, 12.6 mmol) was added EDC (2.89 g, 15.1 mmol), DMAP (153 mg, 1.26 mmol), DCM (15 mL) and DIPEA (11 mL, 62.7 mmol). After 10 min a solution of *(R)*-3-phenylbutanoic acid (2.47 g, 15.1 mmol) in DCM (10 mL) was added. After 20 h, EDC (2.89 g, 15.1 mmol) was added and the reaction stirred for a further 4 h. The reaction was quenched by the addition of saturated $NaHCO_{3(aq)}$ (150 mL) and the resulting mixture was extracted with EtOAc (3 x 50 mL). The combined organic phases were washed with water (50 mL) and brine (50 mL) before being dried over $MgSO_4$, concentrated under reduced pressure and the residue was purified by flash chromatography (Biotage KP-Sil 50 g cartridge, 0-60% EtOAc in PE) to give the title compound (2.93 g, 95%) as a colourless oil. LCMS (Method A, ES^+): R_T = 1.07 min, *m/z* Calcd for $C_{15}H_{20}NO_2$ $[M+H]^+$ 246, found 246. ¹H NMR (300 MHz, $CDCl_3$): δ 7.44-7.13 (m, 5H), 4.30-4.03 (m, 1H), 3.77-3.58 (m, 1H), 3.46 (tdd, 2H), 3.11-2.93 (m, 2H), 2.82-2.61 (m, 4H), 1.86 (m, 1H), 1.77-1.62 (m, 1H), 1.54-1.33 (m, 2H).

Step 1: (*R*)-3-Phenyl-1-(1-oxa-6-azaspiro[2.5]octan-6-yl)butan-1-one: To a solution of trimethylsulfonium iodide (6.09 g, 29.9 mmol) in DMSO (30 mL) was added NaH (1.19 g, 29.9 mmol). The resulting mixture was stirred at rt for 1 h before a solution of *(R)*-1-(3-phenylbutanoyl)piperidin-4-one (2.93 g, 11.9 mmol) in DMSO (15 mL) was added. The reaction mixture was stirred at 50 °C for 2 h before it was allowed to cool to rt, quenched by the addition of water (100 mL) and the resulting mixture was extracted with Et_2O (3 x 50 mL). The combined organic phases were washed with brine (50 mL), dried over Na_2SO_4, concentrated under reduced pressure and the residue was purified by flash chromatography (Biotage KP-Sil 50 g cartridge, 0-70% EtOAc in PE) to give the title compound (2.68 g, 87%) as a colourless oil. LCMS (Method A, ES^+): R_T = 1.16 min, *m/z* Calcd for $C_{16}H_{22}NO_2$ $[M+H]^+$ 260, found 260. ¹H NMR

(300 MHz, $CDCl_3$): δ 7.43-7.14 (m, 5H), 4.30-3.95 (m, 1H), 3.69-3.18 (m, 4H), 2.84-2.47 (m, 4H), 1.87-1.66 (m, 2H), 1.51-1.31 (m, 2H), 1.37 (d, 3H).

Step 3: (*R*)-3-((4-Hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)pyrimidin-4(3*H*)-one: General procedure 1 using pyrimidin-4(*3H*)-one (22 mg, 0.229 mmol), Cs_2CO_3 (94 mg, 0.289 mmol), (*R*)-3-phenyl-1-(1-oxa-6-azaspiro[2.5]octan-6-yl)butan-1-one (50 mg, 0.193 mmol) and DMF (1 mL) gave the title compound (27 mg, 39%) as a white solid. LCMS (Method A, ES^+): R_T = 0.88 min (purity >99% at 254 nm), *m/z* Calcd for $C_{20}H_{26}N_3O_3$ $[M+H]^+$ 356, found 356. ^1H NMR (300 MHz, $CDCl_3$): δ 7.92-8.10 (m, 2H), 7.25 (m, 5H), 6.52 (d, 1H), 4.25-4.50 (m, 1H), 3.48-4.06 (m, 4H), 2.80-3.46 (m, 3H), 2.70 (m, 1H), 2.51 (m, 1H), 1.50 (m, 2H), 1.28 (m, 4H), 0.70 (m, 1H).

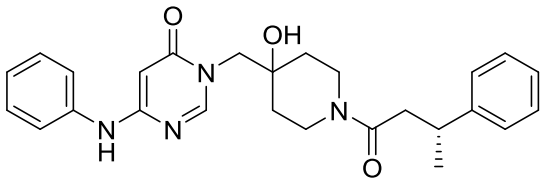
Compound 36: (*R*)-3-((4-Hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-6-(methylamino)pyrimidin-4(3*H*)-one



Step 1: (*R*)-6-Chloro-3-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)pyrimidin-4(3*H*)-one: A suspension of 6-chloropyrimidin-4(3*H*)-one (200 mg, 1.53 mmol), (*R*)-3-phenyl-1-(1-oxa-6-azaspiro[2.5]octan-6-yl)butan-1-one (397 mg, 1.53 mmol) and DIPEA (401 µl, 2.30 mmol) in DMF (3 mL) was heated at 80 °C for 16 h. The reaction mixture was allowed to cool to rt and quenched by the addition of saturated $NH_4Cl_{(aq)}$ (20 mL). The resulting mixture was extracted with EtOAc (3 x 20 mL), the combined organic extracts were dried over Na_2SO_4, concentrated under reduced pressure and the residue was purified by flash chromatography (Biotage KP-Sil 25 g cartridge, 0-100% EtOAc in PE, then 0-30% MeOH in EtOAc) to give the title compound (350 mg, 59%) as a pale yellow solid. LCMS (Method A, ES^+): R_T = 1.11 min, *m/z* Calcd for $C_{20}H_{25}ClN_3O_3$ $[M+H]^+$ 390, 392, found 390, 392. ^1H NMR (300 MHz, methanol-d_4, this molecule appears as conformers in a 2:3 ratio): δ 8.28 (s, 0.4H), 8.24 (s, 0.6H), 7.38-7.13 (m, 5H), 6.58 (s, 1H), 4.27-4.09 (m, 1H), 4.00 (dd, 0.8H), 3.84 (dd, 1.2H), 3.74-3.57 (m, 1H), 3.39-2.86 (m, 3H), 2.86-2.67 (m, 1H), 2.66-2.43 (m, 1H), 1.66-1.20 (m, 6.4H), 0.93-0.79 (m, 0.6H).

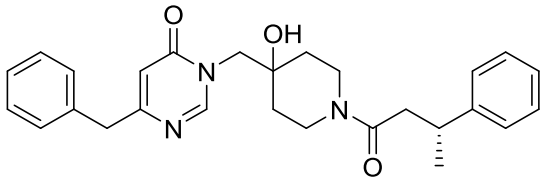
Step 2: (*R*)-3-((4-Hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-6-(methylamino)pyrimidin-4(3*H*)-one: A solution of (*R*)-6-chloro-3-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)pyrimidin-4(3*H*)-one (30 mg, 76.9 µmol) in 2 M $MeNH_2$ in THF (0.5 mL, 1.00 mmol) was heated at 130 °C under microwave irradiation for 1 h. The reaction mixture was concentrated under reduced pressure and the residue was purified by flash chromatography (Biotage KP-NH 11 g cartridge, 0-100% EtOAc in PE, then 0-30% MeOH in EtOAc) to give the title compound (23 mg, 77%) as a colourless solid. LCMS (Method A, ES^+): R_T = 0.84 min (purity >96% at 254 nm), *m/z* Calcd for $C_{21}H_{29}N_4O_3$ $[M+H]^+$ 385, found 385. ^1H NMR (300 MHz, $CDCl_3$, this molecule appears as conformers in a 2:3 ratio): δ 7.73 (s, 0.4H), 7.62 (s, 0.6H), 7.37-7.15 (m, 5H), 5.30-5.23 (m, 1H), 5.11 (d, 1H), 4.49-4.30 (m, 1H), 4.03-3.47 (m, 3H), 3.42-3.16 (m, 2H), 3.07-2.84 (m, 1H), 2.84 (d, 3H), 2.71-2.58 (m, 1H), 2.57-2.42 (m, 1H), 1.92 (br s, 1H), 1.60-1.10 (m, 6.4H), 0.67-0.52 (m, 0.6H).

