Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supporting Information for:

Identification of pyrazolo-pyrimidinones as GHS-R1a antagonists and inverse agonists for the treatment of obesity

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Contents:

Assessment of quinazolinone 2 (YIL-870) in 45% Fat Fed Diet Induced Obese (DIO) GHS-R1a KO mice.

All *in vivo* experiments were conducted in compliance with the relevant laws and institutional guidelines.

Compound Formulated in 1% Pluronic F127 (BASF) in de-ionised water
Formulated every 7 d
Test System: Diet Induced Obese Mouse
Species: C57Bl6 background, GHS-R1a Transgenic (KO), 12 GHS-R1a Wild Type (WT)
No. / Sex: 24 males (12 GHS-R1a Transgenic (KO), 12 GHS-R1a Wild Type (WT)

EXPERIMENTAL PROTOCOL
24 Transgenic mice were secured, housed with their littermates (mixed KO and WT animals) and fed a standard chow diet (SDS Rat and Mouse No. 1 Maintenance (2.61 kcal/g of ration, or 9.2% kcal of fat in ration) up to 7 weeks of age at Alderley Park Breeding facility. At 7 weeks of age all animals are transferred to a high fat diet (Research diets D12451) and fed libitum. Mice are transferred to the experimental facility after 12 weeks of High Fat Diet (19 weeks of age), singly housed in a Scanbur Scantainer and allowed to acclimatise to a 9-21:00 reverse day night cycle for at least 4 weeks. Animals are considered ready for study when their average bodyweight is more than or equal to 35 g. This is typically 16 weeks on high fat diet. After 4 weeks acclimatisation and target bodyweight achieved, animals were randomised into treatment groups according to body weight 1 day prior to the start of the study. All animals were dosed via oral gavage at a volume of 5 mL/kg once daily, depending on treatment group 1 h prior to entering the dark (8am). Compound was formulated in 1% Pluronic F127 (BASF) in de-ionised water every 7 d. Body weight is measured also at this time.

Treatment groups are as follows:
WT Vehicle (Pluronic F127)
WT **YIL-870** at 10 mg/kg
KO Vehicle (Pluronic F127)
KO **YIL-870** at 10 mg/kg

RESULTS:

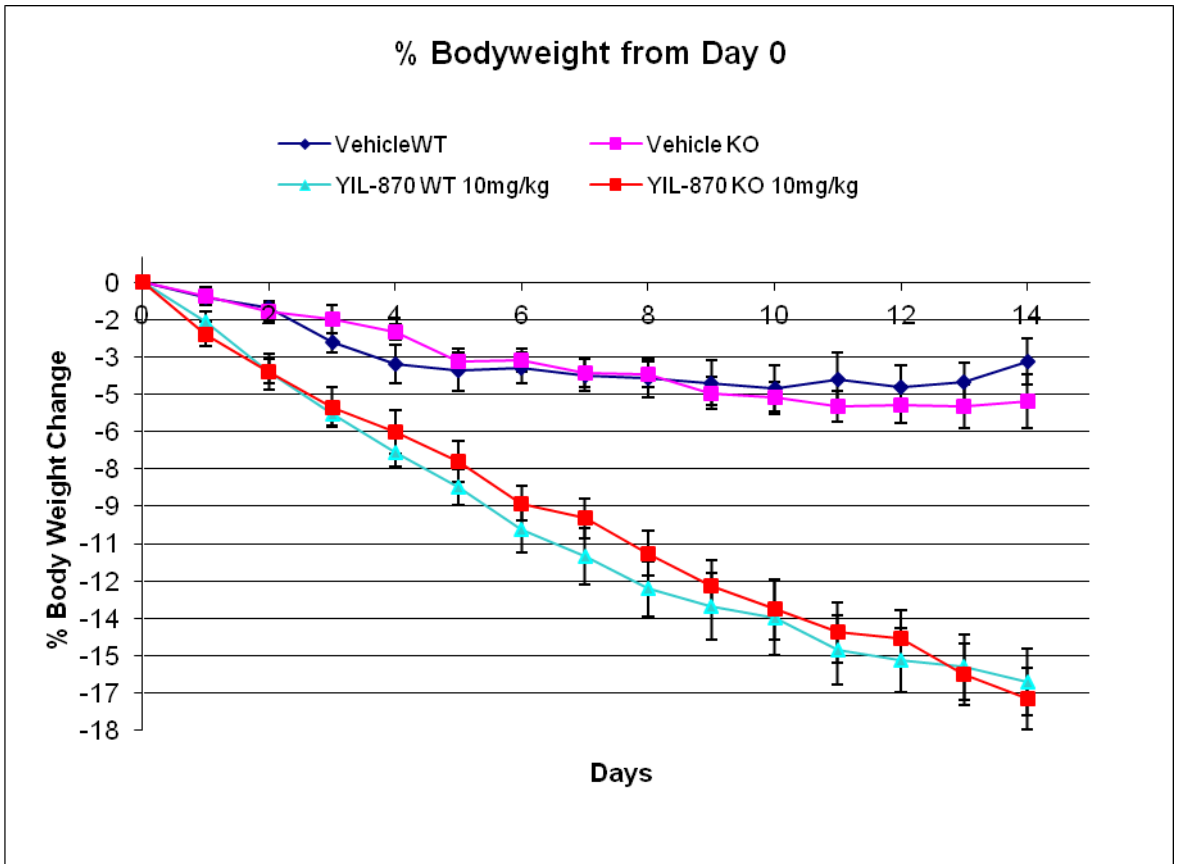


Figure 1: Percentage change in bodyweight from the start of dosing

Figure 1 shows no target specific effect with **YIL-870** as both GHS-R1a KO and Wild type animals show similar absolute bodyweight effects and when compared to their equivalent vehicle controls. Absolute bodyweight loss is 16%

Preparation of compounds:

All solvents and chemicals used were reagent grade. Anhydrous solvents tetrahydrofuran (THF), benzene, 1,2-dichloroethane (DCE), dichloromethane (DCM) and dimethoxyethane (DME) were purchased from Aldrich. Flash column chromatography was carried out using prepacked silica cartridges (from 4 g up to 330 g) from Redisep™, Biotage or Crawford and eluted using an Isco Companion system. Purity and characterization of compounds were established by a combination of liquid chromatography-mass spectroscopy (LC-MS), gas chromatography-mass spectroscopy (GC-MS) and NMR analytical techniques and was >95% for all test compounds. ^{1}H NMR were recorded on a Varian INOVA (600 MHz), Varian Gemini 2000 (300 MHz) or Bruker Avance DPX400 (400 MHz) and were determined in $CDCl_3$ or DMSO-d_6. Chemical shifts are reported in ppm relative to tetramethylsilane (TMS) (0.00 ppm) or solvent peaks as the internal reference. Splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad peak. Elevated temperatures were used where necessary to sharpen broad NMR peaks due to rotamers and the temperature used is noted for such compounds. Merck precoated thin layer chromatography (TLC) plates (silica gel 60 F_{254}, 0.25 mm, art. 5715) were used for TLC analysis. Solutions were dried over anhydrous magnesium sulfate and the solvent was removed by rotary evaporation under reduced pressure. Abbreviations used for preparative HPLC solvents : acetonitrile, MeCN; formic acid, FA.

Methyl 4-amino-2-(4-chlorophenyl)thiazole-5-carboxylate (5)
Methyl 4-amino-2-(methylthio)thiazole-5-carboxylate (1.25 g, 6.12 mmol), 4-chlorophenylboronic acid (1.05 g, 6.73 mmol), copper(I) 2-hydroxy-3-methylbenzoate (1.97 g, 9.18 mmol) and tetrakis(triphenylphosphine)palladium (0.283 g, 0.24 mmol) were added to a round bottom flask. The flask was sealed and degassed several times before adding THF (10 mL). The suspension was again degassed a couple of times. The reaction was heated thermally to 50 °C for 72 h. The reaction mixture was evaporated to dryness, redissolved in EtOAc (100 mL), and washed sequentially with sat. $NaHCO_3$ (3 x 10 mL), water (10 mL), and sat. brine (10 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, 30% EtOAc in heptane. Pure fractions were evaporated to dryness to afford the pure product as a yellow solid. More impure fractions containing the product were collected. They were evaporated under reduced pressure and purified by preparative HPLC on a Kromasil C8 column (10 µm 250 x 50 ID mm) using a gradient of 25-75% acetonitrile in H_2O/MeCN/FA 95/5/0.2 buffer over 22 min with a flow of 100 mL/min. The compounds were detected by UV at 256 nm. All pure fractions were mixed and freeze dried to give the title compound (1.13 g, 69%) as a yellow solid.
m/z (ES+) $[M+H]^+$ = 269;
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.75 (s, 3H), 7.09 (s, 2H), 7.57 (d, 2H), 7.91 (d, 2H).

N-((1-(2-Methoxyethyl)piperidin-3-yl)methyl)-2-methylbenzamide (6)
2-Methylbenzoyl chloride (0.479 mL, 3.67 mmol) dissolved in DCM (10 mL) was added to a solution of (1-(2-methoxyethyl)piperidin-3-yl)methanamine (250 mg, 1.45 mmol) and Et_3N (0.405 mL, 2.90 mmol) in DCM (10 mL). The reaction was stirred overnight at rt. Subsequently the reaction was quenched by addition of K_2CO_3 (aq) (1 M, 25 mL) and the aqueous phase was extracted with DCM. The organic phase was poured on a Rxn CX strong cation exchange column (5 g). The column was washed with THF followed by MeOH, then was eluted with NH_3 saturated MeOH and evaporated to yield the title compound (1.3 g, 78%) as an oil.

m/z (ES+) $[M+H]^+$ = 292.

6-(4-Chlorophenyl)-3-{[(3*R*)-1-(2-methoxyethyl)piperidin-3-yl]methyl}-2-(2-methylphenyl)thieno[3,2-*d*]pyrimidin-4(3*H*)-one (7)

A solution of ethyl 3-amino-5-(4-chlorophenyl)thiophene-2-carboxylate (104 mg, 0.37 mmol), N-((1-(2-methoxyethyl)piperidin-3-yl)methyl)-2-methylbenzamide (107 mg, 0.37 mmol) and phosphoryl trichloride (0.103 mL, 1.11 mmol) in DCE (10 mL) was heated under reflux overnight. The reaction mixture was worked-up by adding K_2CO_3 (1 M, 25 mL) and extracting with DCM. The organic phases were pooled and evaporated. The resulting ethyl 5-(4-chlorophenyl)-3-{[({[1-(2-methoxyethyl)piperidin-3-yl]methyl}amino)(2-methylphenyl)methylidene]amino}thiophene-2-carboxylate was dissolved in DCE (5 mL). Titanium(IV) chloride (0.162 mL, 1.47 mmol) was added and the reaction mixture was heated in a microwave single-node oven to 170 °C for 10 min. The reaction solution was added to MeOH/DCM and filtered. K_2CO_3 (1 M, 25 mL) was added and the aqueous phase was extracted with DCM. The organic phase was poured on a SCX strong cation exchange column (5 g). The column was washed with DCM followed by MeOH, then eluted with NH_3 saturated MeOH and evaporated. Purification by preparative HPLC, instrument: FractionLynx II, mobilphase: gradient 5-95% MeCN in 0.2% NH_3, pH 10, column: Xbridge Prep C18 5 µm OBD 19x150 mm afforded the title compound (113 mg, 60%).
m/z (ES+) $[M+H]^+$ = 508;
HRMS calculated for $C_{28}H_{31}O_2N_3ClS$ $[M+H]^+$ 508.18200, found 508.18234;
^{1}H NMR (600 MHz, DMSO-d_6) δ 0.73 (d, 1H), 1.17 (d, 2H), 1.45 (dd, 3H), 1.71 (s, 1H), 1.82 (s, 1H), 2.13 (s, 3H), 2.24 - 2.33 (m, 2H), 3.11 (d, 3H), 3.20 - 3.30 (m, 2H), 3.49 (s, 2H), 3.95 (s, 1H), 7.29 - 7.38 (m, 2H), 7.40 - 7.49 (m, 2H), 7.56 (t, 2H), 7.80 - 7.90 (m, 3H).