Compound 37: (*R*)-3-((4-Hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-6-(phenylamino)pyrimidin-4(3*H*)-one



A solution of (*R*)-6-chloro-3-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)pyrimidin-4(3*H*)-one (30 mg, 76.9 µmol) in aniline (175 µL, 1.92 mmol) was heated at 130 °C under microwave irradiation for 1 h before the reaction mixture was purified directly by flash chromatography (Biotage KP-NH 11 g cartridge, 0-100% EtOAc in PE then 0-30% MeOH in EtOAc) to give the title compound (22 mg, 64%) as a colourless solid. LCMS (Method A, ES^+): R_T = 1.20 min (purity >95% at 254 nm), *m/z* Calcd for $C_{26}H_{31}N_4O_3$ $[M+H]^+$ 447, found 447. ^1H NMR (300 MHz, methanol-d_4, this molecule appears as conformers in a 2:3 ratio): δ 8.23 (d, 0.4H), 8.08 (d, 0.6H), 7.43-6.92 (m, 10H), 6.56 (s, 0.4H), 5.65 (s, 0.6H), 4.26-3.51 (m, 4H), 3.35-2.85 (m, 3H), 2.85-2.65 (m, 1H), 2.64-2.41 (m, 1H), 1.63-1.20 (m, 6.4H), 0.95-0.73 (m, 0.6H).

Compound 38: (*R*)-6-Benzyl-3-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)pyrimidin-4(3*H*)-one

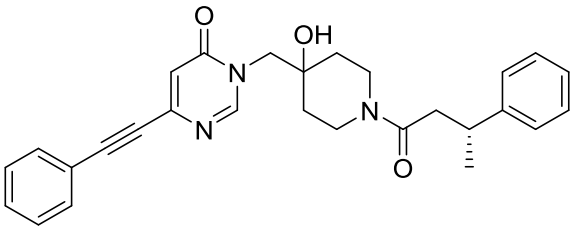


Step 1: *tert*-Butyl 4-((4-chloro-6-oxopyrimidin-1(6*H*)-yl)methyl)-4-hydroxypiperidine-1-carboxylate: A solution of 6-chloropyrimidin-4(3*H*)-one (3.72 g, 28.5 mmol), *tert*-butyl 1-oxa-6-azaspiro[2.5]octane-6-carboxylate[1] (6.08 g, 28.5 mmol) and DIPEA (7.47 mL, 42.7 mmol) in DMF (35 mL) was heated at 80 °C for 16 h. The reaction mixture was allowed to cool to rt before it was quenched by the addition of saturated $NH_4Cl_{(aq)}$ (100 mL) and the resulting mixture was extracted with EtOAc (3 x 50 mL). The combined organic extracts were dried over $MgSO_4$, concentrated under reduced pressure and the residue was purified by flash chromatography (GraceResolv silica 120 g cartridge, 0-100% EtOAc in cyclohexane) to give the title compound (5.87 g, 60%) as an off-white solid. LCMS (Method B, ES^+): R_T = 0.99 min, *m/z* Calcd for $C_{15}H_{23}ClN_3O_4$ $[M+H]^+$ 344, 346, found 344, 346.

Step 2: 6-Benzyl-3-((4-hydroxypiperidin-4-yl)methyl)pyrimidin-4(3*H*)-one: A solution of *tert*-butyl 4-((4-chloro-6-oxopyrimidin-1(6*H*)-yl)methyl)-4-hydroxypiperidine-1-carboxylate (60 mg, 0.174 mmol), potassium benzyltrifluoroborate (38 mg, 0.192 mmol) and triethylamine (36 µL, 0.26 mmol) in toluene (2 mL) and water (0.2 mL) was purged with N_2 before $PdCl_2$(dppf) (12.8 mg, 17.4 µmol) was added. The reaction tube was sealed and the mixture was heated at 110 °C for 16 h. The reaction was allowed to cool to rt, diluted with water (20 mL) and extracted into EtOAc (3 x 20 mL). The combined organic phases were washed with brine (20 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography (GraceResolv silica 12 g cartridge; 10-100% EtOAc in cyclohexane, then 0-15% MeOH in EtOAc) to give a colourless glass. This was dissolved in DCM (1 mL) and TFA (1 mL) and stirred for 10 min before being concentrated under reduce pressure. The residue was dissolved in methanol and added to a 2 g SCX-2 cartridge. The column was flushed with MeOH before being eluted with 2 M NH_3 in MeOH. The NH_3 fractions were concentrated under reduced pressure to give the title compound (11 mg, 21%) as a colourless glass. LCMS (Method A, ES^+): R_T = 0.55 min, *m/z* Calcd for $C_{17}H_{22}N_3O_2$ $[M+H]^+$ 300, found 300.

Step 3: (*R*)-6-Benzyl-3-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)pyrimidin-4(3*H*)-one: General procedure 4 using 6-benzyl-3-((4-hydroxypiperidin-4-yl)methyl)pyrimidin-4(3*H*)-one (11 mg, 36.7 µmol), (*R*)-3-phenylbutanoic acid (6.6 mg, 40.4 µmol), HATU (15.4 mg, 40.4 µmol), DIPEA (7.7 µL, 44.1 µmol) and DCM (1 mL) gave the title compound (11 mg, 67%) as a white solid. LCMS (Method B, ES^+): R_T = 1.14 min (purity >95% at 254 nm), *m/z* Calcd for $C_{27}H_{32}N_3O_3$ $[M+H]^+$ 446, found 446. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.20 (d, 1H), 7.27 (m, 9H), 7.22 (m, 1H), 6.21 (m, 1H), 4.92 (m, 1H), 3.86 (m, 2H), 3.76 (s, 3H), 3.60 (m, 1H), 3.14 (m, 2H), 2.80 (m, 1H), 2.55 (m, 2H), 1.10-1.50 (m, 7H).