2-(4-Chlorophenyl)-6-{[(3*R*)-1-(2-methoxyethyl)piperidin-3-yl]methyl}-5-(2-methylphenyl)[1,3]thiazolo[4,5-*d*]pyrimidin-7(6*H*)-one (8)

A solution of methyl 4-amino-2-(4-chlorophenyl)thiazole-5-carboxylate **5** (100 mg, 0.37 mmol), N-((1-(2-methoxyethyl)piperidin-3-yl)methyl)-2-methylbenzamide (162 mg, 0.56 mmol) and phosphoryl trichloride (0.104 mL, 1.12 mmol) in DCE (10 mL) was heated to 85 °C overnight. The crude was worked-up by addition of K_2CO_3 (1 M, 25 mL) and extraction with DCM. The organic phases were pooled and evaporated. m/z (ES+) $[M+H]^+$ = 542. Methyl 2-(4-chlorophenyl)-4-(((1-(2-methoxyethyl)piperidin-3-yl)methylamino)(o-tolyl)methyleneamino)thiazole-5-carboxylate (201 mg, 0.37 mmol) was dissolved in DCE (5 mL). Titanium(IV) chloride (0.163 mL, 1.49 mmol) was added and the reaction mixture heated in a microwave single-node oven to 170°C for 40 min. The reaction solution was added to MeOH/DCM and filtered. K_2CO_3 (1 M, 25 mL) was added and the aqueous phase was extracted with DCM. The organic phase was poured on an SCX-2 strong cation exchange column (5 g). The column was washed with DCM followed by MeOH, then eluted with NH_3 saturated MeOH and evaporated. Purification by preparative HPLC, instrument: FractionLynx II, mobilphase: gradient 5-95% MeCN in 0.2% NH_3, pH 10, column: Xbridge Prep C18 5 µm OBD 19x150 mm afforded the title compound (30.4 mg, 95%).
m/z (ES+) $[M+H]^+$ = 509;
HRMS calculated for $C_{27}H_{30}O_2N_4ClS$ $[M+H]^+$ 509.17725, found 509.17737;
^{1}H NMR (600 MHz, DMSO-d_6) δ 0.65 - 0.92 (m, 1H), 1.16 - 1.66 (m, 6H), 1.80 (d, 3H), 2.21 (d, 3H), 2.27 - 2.38 (m, 2H), 3.15 (d, 3H), 3.59 (s, 1H), 3.92 - 4.09 (m, 1H), 7.35 - 7.45 (m, 2H), 7.47 - 7.56 (m, 2H), 7.70 (d, 2H), 8.17 (d, 2H).

Methyl 4-nitro-1H-pyrazole-3-carboxylate

Sulfuric acid (95-98%) (1 mL, 31.8 mmol) was added to a stirred suspension of 4-nitro-1H-pyrazole-3-carboxylic acid (5 g, 31.8 mmol) in MeOH (50 mL) at rt. The mixture was stirred for 18 h then a further 1 mL sulfuric acid was added. The reaction was stirred for a further 1 h then concentrated and re-dissolved in EtOAc (200 mL). The solution was neutralised with a sat. solution of $NaHCO_3$ and the organic layer was dried over $MgSO_4$, filtered and concentrated to provide methyl 4-nitro-1H-pyrazole-3-carboxylate (4.39 g, 81%) as a colourless crystalline solid.
m/z (ES-) $(M-H)^-$ = 170;
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.88 (3H, s), 8.91 (1H, s), 14.5 (1H, bs)

Methyl 1-(3-methoxyphenyl)-4-nitro-1H-pyrazole-3-carboxylate (10, Ar=3-MeO-Ph)
Copper(II) acetate (4.78 g, 26.3 mmol) was added to a stirred suspension of methyl 4-nitro-1H-pyrazole-3-carboxylate (3.00 g, 17.5 mmol), 3-methoxyphenylboronic acid (5.33 g, 35.1 mmol) and pyridine (2.84 mL, 35.1 mmol) in DCM (50 mL) at rt. Air was passed through the mixture for 18 h. Silica (~40 g) was added to the reaction mixture and it was concentrated to dryness. The material was purified by flash silica chromatography in DCM. Pure fractions were evaporated to dryness to afford methyl 1-(3-methoxyphenyl)-4-nitro-1H-pyrazole-3-carboxylate (3.34 g, 69%) as a colourless crystalline solid. ^{1}H NMR indicated that there was approx. 25:1 ratio desired product: undesired regioisomer present.
m/z (ES+) $(M+H)^+$ = 278;
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.85 (3H, s), 3.94 (3H, s), 7.04 - 7.07 (1H, m), 7.46 - 7.49 (1H, m), 7.50 - 7.53 (2H, m), 9.75 (1H, s)

Methyl 4-amino-1-(3-methoxyphenyl)-1H-pyrazole-3-carboxylate (11, Ar=3-MeO-Ph)
c.HCl (15 drops) was added to a suspension of methyl 1-(3-methoxyphenyl)-4-nitro-1H-pyrazole-3-carboxylate (3.43 g, 12.4 mmol) and iron (3.45 g, 61.8 mmol) in EtOH (30 mL) and water (30 mL). The mixture was heated at 100 °C for 40 min. After cooling the mixture, it was filtered and washed with EtOAc (50 mL). The mixture was neutralised by the addition of sat. $NaHCO_3$ (50 mL) and water (100 mL). The organic layer was separated and the aqueous phase was extracted with EtOAc (3 x 100 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated to provide methyl 4-amino-1-(3-methoxyphenyl)-1H-pyrazole-3-carboxylate (2.49 g, 81%) as a lilac solid.
m/z (ES+) $[M+H]^+$ = 248;
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.83 (6H, s), 4.87 (2H, s), 6.89 - 6.92 (1H, m), 7.31 - 7.36 (2H, m), 7.38 (1H, d), 7.85 (1H, s)

Methyl 4-amino-1-phenyl-1H-pyrazole-3-carboxylate (11, Ar=Ph)
Methyl 1-(4-chlorophenyl)-4-nitro-1H-pyrazole-3-carboxylate (10.2 g, 36.2 mmol) and palladium (10% on carbon, wet) (1.5 g, 1.41 mmol) in MeOH (300 mL) were stirred under an atmosphere of hydrogen at 1 atm and rt for 40 h. The reaction mixture was filtered through celite and the filtrate was evaporated *in vacuo* to yield crude product. The product was partitioned between EtOAc (300 mL) and sat $NaHCO_3$ (300 mL). The layers were separated and the aqueous layer was extracted with EtOAc (100 mL). The combined organic layers were washed with sat. brine (100 mL), dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 20 to 30% EtOAc in DCM. Pure fractions were evaporated to dryness to afford methyl 4-amino-1-phenyl-1H-pyrazole-3-carboxylate (6.61 g, 84%) as a brown oil which solidified on standing.
m/z (ES+) $[2M+Na]^+$ = 457;