Compound 39: (*R*)-3-((4-Hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-6-(phenylethynyl)pyrimidin-4(3*H*)-one

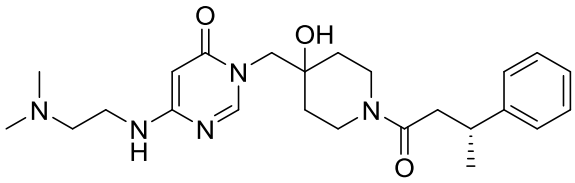


Step 1: 3-((4-Hydroxypiperidin-4-yl)methyl)-6-(phenylethynyl)pyrimidin-4(3*H*)-one: A solution of *tert*-butyl 4-((4-chloro-6-oxopyrimidin-1(6*H*)-yl)methyl)-4-hydroxypiperidine-1-carboxylate (100 mg, 0.291 mmol), phenylacetylene (70 µL, 0.640 mmol) and trimethylamine (0.24 mL, 1.75 mmol) in DMF (1 mL) was purged with N_2 before $AuCl(PPh_3)$ (7.2 mg, 14.5 µmol) and $PdCl_2(PPh_3)_2$ (10.2 mg, 14.5 µmol) were added. The reaction tube was sealed and the reaction was heated at 60 °C for 16 h before being allowed to cool to rt. The mixture was diluted with water (20 mL) and extracted with EtOAc (3 x 20 mL). The combined organic phases were washed with brine (20 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography (GraceResolv 12 g cartridge, 0-100% EtOAc in cyclohexane) to give an orange syrup. This was dissolved in DCM (1 mL) and TFA (1

mL) and stirred for 5 min before being concentrated under reduced pressure. The residue was dissolved in MeOH and added to a 2 g SCX-2 cartridge. The column was flushed with MeOH before being eluted with 2 M NH_3 in MeOH. The NH_3 fractions were concentrated under reduced pressure to give the title compound (60 mg, 66%). LCMS (Method A, ES^+): R_T = 0.73 min, *m/z* Calcd for $C_{18}H_{20}N_3O_2$ $[M+H]^+$ 310, found 310.

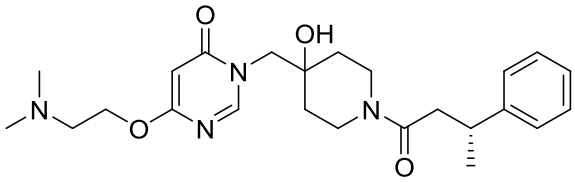
Step 2: (*R*)-3-((4-Hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-6-(phenylethynyl)pyrimidin-4(3*H*)-one: General procedure 4 using 3-((4-hydroxypiperidin-4-yl)methyl)-6-(phenylethynyl)pyrimidin-4(3*H*)-one (58 mg, 0.187 mmol), (*R*)-3-phenylbutanoic acid (33.9 mg, 0.206 mmol), DIPEA (0.043 mL, 0.244 mmol), HATU (82 mg, 0.216 mmol) and DCM (2 mL) gave the title compound (9 mg, 10%) as a colourless glass. LCMS (Method B, ES^+): R_T = 1.25 min (purity >95% at 254 nm), *m/z* Calcd for $C_{28}H_{30}N_3O_3$ $[M+H]^+$ 456, found 456. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.28 (d, 1H), 7.60 (d, 2H), 7.50 (m, 3H), 7.26 (m, 4H), 7.22 (m, 1H), 6.68 (m, 1H), 4.96 (m, 1H), 4.00 (m, 1H), 3.94 (m, 2H), 3.71 (m, 1H), 3.21 (m, 2H), 2.90 (m, 1H), 2.55 (m, 2H), 1.05-1.55 (m, 7H).

Compound 40: (*R*)-6-((2-(Dimethylamino)ethyl)amino)-3-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)pyrimidin-4(3*H*)-one



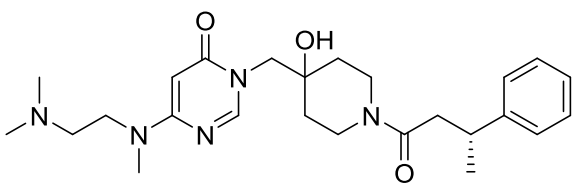
A mixture of (*R*)-6-chloro-3-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)pyrimidin-4(3*H*)-one (25 mg, 64.1 µmol), *N*,*N*-dimethylethylenediamine (85 mg, 0.962 mmol) and ethanol (0.5 mL) was heated at 120 °C under microwave irradiation for 20 min before the reaction mixture was purified directly by flash chromatography (Biotage KP-NH 11 g cartridge, 0-100% EtOAc in PE, then 0-30% MeOH in EtOAc) to give the title compound (22 mg, 77%) as a pale yellow solid. LCMS (Method A, ES^+): R_T = 0.60 min (purity >96% at 254 nm), *m/z* Calcd for $C_{24}H_{36}N_5O_3$ $[M+H]^+$ 442, found 442. ^{1}H NMR (300 MHz, $CDCl_3$, this molecule appears as conformers in a 2:3 ratio): δ 7.71 (s, 0.4H), 7.60 (s, 0.6H), 7.36-7.15 (m, 5H), 5.68-5.58 (m, 1H), 5.29-5.23 (m, 1H), 4.49-4.32 (m, 1H), 4.00-3.48 (m, 3H), 3.43-3.07 (m, 4H), 3.05-2.84 (m, 1H), 2.71-2.59 (m, 1H), 2.56-2.42 (m, 3H), 2.25 (s, 6H), 1.61-1.11 (m, 6.4H), 0.62-0.49 (m, 0.6H).

Compound 41: (*R*)-6-(2-(Dimethylamino)ethoxy)-3-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)pyrimidin-4(3*H*)-one



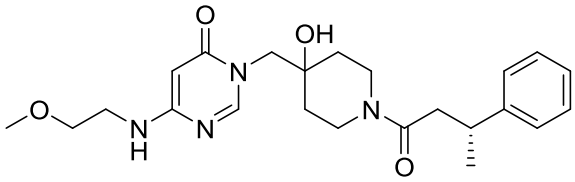
A mixture of (*R*)-6-chloro-3-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)pyrimidin-4(3*H*)-one (25 mg, 64.1 µmol) and 2-(dimethylamino)ethanol (200 µL, 1.99 mmol) was heated at 150 °C under microwave irradiation for 15 min before the reaction mixture was purified directly by flash chromatography (Biotage KP-NH 11 g cartridge, 0-100% EtOAc in PE, then 0-30% MeOH in EtOAc) to give the title compound (17 mg, 59%) as a pale yellow solid. LCMS (Method A, ES^+): R_T = 0.58 min (purity >96% at 254 nm), *m/z* Calcd for $C_{24}H_{35}N_4O_4$ $[M+H]^+$ 443, found 443. ^{1}H NMR (300 MHz, $CDCl_3$, this molecule appears as conformers in a 2:3 ratio): δ 7.95 (s, 0.4H), 7.83 (s, 0.6H), 7.37-7.16 (m, 5H), 5.78 (s, 1H), 4.47-4.23 (m, 3H), 4.11-4.02 (m, 0.6H), 3.90-3.69 (m, 1.4H), 3.64-3.49 (m, 1H), 3.42-3.16 (m, 2H), 3.10-2.85 (m, 1H), 2.75-2.58 (m, 3H), 2.56-2.42 (m, 1H), 2.33 (s, 3H), 2.31 (s, 3H), 1.58-1.16 (m, 6.4H), 0.68-0.52 (m, 0.6H).

Compound 42: (*R*)-6-((2-(Dimethylamino)ethyl)(methyl)amino)-3-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)pyrimidin-4(3*H*)-one



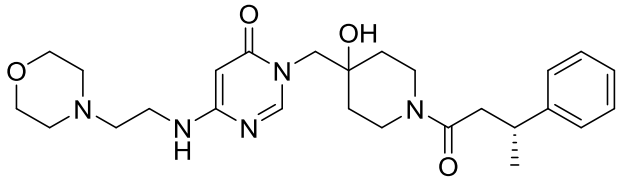
A mixture of (*R*)-6-chloro-3-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)pyrimidin-4(3*H*)-one (25 mg, 64.1 µmol), *N*,*N*,*N*′-trimethylethylenediamine (83 µL, 0.641 mmol) and 1,4-dioxane (0.5 mL) was heated at 150 °C under microwave irradiation for 15 min before the reaction mixture was purified directly by flash chromatography (Biotage KP-NH 11 g cartridge, 0-100% EtOAc in PE, then 0-30% MeOH in EtOAc) to give the title compound (24 mg, 82%) as a pale yellow solid. LCMS (Method A, ES^+): R_T = 0.64 min (purity >96% at 254 nm), *m/z* Calcd for $C_{25}H_{38}N_5O_3$ $[M+H]^+$ 456, found 456. ^{1}H NMR (300 MHz, $CDCl_3$, this molecule appears as conformers in a 2:3 ratio): δ 7.74 (s, 0.4H), 7.63 (s, 0.6H), 7.36-7.15 (m, 5H), 5.38-5.30 (m, 1H), 4.50-4.33 (m, 1H), 4.00-3.17 (m, 8H), 3.08-2.84 (m, 4H), 2.72-2.59 (m, 1H), 2.57-2.40 (m, 3H), 2.28 (d, 6H), 1.62-1.10 (m, 6.4H), 0.63-0.50 (m, 0.6H).

Compound 43: (*R*)-3-((4-Hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-6-((2-methoxyethyl)amino)pyrimidin-4(3*H*)-one



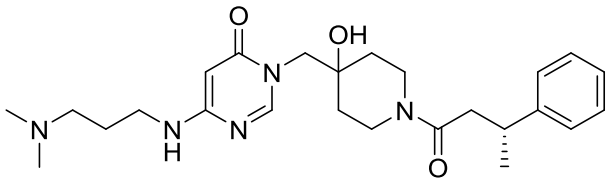
A mixture of (*R*)-6-chloro-3-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)pyrimidin-4(3*H*)-one (25 mg, 64.1 µmol), 2-methoxyethanamine (56 µL, 0.641 mmol) and 1,4-dioxane (0.5 mL) was heated for 15 min at 150 °C under microwave irradiation. The reaction mixture was diluted with brine (15 mL) and extracted with DCM (10 mL) using a Biotage phase separator. The organic layer was concentrated under reduced pressure and the residue was purified by flash chromatography (Biotage KP-NH 11 g cartridge, 0-100% EtOAc in PE, then 0-30% MeOH in EtOAc) to afford the title compound (17 mg, 61%) as colourless solid. LCMS (Method A, ES^+): R_T = 0.85 min (purity >96% at 254 nm), *m/z* Calcd for $C_{23}H_{33}N_4O_4$ $[M+H]^+$ 429, found 429. ^{1}H NMR (500 MHz, DMSO-d_6, this molecule appears as conformers in a 1:1 ratio): δ 7.98 (s, 0.5H), 7.96 (s, 0.5H), 7.31-7.21 (m, 4H), 7.20-7.12 (m, 1H), 6.95 (s, 1H), 5.09 (s, 1H), 4.99 (s, 0.5H), 4.99 (s, 0.5H), 4.03-3.94 (m, 1H), 3.84-3.58 (m, 4H), 3.42 (t, 2H), 3.25 (s, 3H), 3.28-3.12 (m, 3H), 2.93-2.86 (m, 1H), 2.62-2.53 (m, 2H), 1.48-1.22 (m, 4H), 1.20 (d, 3H).

Compound 44: (*R*)-3-((4-Hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-6-((2-morpholinoethyl)amino)pyrimidin-4(3*H*)-one



A mixture of (*R*)-6-chloro-3-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)pyrimidin-4(3*H*)-one (25 mg, 64.1 µmol), 2-morpholinoethan-1-amine (84 µL, 0.641 mmol) and 1,4-dioxane (0.5 mL) was heated for 15 min at 150 °C under microwave irradiation. The reaction mixture was diluted with brine (15 mL) and extracted with DCM (10 mL) using a Biotage phase separator. The organic layer was concentrated under reduced pressure and the residue was purified by flash chromatography (Biotage KP-NH 11 g cartridge, 0-100% EtOAc in PE, then 0-30% MeOH in EtOAc) to afford the title compound (25 mg, 80%) as pale yellow solid. LCMS (Method B, ES^+): R_T = 0.66 min (purity >98% at 254 nm), *m/z* Calcd for $C_{26}H_{38}N_5O_4$ $[M+H]^+$ 484, found 484. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.01-7.93 (m, 1H), 7.31-7.21 (m, 4H), 7.20-7.13 (m, 1H), 6.74 (br s, 1H), 5.12-5.03 (m, 1H), 5.03-4.92 (m, 1H), 4.04-3.92 (m, 1H), 3.85-3.68 (m, 2H), 3.66-3.58 (m, 1H), 3.59-3.52 (m, 4H), 3.28-3.09 (m, 4H), 2.95-2.85 (m, 1H), 2.62-2.53 (m, 2H), 2.46-2.42 (m, 2H), 2.41-2.36 (m, 4H), 1.48-1.22 (m, 4H), 1.20 (d, 3H).

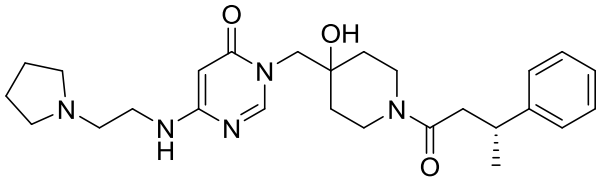
Compound 45: (*R*)-6-((3-(Dimethylamino)propyl)amino)-3-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)pyrimidin-4(3*H*)-one



A mixture of (*R*)-6-chloro-3-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)pyrimidin-4(3*H*)-one (25 mg, 64.1 µmol), N^1,N^1-dimethylpropane-1,3-diamine (81 µL, 0.641 mmol) and 1,4-dioxane (0.5 mL) was heated for 15 min at 150 °C under microwave irradiation. The reaction mixture was diluted with brine (15 mL) and extracted with DCM

(10 mL) using a Biotage phase separator. The organic layer was concentrated under reduced pressure and the residue was purified by flash chromatography (Biotage KP-NH 11 g cartridge, 0-100% EtOAc in PE, then 0-30% MeOH in EtOAc) to afford the title compound (24 mg, 82%) as pale yellow solid. LCMS (Method B, ES^+): R_T = 0.66 min (purity >97% at 254 nm), *m/z* Calcd for $C_{25}H_{38}N_5O_3$ $[M+H]^+$ 456, found 456. ^1H NMR (500 MHz, DMSO-d_6): δ 7.99-7.90 (m, 1H), 7.34-7.19 (m, 4H), 7.20-7.13 (m, 1H), 7.03-6.93 (m, 1H), 5.08-4.93 (m, 2H), 4.03-3.93 (m, 1H), 3.84-3.69 (m, 2H), 3.68-3.58 (m, 1H), 3.26-2.96 (m, 4H), 2.94-2.85 (m, 1H), 2.61-2.52 (m, 2H), 2.27-2.17 (m, 2H), 2.13-2.07 (m, 6H), 1.68-1.57 (m, 2H), 1.55-1.23 (m, 4H), 1.20 (d, 3H).