^{1}H NMR (400 MHz, DMSO-d_6) δ 3.83 (3H, s), 4.88 (2H, s), 7.31 - 7.35 (1H, m), 7.46 - 7.51 (2H, m), 7.76 - 7.79 (2H, m), 7.83 (1H, s)

Methyl 1-(4-methoxyphenyl)-4-nitro-1H-pyrazole-3-carboxylate (10, Ar=4-MeO-Ph)
Pyridine (9.85 mL, 116 mmol) was added to methyl 4-nitro-1H-pyrazole-3-carboxylate (9.91 g, 57.9 mmol), 4-methoxyphenylboronic acid (17.6 g, 116 mmol) and copper(II) acetate (15.8 g, 86.9 mmol) in DCM (222 mL) at rt under air. The resulting suspension was stirred for 16 h. The crude product was purified by flash silica chromatography, elution gradient 0 to 100% EtOAc in DCM. Pure fractions were evaporated to dryness to afford crude material. The crude product was purified by crystallisation from Et_2O to afford methyl 1-(4-methoxyphenyl)-4-nitro-1H-pyrazole-3-carboxylate (3.57 g, 22%) as a pale yellow crystalline solid.
m/z (ES+) $(M\text{-}OMe)^+$ = 246;
^{1}H NMR (400 MHz, $CDCl_3$, 30 °C) δ 3.87 (3H, s), 4.02 (3H, s), 6.85 - 7.08 (2H, m), 7.45 - 7.77 (2H, m), 8.51 (1H, s).

Methyl 4-amino-1-(4-methoxyphenyl)-1H-pyrazole-3-carboxylate (11, Ar=4-MeO-Ph)
Methyl 1-(4-methoxyphenyl)-4-nitro-1H-pyrazole-3-carboxylate (3.57 g, 12.9 mmol) and palladium (10% on carbon, wet type) (0.356 g, 0.33 mmol) in MeOH (120 mL) were stirred under an atmosphere of hydrogen at 1 atm at 22 °C for 2 h. The reaction mixture was filtered through celite, washed with MeOH (3 x 50 mL) and evaporated to dryness. The crude product was purified by flash silica chromatography, elution gradient 30 to 60% EtOAc in isohexane. Pure fractions were evaporated to dryness to afford methyl 4-amino-1-(4-methoxyphenyl)-1H-pyrazole-3-carboxylate (0.745 g, 23%) as an orange crystalline solid.
m/z (ES+) $[M+H]^+$ = 248;
^{1}H NMR (400 MHz, DMSO-d_6, 30 °C) δ 3.85 (3H, s), 3.87 (3H, s), 4.90 (2H, br), 7.05 - 7.12 (2H, m), 7.71 - 7.78 (2H, m), 7.77 - 7.81 (1H, m)

Methyl 1-(4-fluorophenyl)-4-nitro-1H-pyrazole-3-carboxylate (10, Ar=4-F-Ph)
Methyl 4-nitro-1H-pyrazole-3-carboxylate (1 g, 5.84 mmol), 4-fluorophenylboronic acid (1.64 g, 11.7 mmol) and 1.59 g of copper(II) acetate were suspended in 12 mL DCM. Pyridine (0.994 mL, 11.7 mmol) was added and the agitation was maintained at rt under air. The reaction was stopped after 70 h by diluting the crude in 50 mL DCM and directly adsorbing it on 10 mL SiO_2 60-200Å. The crude was then purified by flash chomatography on silica (9.5x4.5cm) using 20% EtOAc in heptane as eluent. Purest fraction were concentrated to give methyl 1-(4-fluorophenyl)-4-nitro-1H-pyrazole-3-carboxylate (1.21 g, 78%) as a white solid.
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.92 (s, 3H), 7.28 - 7.53 (m, 2H), 7.78 - 8.12 (m, 2H), 9.69 (s, 1H).

Methyl 4-amino-1-(4-fluorophenyl)-1H-pyrazole-3-carboxylate (10, Ar=4-F-Ph)
Methyl 1-(4-fluorophenyl)-4-nitro-1H-pyrazole-3-carboxylate (1.18 g, 4.47 mmol) was dissolved in 20 mL abs. EtOH. 20 mL water were added followed by iron (0.998 g, 17.9 mmol) and finally c. HCl (5 drops). The crude mixture was then agitated 15 min at 100 °C. The crude mixture was then cooled to rt and filtered over paper. The resulting solution was evaporated to dryness and retaken in 200 mL EtOAc/$NaHCO_3$ 1M : 1/1 (v/v). The aqueous phase was reextracted twice with EtOAc (2 x 50 mL) and the combined organic phases were washed with brine (100 mL), dried over $MgSO_4$, filtrated and concentrated. The crude was retaken in 5 mL DCM and purified using a prepacked 10 g NH_2 SPE column conditionned in MeOH. The product was eluted with pure DCM (100 mL) and evaporation yielded methyl 4-amino-1-(4-fluorophenyl)-1H-pyrazole-3-carboxylate (0.92 g, 88%) as a solid.
m/z (ES+) $[M+H]^+$ = 236;

^{1}H NMR (600 MHz, DMSO-d_6) δ 3.78 (s, 3H), 4.86 (d, 2H), 7.27 - 7.33 (m, 2H), 7.71 - 7.90 (m, 3H).

Methyl 1-(3-chlorophenyl)-4-nitro-1H-pyrazole-3-carboxylate (10, Ar=3-Cl-Ph)
Procedure as for **10** (Ar=4-F-Ph)
Methyl 1-(3-chlorophenyl)-4-nitro-1H-pyrazole-3-carboxylate (0.98 g, 59%) isolated as a white solid.
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.05 (s, 3H), 7.43 - 7.53 (m, 2H), 7.60 - 7.66 (m, 1H), 7.81 (t, 1H), 8.63 (s, 1H).