Compound 46: (*R*)-3-((4-Hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-6-((2-(pyrrolidin-1-yl)ethyl)amino)pyrimidin-4(3*H*)-one



A mixture of (*R*)-6-chloro-3-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)pyrimidin-4(3*H*)-one (25 mg, 64.1 µmol), 2-(pyrrolidin-1-yl)ethanamine (81 µL, 0.641 mmol) and 1,4-dioxane (0.5 mL) was heated at 150 °C under microwave irradiation for 15 min before the reaction mixture was purified directly by flash chromatography (Biotage KP-NH 11 g cartridge, 0-100% EtOAc in PE, then 0-30% MeOH in EtOAc) to give the title compound (22 mg, 73%) as a pale yellow solid. LCMS (Method A, ES+): R_T = 0.61 min (purity >98% at 254 nm), *m/z* Calcd for $C_{26}H_{38}N_5O_3$ $[M+H]^+$ 468, found 468. ^1H NMR (500 MHz, DMSO-d_6): δ 8.06-7.93 (m, 1H), 7.37-7.19 (m, 4H), 7.19-7.13 (m, 1H), 6.79 (br s, 1H), 5.05 (s, 1H), 5.00 (s, 1H), 4.02-3.94 (m, 1H), 3.86-3.68 (m, 2H), 3.66-3.58 (m, 1H), 3.25-3.10 (m, 4H), 2.94-2.86 (m, 1H), 2.65-2.52 (m, 4H), 2.48-2.35 (m, 4H), 1.77-1.61 (m, 4H), 1.50-1.22 (m, 4H), 1.20 (d, 3H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 169.0 + 169.0 (conformers), 161.9, 161.5, 152.2, 146.6 + 146.6 (conformers), 128.2 + 128.2 (conformers), 126.8, 125.9 + 125.9 (conformers), 85.6 (very broad), 69.0 + 68.9 (conformers), 53.5, 52.8, 41.1 + 41.0 (conformers), 40.3, 37.0, 36.1 + 36.0 (conformers), 35.0 + 34.9 (conformers), 34.3 + 34.2 (conformers), 23.1, 22.0 + 21.8 (conformers). HRMS (FTMS ES^+): *m/z* Calcd for $C_{26}H_{38}N_5O_3$ $[M+H]^+$ 468.2969, found 468.2963.

3. USP7 surface plasmon resonance (SPR)

Fragment Library Screening:

SPR fragment library screening was performed by Beactica (Sweden) using Biacore 4000, S51 and T200 instruments (GE Healthcare/Biacore, Uppsala, Sweden). USP7 (His_6-USP7CD (HAUSP cat domain), catalytic domain, aa 213-548, Boston Biochem, Lot # DBCW0111101) was immobilized by amine coupling using materials provided by GE Healthcare. The protein was prepared as a 0.1–0.2 mg/mL solution in coupling buffer (USP7: 50 mM acetate pH 5.5 supplemented with 2 mM DTT, 0.5 mM EDTA) and injected for 5 min over activated surfaces (50 mM N-hydroxysuccinimide, 200 mM N-ethyl-N´-((dimethylamino)propyl)carbodiimide) of CM7 or CM5 chips. The surfaces were subsequently deactivated by 1 M ethanolamine, pH 8.0.

Compound characterization: All interaction experiments were performed at 25 °C. Experiments were conducted in HBS buffer (10 mM HEPES, pH 7.4, 150 mM NaCl), supplemented with 0.05% Pluronic 127, 2 mM DTT, 0.5 mM EDTA and 5 % (v/v) DMSO (all Sigma). The test compounds were diluted in the running buffer in two- or three-fold dilution series up to 300 μM and injected for 15-25 s over the immobilized Target Proteins as well as three Reference Targets (GST (Schistosoma japonicum, Sigma), anti-His antibody (Pierce), serine protease (Sprint™)).

Data analysis: Report points from sensorgrams were extracted 5-15 s after initiation of the association phase. Responses were referenced with responses from unmodified reference surfaces, corrected for bulk shifts arising from differences in DMSO concentrations between samples and running buffer (solvent correction) and blank-referenced. For competition analysis, responses of competitor-Test Compound mixtures were compared to the sum of responses of Test Compound and competitor alone. Competition values were determined as normalised deviation from theoretical responses for independent interaction (additivity of responses). Responses of mixtures 3 × σ lower or higher than predicted responses were classified as potential competitive or synergistic, respectively. For K_D determination, the data was globally fitted to the sum of a Langmuir term and a linear term to compensate for non-specific binding using the Sprint™ evaluation software.

SPR K_D evaluation of compound 1:

SPR K_D evaluations were carried out on a SensíQ Pioneer instrument using COOH5 sensor chips, Qdat™ software and coupling reagents from Pall ForteBio. EDC (1-ethyl 3-(3-dimethylaminopropyl)-carbodiimide hydrochloride), sulfo-NHS (sulfo-N-hydroxysuccinimide), sodium acetate pH4.5 and ethanolamine pH8.9 were from GE Health Sciences. Catalytic domain USP7 was procured from Boston Biochem.

A COOH5 sensor chip was installed in the SensíQ Pioneer system, normalized with air, followed by DMSO, and primed with running buffer (10 mM HEPES, 150 mM NaCl, pH 7.4; HBS). Catalytic domain of USP 7 was immobilized at 25 ºC onto a COOH5 sensor chip using standard amine-coupling methods and HBS as the running buffer. The surface was activated with 0.5 mM EDC and 0.2 mM sulfo-NHS for 5 min, followed by a 12-min injection of USP7 CD at 100 μg/mL in 10 mM sodium acetate, pH 4.5, and finally a 5-min blocking step of 1 M ethanolamine, pH 8.9. This coupling method resulted in a density of 9,000 RU (resonance units) of USP7 on one flow cell of the COOH5 chip. OneStep screening using Taylor dispersion concentration gradient of compound **1** at concentrations in a three-fold dilution series starting at 5 mM were completed at a flow rate of 75 μL/min for 60 s. The response data were processed using Qdat™ software using a reference surface to correct for any bulk refractive index changes and blank injections for double referencing. The binding profiles were fitted globally to a 1:1 interaction model.

4. USP7 NMR-binding studies:

Human USP7 catalytic domain (amino acids 208-555, Crelux GmbH) was delivered in PBS (137 mM NaCl, 2.7 mM KCl, 1 mM NaH_2PO_4/Na_2HPO_4), pH 7.4 at a concentration of 13.9 mg/mL (331 μM). The protein was cloned and expressed in *E. coli* using an *N*-terminal 6-histidine tag. Purification of cell lysate was performed by Ni-NTA column, followed by Superdex size exclusion chromatography (SEC). Purity was determined to be >85% using SDS page.

Reference ^{1}H spectrum for compound **1** was collected at 1 mM concentration, formulated in PBS buffer identical to that the protein was supplied in. The compound tested negative for self-aggregation using the WaterLOGSY pulse sequence with no macroscopically visible precipitate or diminished signals in the proton spectrum. Compound **1** was included in a cocktail of other compounds, including a positive and negative control. The cocktail was comprised of 10 μM USP7 and a total of 6 compounds at a concentration of 200 μM each, resulting in a 1:20 USP7:compound ratio. Each cocktail was subjected to a trio of ^{1}H, ligand-observed experiments, namely saturation transfer difference (STD), Carr-Purcell-Meiboom-Gill (CPMG) T2, and WaterLOGSY experiments.

NMR Hardware and Processing: All experiments were performed in-house on a 500 MHz Bruker AVANCE spectrometer equipped with TCI Cryoprobe, using standard pulse programs included in Bruker's experiment library. Spectra were collected at 25 °C, and processed using Bruker's TopSpin software program.

5. Biochemical and cellular assays:

The USP7 biochemical assay was performed using materials and conditions outlined in reference 1.

Target Engagement Assay in HCT116 Cells

HCT116 cells were treated with vehicle (DMSO) or USP7 inhibitor for 2 h. Following incubation, cells were washed extensively with 1 x PBS and harvested in TE lysis buffer containing 50 mM TRIS-HCl (pH7.4), 150 mM NaCl, 5 mM $MgCl_2$, 0.5 mM EDTA, 0.5% NP40, 10% Glycerol, 2 mM DTT and clarified cell lysates (40 μg) incubated with the ubiquitin-propargylamine probe (Ub-PA; 8 μg/ml final concentration) in assay buffer containing 50 mM TRIS-HCl (pH7.6), 5 mM $MgCl_2$, 250 mM Sucrose, 0.5 mM EDTA, 2 mM DTT for 30 min. The reaction was terminated by the addition of LDS sample buffer (Life Technologies) and heated to 70 ^{o}C. Samples were then analyzed by western blotting using the Cell Signalling anti-USP7 Ab (#4833; 1/1000 dilution). EC_{50} values were determined upon densitometry analysis. Band intensities were quantified using ImageJ software where the upper bands (USP7-Ub) and lower bands (USP7) were calculated as a percentage of the corresponding DMSO controls (-/+ Ub-PA) and values were then normalized to the sum of the lower and upper bands for each concentration.

6. DUB selectivity assay on compound 46:

Selectivity assays were performed against all USPs included in the DUBprofiler™ panel (Ubiquigent Ltd). Screening was performed at a fixed inhibitor concentration of 10 μM. Data generated is displayed as a percentage inhibition of total enzyme activity for each enzyme. Under the conditions of this screen, **46** exhibited an IC_{50} value of 140 ± 22 nM. Data reported as the mean of 2 independent experiments.

7. Protein production, crystallization, data collection and structure determination:

The USP7 catalytic domain (residues 207-560), genetically fused with a C-terminal hexa-histidine tag, was expressed in E.coli. BL21 cells were transformed with the corresponding expression plasmid and grown in Terrific broth (TB) and protein expression induced with 0.25 mM IPTG overnight at 16°C. After harvesting by centrifugation, cell pellets were resuspended in Lysis Buffer (40 mM TRIS-HCl, 500 mM NaCl, 1 mM AEBSF, 2 mM TCEP, 5 mM Imidazole, 0.1% Tween 20, pH 7.5) and lysed by sonication on ice. The soluble fraction was then loaded directly onto an IMAC column (5 mL HisTrap HP) pre-equilibrated with Lysis Buffer and the protein eluted with IMAC Buffer B (40 mM TRIS-HCl, 500 mM NaCl, 1 mM AEBSF, 2 mM TCEP, 300 mM Imidazole, 0.1% Tween 20, pH 7.5). Fractions

containing the desired protein were pooled and buffer exchanged by disalysis (MWCO 8,000-10,000 Da) against anion exchange (AEX) Buffer A (20 mM TRIS-HCl, 30 mM NaCl, 1 mM EDTA, 4 mM DTT, pH 8.0).

The protein was then loaded onto a YMC-BioPro ion exchange column (15 x 120, 7.4 mL) pre-equilibrated with AEX Buffer A and eluted over 30 CV with a gradient of 0-50% AEX Buffer B (20 mM TRIS-HCl, 1M NaCl, 1 mM EDTA, 4 mM DTT, pH 8.0).

Fractions were analyzed by SDS-PAGE and those containing the desired protein were pooled and then further purified by SEC (HighLoad Superdex 75 column) using a running buffer of 10 mM TRIS-HCl, 100 mM NaCl, 4 mM DTT, pH 8. SEC fractions were analyzed by SDS-PAGE and the pure fractions pooled and concentrated (Vivaspin column, MWCO 12KDa) to 5.3 mg / mL as measured by UV A_{280nm}.

Crystals of USP7 in complex with **46** were grown by hanging drop vapour diffusion. USP7 (14.2 mg/ml in 10 mM TRIS-HCl, 100 mM NaCl, 4mM TCEP, pH 8.0) was pre-incubated with an 8.9-fold molar excess of **46** (150 mM in DMSO) for 2 h. 0.7 µl of the protein solution was then mixed with 0.7 µl of reservoir solution containing 100 mM TRIS-HCl (pH 7.75), 200 mM Li_2SO_4, 25% (w/v) PEG4000 and equilibrated at 20°C over 0.4 mL of reservoir solution. Crystals appeared within 4 days.

Diffraction data at 2.2 Å resolution for a USP7/**46** crystal was collected at the ESRF synchrotron radiation source, id30a1, Grenoble. The structure was solved via molecular replacement using the PDB structure 5N9R as a template. Iterative manual modelling in Coot and refinement using REFMAC5 resulted in the final model. 97.5% of backbone torsions for the final model are within the Ramachandran favoured regions, with 2.5% in the allowed regions. The crystallography data collection and refinement statistics are provided in **Supplementary Table 1** below.

Data collection statistics	
Space group	$P2_1$
Unit cell *a*, *b*, *c* (Å) α, β, γ, (°)	 74.9. 67.3, 80.7 90, 105.1, 90
# molecules per au	2
Resolution (Å)	28.22-2.16 (2.28-2.16)
# unique reflections	39952 (5872)
Completeness (%)	95.9 (96.8)
Redundancy	2.4 (2.4)
R_{merge}	0.110 (0.670)
I/σ	6.4 (1.4)
Refinement statistics	
Resolution (Å)	29.95-2.16 (2.22-2.16)
R_{work} / R_{free}	0.202 / 0.262

Supplementary Table 1. Crystallography data collection and refinement statistics for compound 46 and USP7

8. *In vitro* ADME & physicochemical methods:

Kinetic Solubility:

Test compounds (5 μL; 10 mM DMSO stock) were added to 245 μL of PBS buffer pH 7.4 (Dulbecco A) in a Millipore MultiScreen® Solubility Filter plate and mixed at 300 rpm at rt on a plate shaker for 90 min. Meanwhile 5-points calibration curves for each compound were established in a mixture of acetonitrile/PBS buffer (top concentration 200 μM). After filtration and matrix match, the calibration and assay plates were analyzed on a Bioteck Synergy 4 plate reader (240-400 nm). Final concentration of the test compound in the filtrate was calculated using the slope of the calibration curve. Two markers were used as controls: hydrocortisone (high solubility KSol >180 μM) and reserpine (low solubility KSol < 25 μM).

Caco-2 permeability:

Caco-2 permeability measurements were performed at Cyprotex Ltd. Caco-2 cells obtained from the ATCC are used between passage numbers 40-60. Cells are seeded on to Millipore Multiscreen Caco-2 plates at 1 x 10^5 cells/cm^2. They are cultured for 20 days in DMEM and media is changed every two or three days. On day 20 the permeability study is performed.

Hanks Balanced Salt Solution (HBSS) pH 7.4 buffer with 25 mM HEPES and 4.45 mM glucose at 37 °C is used as the medium in the permeability studies. Incubations are carried out in an atmosphere of 5% CO_2 with a relative humidity of 95% at 37 °C.

On day 20, the monolayers are prepared by rinsing both basolateral and apical surfaces twice with HBSS at 37 °C. Cells are then incubated with HBSS in both apical and basolateral compartments for 40 min to stabilise physiological parameters.

HBSS is then removed from the apical compartment and replaced with test compound dosing solutions. The solutions are made by diluting 10 mM test compound in DMSO with HBSS to give a final test compound concentration of 10 μM (final DMSO concentration 1%). The fluorescent integrity marker Lucifer yellow is also included in the dosing solution. Analytical standards are made from dosing solutions. The apical compartment inserts are then placed into 'companion' plates containing fresh HBSS. For basolateral to apical (B-A) permeability determination the experiment is initiated by replacing buffer in the inserts then placing them in companion plates containing dosing solutions. At 120 min, the companion plate is removed and apical and basolateral samples diluted for analysis by LC-MS/MS. Test compound permeability is assessed in duplicate. On each plate compounds of known permeability characteristics are run as controls.