Methyl 4-amino-1-(3-chlorophenyl)-1H-pyrazole-3-carboxylate (11, Ar=3-Cl-Ph)
Procedure as for **11 (**Ar=4-F-Ph)
Methyl 4-amino-1-(3-chlorophenyl)-1H-pyrazole-3-carboxylate (0.8 g, 87%) isolated as yellow solid.
m/z (ES+) $(M+H)^+$ = 252;
^{1}H NMR (600 MHz, DMSO-d_6) d 3.80 (s, 3H), 4.90 (d, 2H), 7.36 (d, 1H), 7.48 (t, 1H), 7.75 (d, 1H), 7.85 (s, 1H), 7.88 (s, 1H).

Methyl 1-(3-cyanophenyl)-4-nitro-1H-pyrazole-3-carboxylate (10, Ar=3-CN-Ph)

Procedure as for **10** (Ar=4-F-Ph)
Methyl 1-(3-cyanophenyl)-4-nitro-1H-pyrazole-3-carboxylate (0.62 g, 39%).
m/z (ES-) $(M-H)^-$ = 271;
^{1}H NMR (400MHz, DMSO-d_6) δ 3.94 (s, 3H), 7.80 (t, 1H), 7.97 (m, 1H), 8.28 (ddd, 1H), 8.48 (m, 1H), 9.82 (s, 1H).

Methyl 4-amino-1-(3-cyanophenyl)-1H-pyrazole-3-carboxylate (11, Ar=3-CN-Ph)
Procedure as for **11 (**Ar=4-F-Ph)
Methyl 4-amino-1-(3-cyanophenyl)-1H-pyrazole-3-carboxylate was isolated as mixture of amine and N-hydroxylamine (1/1, 540 mg) and was used as such in the next step.
m/z (ES+) $(M+H)^+$ = 243

Ethyl 5-(4-chlorophenyl)-3-(2-methylbenzamido)thiophene-2-carboxylate
2-Methylbenzoic acid (340 mg, 2.5 mmol) was dissolved in toluene (8 mL) under nitrogen. Oxalyl dichloride (666 µl, 7.5 mmol) was added followed by 2 drops of DMF. The reaction was then agitated for 1 h at 120 °C. Volatiles were evaporated and the crude material co-evaporated twice with toluene (50 mL). The resulting orange oil was briefly dried under vacuum and dissolved in 20 mL DCM. Ethyl 3-amino-5-(4-chlorophenyl)thiophene-2-carboxylate (564 mg, 2 mmol) was added as a solid in one portion. Immediate precipitation occured and the reaction became clear again upon addition of triethylamine (416 µl, 3 mmol) addition. The agitation was maintained at rt for 18 h. Volatiles were then evaporated and the crude retaken in 100 mL EtOAc and washed sequentially with water (100 mL), aq. NaOH 1N (100 mL), aq. HCl 0.1N (100 mL) and brine (100 mL). The crude product was dried over $MgSO_4$, filtrated and adsorbed on 20 mL SiO_2 60-200Å and purified by flash chromatography on silica using 15% EtOAc in heptane as eluent. The purest fractions were evaporated to yield ethyl 5-(4-chlorophenyl)-3-(2-methylbenzamido)thiophene-2-carboxylate (472 mg, 59%) as a white solid.
m/z (ES+) $(M+H)^+$ = 400;
^{1}H NMR (600 MHz, $CDCl_3$) δ 1.40 (t, 3H), 2.59 (s, 3H), 4.37 (q, 2H), 7.31 (t, 2H), 7.41 (t, 3H), 7.64 (d, 1H), 7.67 (d, 2H), 8.56 (s, 1H), 10.65 (s, 1H).

6-(4-Chlorophenyl)-3-((1-isopropylpiperidin-3-yl)methyl)-2-o-tolylthieno[3,2-d]pyrimidin-4(3H)-one (23)

(1-isoPropylpiperidin-3-yl)methanamine (117 mg, 0.75 mmol) was dissolved in DCE (3 mL). Trimethylaluminum (2M in toluene) (0.385 mL, 0.77 mmol) was added under nitrogen and agitation maintained at rt for 25 min. Ethyl 5-(4-chlorophenyl)-3-(2-methylbenzamido)thiophene-2-carboxylate (100 mg, 0.25 mmol) in DCE (4 mL) was added under nitrogen and the resulting mixture stirred for 5 min at rt before the reaction was placed in preheated oil bath at 100 °C. The reaction was stirred for 4 hours during which time no ring closure was observed. The reaction was diluted in EtOAc (50 mL) and washed with water (50 mL+5 mL NaOH 1N to help the phase separation) and brine (50 mL). The crude was dried over $MgSO_4$ and concentrated to a yellowish oil which was retaken in DCE (5 mL). Triethylamine (2.08 mL, 15mmol) was added under N_2 followed TMSCl (635 µl, 5 mmol). The resulting mixture was stirred at 80 °C for 18 h and 110 °C for 4.5 h. The mixture was then diluted in EtOAc/water (1/1 : v/v, (100 mL)). The aqueous phase was basified to pH14 using NaOH 1N (10 mL) and re-extracted with EtOAc (30 mL). The combined organic phases were washed with brine (30 mL), dried over $MgSO_4$, filtrated, concentrated and purified by preparative HPLC on a Kromasil C8 column (10 µm 250 x 50 ID mm) using a gradient of 15 to 75% Acetonitrile in H_2O/MeCN/FA 95/5/0.2 buffer over 30 min with a flow of 100 mL/min. The compounds were detected by UV at 270nm. Freeze drying of purest fractions yielded the title compound (23 mg, 17%) as a white solid.

m/z (ES+) $[M+H]^+$ = 492;

HRMS calculated for $C_{28}H_{31}ON_6$ $[M+H]^+$ 492.1871, found 467.1844

^{1}H NMR (600 MHz, DMSO-d_6) δ 0.76 - 0.92 (m, 6H), 1.21 (s, 1H), 1.35 (s, 1H), 1.44 (s, 1H), 1.61 (s, 1H), 1.76 (s, 2H), 1.99 (d, 1H), 2.13 (d, 3H), 2.35 (s, 1H), 2.59 (dd, 2H), 3.98 (s, 2H), 7.29 - 7.4 (m, 2H), 7.44 (dd, 2H), 7.54 (d, 2H), 7.81 - 7.91 (m, 3H)

2-(4-Chlorophenyl)-5-(2-methylphenyl)-6-{[1-(propan-2-yl)piperidin-3-yl]methyl}-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (24)

Phosphoryl trichloride (0.063 mL, 0.68 mmol) was added in one portion to methyl 4-amino-1-(4-chlorophenyl)-1H-pyrazole-3-carboxylate (57.0 mg, 0.23 mmol), and N-((1-isopropylpiperidin-3-yl)methyl)-2-methylbenzamide (93 mg, 0.34 mmol) in DCE (9 mL) at rt under air. The resulting suspension was stirred at 85 °C for 16 h. The reaction mixture was evaporated to dryness, redissolved in EtOAc (50 mL), and washed sequentially with sat. $NaHCO_3$ (20 mL), and sat. brine (10 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude methyl 1-(4-chlorophenyl)-4-{[(2-methylphenyl)({[1-(propan-2-yl)piperidin-3-yl]methyl}amino)methylidene]amino}-1*H*-pyrazole-3-carboxylate as a brown foam. Titanium(IV) chloride (0.132 mL, 1.20 mmol) was added to methyl 1-(4-chlorophenyl)-4-(((1-isopropylpiperidin-3-yl)methylamino)(o-tolyl)methyleneamino)-1H-pyrazole-3-carboxylate (152 mg, 0.3 mmol) in DCE (3 mL) sealed into a microwave tube. The reaction was heated to 150 °C for 10 min in the microwave reactor and cooled to RT. The reaction mixture was poured into a mix of DCM (10 mL) and sat $NaHCO_3$ (10 mL), a white solid was removed by filtration. The organic layer was extracted and the aqueous re-extraced with DCM (2 x 15 mL). The organic layers were collected together, washed with brine (5 mL) and dried over $MgSO_4$. A yellow oil was obtained. The compound was purified by preparative HPLC on a XBridge C18 column (10 µm 250x19 ID mm) using a gradient of 45 - 85% MeCN in H_2O/MECN/NH_3 95/5/0.2 buffer over 20 min with a flow of 19 mL/min. The compounds were detected by UV at 251 nm. The pure fractions were mixed together and freeze dried overnight to give the title compound (33 mg, 23%) as a white solid.

m/z (ES+) $[M+H]^+$ = 476;

HRMS calculated for $C_{27}H_{31}ON_5$ $[M+H]^+$ 476.22116, found 476.22061
^{1}H NMR (400 MHz, DMSO-d_6) δ 0.63 - 0.74 (m, 1H), 0.76 - 0.86 (m, 6H), 1.10 - 1.27 (m, 2H), 1.30 - 1.49 (m, 2H), 1.50 - 1.71 (m, 2H), 1.86 - 2.00 (m, 1H), 2.15 (d, 3H), 2.21 - 2.33 (m, 1H), 2.51 - 2.59 (m, 1H), 3.34 - 3.54 (m, 1H), 3.87 - 4.03 (m, 1H), 7.30 - 7.38 (m, 2H), 7.42 (t, 2H), 7.67 (d, 2H), 8.08 (d, 2H), 9.13 (s, 1H).

N-((1-Isopropylpiperidin-3-yl)methyl)-2-methylbenzamide
(1-isoPropylpiperidin-3-yl)methanamine (220 mg, 1.41 mmol) was dissolved in 15 mL DCM. PS-DIEA (1.09 g, 4.22 mmol) was added and finally 2-methylbenzoyl chloride (0.2 mL, 1.53 mmol). The reaction was stirred for 24 h at rt, the resin was filtered on a fritted glass and washed extensively with DCM and MeOH. Crude was concentrated to a yellowish oil that was retaken in MeOH (5 mL) and purified on a 10 g SCX column eluting first with 100 mL MeOH followed by 2M NH_3 in EtOH (100 mL). N-((1-isopropylpiperidin-3-yl)methyl)-2-methylbenzamide was isolated as an orange oil in quantitative yield.
m/z (ES+) $[M+H]^+$ = 275.
^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (dd, 7H), 1.04 (t, 1H), 1.37 (dd, 1H), 1.54 - 1.76 (m, 3H), 1.82 (t, 1H), 2.07 (dd, 1H), 2.30 (s, 3H), 2.65 (dd, 2H), 2.75 (d, 1H), 2.99 - 3.19 (m, 2H), 7.15 - 7.34 (m, 4H), 8.21 (t, 1H).

Methyl 4-amino-1-(4-chlorophenyl)-1H-pyrazole-3-carboxylate (11, Ar = 4-Cl-Ph)
c. HCl (10 drops) was added to methyl 1-(4-chlorophenyl)-4-nitro-1H-pyrazole-3-carboxylate (3.14 g, 11.2 mmol) and iron (3.74 g, 66.9 mmol) in EtOH (60 mL) and water (60.0 mL) at rt under air. The resulting suspension was stirred at 100 °C for 30 min. A change of colour from a white suspension to a brown one was observed, and complete conversion obtained after 30 min. The hot reaction mixture was filtered through celite and the celite cake washed again with a hot mixture of EtOH/Water (1:1, 100 mL). Most of the solvent was then evaporated under reduced pressure. The reaction mixture was basified with sat. $NaHCO_3$ (30 mL). The reaction mixture was then diluted with EtOAc (125 mL), and washed sequentially with water (15 mL), and saturated brine (25 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford methyl 4-amino-1-(4-chlorophenyl)-1H-pyrazole-3-carboxylate (82%, 2.29 g).
m/z (ES+) $(M+H)^+$ = 252;
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.81 (s, 3H), 4.92 (s, 2H), 7.53 (d, 2H), 7.82 (d, 2H), 7.84 (s, 1H).