Test and control compounds are quantified by LC-MS/MS cassette analysis using a 5-point calibration with appropriate dilution of the samples. Cyprotex generic analytical conditions are used. The starting concentration (C_0) is determined from the dosing solution and experimental recovery calculated from C_0 and both apical and basolateral compartment concentrations.

The integrity of the monolayers throughout the experiment is checked by monitoring Lucifer yellow permeation using fluorimetric analysis. Lucifer yellow permeation is low if monolayers have not been damaged. If a Lucifer yellow P_{app} value is above QC limits in one individual test compound well, then an n=1 result is reported. If Lucifer yellow P_{app} values are above QC limits in both replicate wells for a test compound, the compound is re-tested. If on repeat, high Lucifer yellow permeation is observed in both wells then toxicity or inherent fluorescence of the test compound is assumed. No further experiments are performed in this instance.

Data Analysis:

The permeability coefficient for each compound (P_{app}) is calculated from the following equation:

$$P_{app} = \left(\frac{dQ/dt}{C_0 \times A}\right)$$

Where dQ/dt is the rate of permeation of the drug across the cells, C_0 is the donor compartment concentration at time zero and A is the area of the cell monolayer. C_0 is obtained from analysis of dosing solution at the start of the experiment. An efflux ratio (ER) is derived as follows:

$$ER = \frac{P_{app(B-A)}}{P_{app(A-B)}}$$

An efflux ratio greater than two shows efflux from the Caco-2 cells, which indicates that the compound may have potential absorption problems *in vivo*.

The apparent permeability ($P_{app\ (A\text{-}B)}$) values of test compounds are compared to those of control compounds, atenolol and propranolol, which have human absorption of approximately 50 and 90% respectively. Talinolol (a known P-gp substrate[2]) is also included as a control compound to assess whether functional P-gp is present in the Caco-2 cell monolayer.

$LogD_{7.4}$:

Test compounds (8 μL; 10 mM DMSO stock) was added to 392 μL of PBS buffer pH 7.4 (Dulbecco A pre-saturated with octanol) and 400 μL of octanol (pre-saturated with PBS buffer). The plate was shaken at rt for 4 h. The layers were allowed to separate before being analyzed by HPLC.

Samples were analyzed on an Agilent 1260 HPLC fitted with a Phenomenex Kinetex XB-C18 100A 2.6 μm 2.1x50 mm column. Mobile phases were water and acetonitrile containing 0.1% formic acid as modifier. The relative drug concentration in each phase was determined by the peak area measurement from LC-UV analysis, UV detection at 254 nm and 210 nm.

Log $D_{7.4}$ is calculated as follows:

$$LogD = Log\left[\frac{\text{peak area of compound in octanol x injection volume of buffer phase}}{\text{peak area of compound in buffer x injection volume of octanol phase}}\right]$$

Three markers were used as controls: caffeine ($LogD_{7.4} \sim 0$), furosemide ($LogD_{7.4} < -0.5$) and reserpine ($LogD_{7.4} > 3$).

Microsomal stability:

Test compounds (final concentration = 1 μM; final DMSO concentration = 0.1%) were incubated in 0.1 M phosphate buffer pH 7.4 with liver microsomes (human, mouse or rat; 0.5 mg of protein/mL) at 37 °C. Reactions were started by addition of NADPH in 0.1 M phosphate buffer pH 7.4 (final concentration 1 mM). 40 μL aliquots were removed at 2, 5, 10, 15, 20, 30, 40 and 50 min. Reactions were quenched in 80 μL of ice-cold methanol containing internal standard. Samples were subsequently frozen overnight then centrifuged at 3500 rpm for 20 min at 4 °C. The supernatants were removed and transferred into analytical plates and analyzed by LC-MS/MS.

LC-MS/MS method: All samples were analyzed on a Waters Acquity I-Class coupled to a Waters Xevo TQD mass spectrometer. A Waters BEH C18 2.1 x 50 mm 1.7 μm column was used and mobile phases were water and methanol containing 0.1 % formic acid as modifier. Analysis was by multiple reaction monitoring and conditions were optimised for each test compound.

Data analyses: From a plot of ln peak area against time, the gradient of the line is determined. Subsequently, half-life and intrinsic clearance are calculated using the equations below:

Eliminated rate constant (k) = (- gradient)

Half-life ($t_{½}$) (min) = $\frac{0.693}{k}$

Intrinsic Clearance (CL_{int})(μL/min/mg protein) = $V\ x\ \frac{0.693}{t½}$

Where V = Incubation volume (μL)/mg of protein

Four markers were used as controls for each assay:

Human Liver Microsomes	Dextromethorphan	Moderate-high CL_{int}
	Quinidine	Low-moderate CL_{int}
	Tolbutamide	Low CL_{int}
	Verapamil	High CL_{int}
Mouse Liver Microsomes	Diphenydramine	High CL_{int}
	Metroprolol	Low-moderate CL_{int}
	Diclofenac	Moderate CL_{int}
	Verapamil	High CL_{int}

9. Pharmacokinetic Profiling of Compounds 46 & 47:

Pharmacokinetics of compounds **46** and **47** were evaluated in healthy male CD-1 mice following a single oral, intravenous or intraperitoneal administration. Dosing solutions for intravenous administration were prepared using DMSO: 20% 2-hydroxypropyl β cyclodextrin (2:98) and administered at a dose level of 1 mg/kg. Dosing solutions for oral administration were prepared using 0.5 % methylcellulose and administered at a dose level of 30 mg/kg. Dosing solutions for intraperitoneal administration were prepared using saline and administered at a dose level of 10 mg/kg. Blood samples were collected up to 24 h post-dose. In-vivo experiments were carried out at Axis BioServices and pharmacokinetic parameters were measured from blood by XenoGesis Ltd.

A total of 27 male CD-1 mice aged 5-8 weeks, weighing approximately 30-35g were used for the study (animals were bred in-house at Axis BioServices). Mice were housed in IVC cages (5 per cage) with individual mice identified by tail mark. The holding room was maintained under standard conditions: 20-24 °C, 40-70 % humidity and a 12 h light/dark cycle. Animals were fed a standard certified commercial laboratory rodent diet except for overnight fasting immediately prior to dosing; animals were allowed access to food 2 hours after test compound administration. Animals were allowed free access to water at all times during the study.

Dosing solutions were freshly prepared as follows:

For IV dosing solutions:

1.0 mg compound **46** was weighed and mixed with DMSO (40 μL). 1960 μL of 20% 2-hydroxypropyl β cyclodextrin was added to give a final concentration of 0.5 mg/mL.

1.3 mg compound **47** was weighed and mixed with DMSO (52 μL). 2548 μL of 20% 2-hydroxypropyl β cyclodextrin was added to give a final concentration of 0.5 mg/mL.

For PO dosing solutions:

12.2 mg compound **46** was weighed and mixed with 1mL 0.5% methylcelluose with a pestle and mortar. A further 1.03 mL 0.5% methylcellulose was used to completely wash any compound into the tube, giving a final concentration of 6 mg/mL.

15.2 mg compound **47** was weighed and mixed with 1mL 0.5% methylcelluose with a pestle and mortar. A further 1.533 mL 0.5% methylcellulose was used to completely wash any compound into the tube, giving a final concentration of 6 mg/mL.

For IP dosing solutions:

4.5 mg compound **46** was weighed and mixed with 1mL sterile saline with a pestle and mortar. A further 3.5 mL saline was added to completely wash any compound into the tube, giving a final concentration of 1 mg/mL.