Methyl 1-(4-chlorophenyl)-4-nitro-1H-pyrazole-3-carboxylate (10, Ar = 4-Cl-Ph)
Pyridine (2.48 mL, 29.2 mmol) was added in one portion to methyl 4-nitro-1H-pyrazole-3-carboxylate (2.5 g, 14.6 mmol), 4-chlorophenylboronic acid (4.57 g, 29.2 mmol) and copper(II) acetate (3.98 g, 21.9 mmol) in DCM (28.6 mL) at rt under air. The resulting suspension was stirred at rt for 48 h. The reaction mixture was evaporated to dryness and redissolved in DCM (75 mL). It was then filtered through a plug of silica, washing with DCM (300 mL). The solvent was evaporated under reduced pressure to afford a white solid.
The crude product was purified by flash silica chromatography. The solid was first dry loaded on silica and was purified eluting with 10% EtOAc in heptane. Pure fractions were evaporated to dryness to afford methyl 1-(4-chlorophenyl)-4-nitro-1H-pyrazole-3-carboxylate (3.32 g, 81%).
m/z (ES+) $(M+H)^+$ = 282;
^{1}H NMR (400.13 MHz, DMSO-d_6) δ 3.93 (3H, s), 7.60 - 7.70 (2H, m), 7.90 - 8.00 (2H, m), 9.74 (1H, s)

2-(4-Chlorophenyl)-6-{[(3*R*)-1-(2-methoxyethyl)piperidin-3-yl]methyl}-5-(2-methylphenyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (25)

A solution of methyl 4-amino-1-(4-chlorophenyl)-1H-pyrazole-3-carboxylate (100 mg, 0.40 mmol), N-((1-(2-methoxyethyl)piperidin-3-yl)methyl)-2-methylbenzamide (173 mg, 0.60 mmol) and phosphoryl trichloride (0.111 mL, 1.19 mmol) in DCE (10 mL) was heated to 85 °C overnight. The reaction was worked-up by addition of K_2CO_3 (1 M, 25 mL) and extraction with DCM. The organic phases were pooled and evaporated.

Methyl 1-(4-chlorophenyl)-4-(((1-(2-methoxyethyl)piperidin-3-yl)methylamino)(o-tolyl)methyleneamino)-1H-pyrazole-3-carboxylate (208 mg, 0.40 mmol) was dissolved in DCE (5 mL). Titanium(IV) chloride (0.174 mL, 1.59 mmol) was added and the reaction mixture heated in a microwave single-node oven to 150 °C for 10 min. The reaction solution was added to MeOH/DCM and filtered. K_2CO_3 (1 M, 25 mL) was added and the aqueous phase was extracted with DCM. The organic phase was poured on a SCX-2 strong cation exchange column (5 g). The column was washed with DCM followed by MeOH. Subsequently the product was eluted with NH_3 saturated MeOH and evaporated. Purification by preparative HPLC, instrument: FractionLynx II, mobilphase: gradient 5-95% MeCN in 0.2% NH_3, pH 10, column: Xbridge Prep C18 5 µm OBD 19 x 150 mm afforded the title compound (58 mg, 29%).

m/z (ES+) $[M+H]^+$ = 492;

HRMS calculated for $C_{27}H_{31}O_2N_5Cl$ $[M+H]^+$ 492.21608, found 492.21573;

^{1}H NMR (600 MHz, DMSO-*d*$_6$) δ ^{1}H NMR (600 MHz, DMSO-d$_6$) δ 0.6 - 0.93 (m, 2H), 1.14 - 1.31 (m, 3H), 1.31 - 1.63 (m, 3H), 1.76 (d, 2H), 2.18 (s, 3H), 2.33 (ddd, 2H), 3.15 (d, 3H), 4.00 (d, 1H), 7.33 - 7.41 (m, 2H), 7.43 - 7.52 (m, 2H), 7.68 - 7.75 (m, 2H), 8.08 - 8.15 (m, 2H), 9.16 (d, 1H).

2-(4-Fluorophenyl)-6-((1-isopropylpiperidin-3-yl)methyl)-5-o-tolyl-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (26)

N-((1-isoPropylpiperidin-3-yl)methyl)-2-methylbenzamide (0.071 g, 0.26 mmol), methyl 4-amino-1-(4-fluorophenyl)-1H-pyrazole-3-carboxylate (0.061 g, 0.26 mmol) and phosphoryl trichloride (0.072 mL, 0.78 mmol) were heated in DCE (3 mL) at 85 °C overnight. Volatiles were evaporated and the crude retaken in EtOAc (20 mL). The organic phase was sequentially washed with sat. $NaHCO_3$ (20 mL), 2M aq. NaOH (20 mL) and brine (30 mL), dried over $MgSO_4$ filtrated and concentrated to yield 150 mg of an oil. The crude material and potassium carbonate (127 mg, 0.92 mmol) were suspended in DMF (2 mL) and heated at 130 °C for 20 min under microwave conditions. The crude was filtered, concentrated and purified by preparative HPLC using the following gradient : 5-95% MeCN in 0.2% aq. NH_3, pH 10 (column:Xbridge Prep C18 5µm OBD 19x150 mm). Freeze drying of the purest fractions yielded the title compound (42 mg, 30%).

m/z (ES+) $[M+H]^+$ = 460;

HRMS calculated for $C_{27}H_{31}ON_5F$ $[M+H]^+$ 460.25072, found 460.25031;

^{1}H NMR (600 MHz, DMSO-d$_6$) δ 0.6-0.76 (m, 1H), 0.76-0.84 (m, 6H), 1.10-1.22 (m, 2H), 1.30-1.47 (m, 2H), 1.50-1.58 (m, 2H), 1.60-1.67 (m, 2H), 1.88-1.93 (m, 1H), 2.14 (d, 3H), 3.48 (m, 1H), 3.92 (m, 1H), 7.27 - 7.39 (m, 2H), 7.39 - 7.48 (m, 4H), 8.06 (ddd, 2H), 9.07 (s, 1H).

4-(6-((1-Isopropylpiperidin-3-yl)methyl)-7-oxo-5-o-tolyl-6,7-dihydro-2H-pyrazolo[4,3-d]pyrimidin-2-yl)benzonitrile (27)