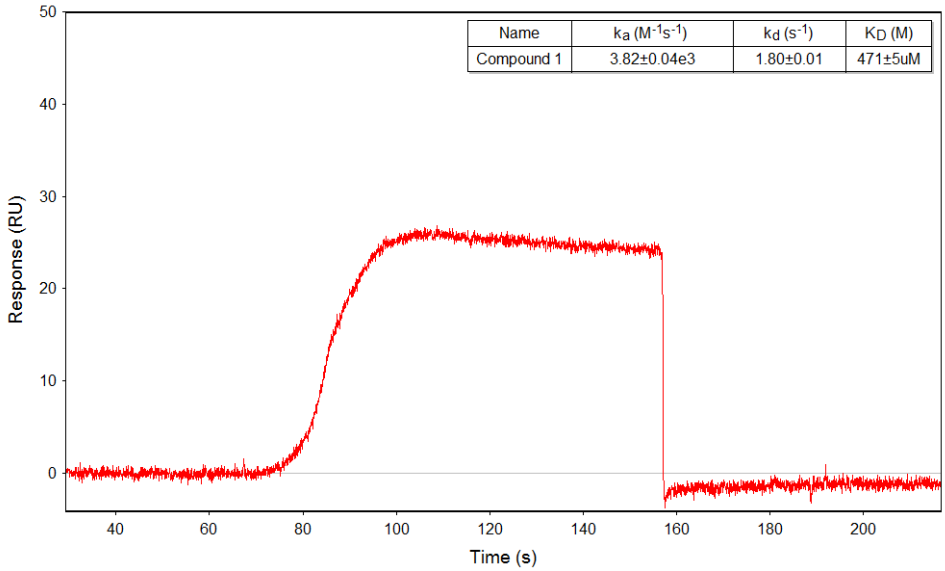
3.8 mg compound **47** was weighed and mixed with 1mL sterile saline with a pestle and mortar. A further 2.8 mL saline was added to completely wash any compound into the tube, giving a final concentration of 1 mg/mL.

The dosing volumes were 2 mL/kg for IV dosing, 5 mL/kg for PO dosing and 10 mL/kg for IP dosing with individual dose calculated from the bodyweight recorded on the day of dosing. At the required time points 100 uL whole blood was removed from the lateral vein into tubes coated with K_2-EDTA. Blood samples were diluted 1:1 with ultrapure water and stored at -80°C before being transported to XenoGesis Ltd on dry ice for bioanalysis. The time points for blood sampling were 5min, 15min, 30min, 1h, 2h, 4h, 8h and 24h for the IV and IP routes. They were 15min, 30min, 1h, 2h, 4h, 6h, 8h and 24h for the PO route. PK parameters were calculated using Phoenix WinNonlin software.

10. Computational Chemistry:

Docking studies and images were carried out or created using Molecular Operating Environment (MOE, 2015.1001 or 2016.0802; Chemical Computing Group ULC, 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7 (2017)) and/or Maestro (Schrödinger Release 2016-4: Maestro, Schrödinger LLC, New York, NY (2016).

11. Supporting Figure S1: USP7 SPR sensogram of compound **1**:



12. Supporting Figure S2: USP7 NMR spectra of compound **1**:

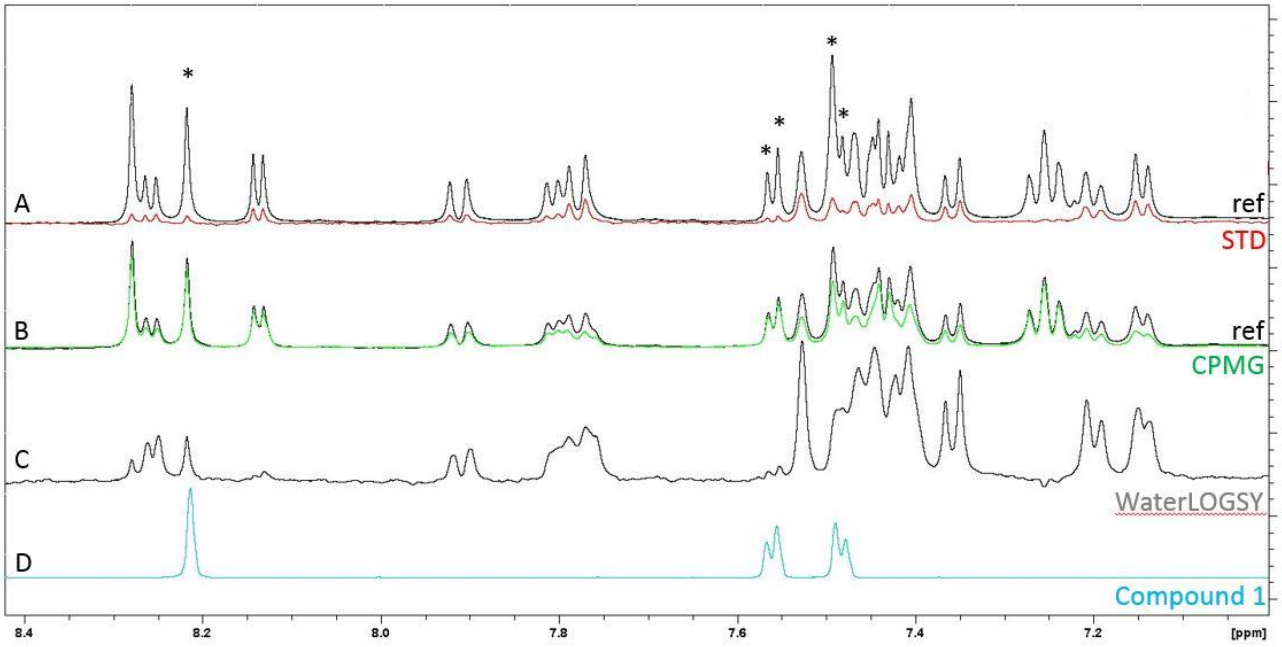
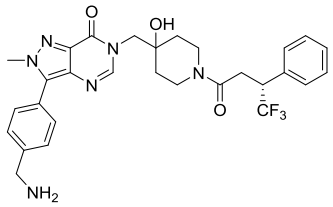


Figure S2: A-C Aromatic region expansion of 1D ^{1}H NMR spectra illustrating binding of compound **1** to USP7 in cocktail containing 10 μM USP7, 200 μM compound **1** in PBS pH 7.4, collected at 25 °C. **A.** Cocktail reference (black trace) and saturation transfer difference spectra (STD, red trace) illustrating positive STD signal of compound **1**, indicated by asterisks. **B.** Reference (black trace) and T2 Carr-Purcell-Meiboom-Gill (CPMG, green trace) spectra illustrating line broadening of signals of compound **1**. **C.** WaterLOGSY spectrum (black trace) of cocktail illustrating binding of compound **1**. **D.** Aromatic region expansion of reference 1D ^{1}H NMR spectrum (blue trace) of 1 mM compound **1** in PBS pH 7.4.

13. Supporting Figure S3: Structure of trifluromethyl analogue **47**:



References:

1. Gavory, G.; O'Dowd, C. R.; Helm, M. D.; Flasz, J.; Arkoudis, E.; Dossang, A.; Hughes, C.; Cassidy, E.; McClelland, K.; Odrzywol, E.; Page, N.; Barker, O.; Miel, H.; Harrison, T. Discovery and Characterisation of Highly Potent and Selective Allosteric Inhibitors of USP7. *Nat. Chem. Biol.* **2018**, *14*, 118-125.