Methyl 4-amino-1-(4-cyanophenyl)-1H-pyrazole-3-carboxylate (0.135 g, 0.5 mmol) and N-((1-isopropylpiperidin-3-yl)methyl)-2-methylbenzamide (0.195 g, 0.71 mmol) were dissolved in DCE (10 mL). Phosphoryl trichloride (0.186 mL, 2.00 mmol) was added under nitrogen

and the vial was placed in a preheated oil bath (85 °C) and agitation maintained at this temperature. Immediate formation of precipate upon heating was observed. After 1 h the yellow thick precipitate was hindering agitation, but the mixture went gradually back to a clear orange solution. The reaction was cooled to rt and diluted with 30 mL DCM and washed sequentially with Na_2CO_3 (1M, 30 mL) and brine (30 mL), dried over $MgSO_4$, filtered, concentrated and dried under vacuum for 1h. Crude material was dissolved in 5 mL DCE under nitrogen. Titanium(IV) chloride (0.220 mL, 2.00 mmol) was then added under nitrogen and the resulting suspension was irradiated for 10 min at 150 °C in the microwave. The mixture was diluted with MeOH (5 mL) and agitation maintained for 10 min at rt. It was then diluted with DCM (30 mL) and washed with aq. NaOH (1N, 30 mL) and the aqueous phase re-extracted with DCM (10 mL). The combined organic phases were washed with brine (50 mL), dried over $MgSO_4$ filtrated, concentrated and dried under vacuum. The crude was retaken in DMSO (4 mL) and purified by preparative HPLC on a XBridge C18 column (10 µm 250x50 ID mm) using a gradient of 50-95% MeCN in H_2O/MeCN/NH_3 95/5/0.2 buffer over 30 min with a flow of 100 mL/min. The compounds were detected by UV at 257nm. Freeze drying of the purest fractions yielded the title compound (40 mg, 17%) as a white solid.
m/z (ES+) $[M+H]^+$ = 467;
HRMS calculated for $C_{28}H_{31}ON_6$ $[M+H]^+$ 467.25539, found 467.25522;
^{1}H NMR (400 MHz, DMSO-d_6) δ 0.56 - 0.71 (m, 1H), 0.71 - 0.84 (m, 6H), 1.10-1.40 (m, 3H), 1.45-1.65 (m, 2H), 1.87 (s, 1H), 2.11 (d, 3H), 2.15-2.40 (m, 1H), 2.46 - 2.55 (m, 2H), 3.38 (m, 1H), 3.91 (m, 1H), 7.23 - 7.34 (m, 2H), 7.38 (t, 2H), 8.05 (d, 2H), 8.22 (d, 2H), 9.22 (s, 1H).

6-((1-Isopropylpiperidin-3-yl)methyl)-2-(4-methoxyphenyl)-5-o-tolyl-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (28)
Procedure as for **26**
6-((1-isoPropylpiperidin-3-yl)methyl)-2-(4-methoxyphenyl)-5-o-tolyl-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (64 mg, 46%)
m/z (ES+) $[M+H]^+$ = 472;
HRMS calculated for $C_{28}H_{34}O_2N_5$ $[M+H]^+$ 472.27070, found 472.27057;
^{1}H NMR (600 MHz, DMSO-d_6) δ 0.6 - 0.76 (m, 1H), 0.76 - 0.87 (m, 6H), 1.07 - 1.36 (m, 2H), 1.37 - 1.48 (m, 1H), 1.49 - 1.7 (m, 2H), 1.90 (d, 1H), 2.13 (d, 3H), 3.47 (s, 1H), 3.80 (d, 3H), 3.95 (dd, 1H), 7.08 - 7.17 (m, 2H), 7.27 - 7.37 (m, 2H), 7.41 (ddd, 2H), 7.85 - 7.98 (m, 2H), 8.93 - 9.03 (m, 1H).

2-(3-Chlorophenyl)-6-((1-isopropylpiperidin-3-yl)methyl)-5-o-tolyl-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (29)
Methyl 4-amino-1-(3-chlorophenyl)-1H-pyrazole-3-carboxylate (0.088 g, 0.35 mmol) and N-((1-isopropylpiperidin-3-yl)methyl)-2-methylbenzamide (0.144 g, 0.52 mmol) were dissolved in DCE (10 mL). Phosphoryl trichloride (0.130 mL, 1.40 mmol) was added under nitrogen and the resulting mixture was placed in a preheated oil bath (85 °C) and stirred for 16 h at this temperature. The mixture was allowed to cool to rt, diluted with DCM (20 mL) and washed with aq. Na_2CO_3 (1M, 20 mL), brine (20 mL). The organic phase was dried over $MgSO_4$, filtered, concentrated and dried under vacuum for 1h. The crude material was dissolved in DCE (3 mL) under nitrogen. Titanium(IV) chloride (0.154 mL, 1.40 mmol) was then added under nitrogen and the resuting suspension was agitated for 10 min at 150 °C in the microwave. The reaction was diluted with MeOH (5 mL) and stirred for 10 mins at rt. The reaction was then diluted with DCM (30 mL) and washed with aq. NaOH 1N (30 mL), brine (30 mL), dried over $MgSO_4$ filtered, concentrated and dried under vacuum. The crude material was retaken in DMSO (4 mL) and purified by preparative HPLC on a XBridge C18

column (10 μm 250x50 ID mm) using a gradient of 50-90% Acetonitrile in H_2O/MeCN/NH_3 95/5/0.2 buffer over 20 min with a flow of 100 mL/min. The compounds were detected by UV at 249 nm. Freeze drying of purest fractions yielded the title compound (66 mg, 40%) as a white solid.
m/z (ES+) $[M+H]^+$ = 476;
HRMS calculated for $C_{27}H_{31}ON_5Cl$ $[M+H]^+$ 476.22116, found 476.22101;
^{1}H NMR (400 MHz, DMSO-d_6) δ 0.71 (d, 1H), 0.74 - 0.86 (m, 6H), 1.15-1.50 (m, 3H), 1.50 - 1.70 (m, 2H), 1.91 (t, 1H), 2.15 (d, 3H), 2.20-2.45 (m, 1H), 2.51 - 2.57 (m, 2H), 3.41 (m, 1H), 3.95 (t, 1H), 7.34 (dd, 2H), 7.42 (t, 2H), 7.53 (dd, 1H), 7.62 (t, 1H), 8.04 (dd, 1H), 8.15 (t, 1H), 9.17 (s, 1H).

6-((1-Isopropylpiperidin-3-yl)methyl)-2-(3-methoxyphenyl)-5-o-tolyl-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (30)
Procedure as for **26**
6-((1-isoPropylpiperidin-3-yl)methyl)-2-(3-methoxyphenyl)-5-o-tolyl-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (72 mg, 52%)
m/z (ES+) $[M+H]^+$ = 472;
HRMS calculated for $C_{28}H_{34}O_2N_5$ $[M+H]^+$ 472.27070, found 472.27045;
^{1}H NMR (600 MHz, DMSO-d_6) δ 0.65-0.75 (m, 1H), 0.76-0.81 (m, 6H), 1.10-1.32 (m, 2H), 1.32-1.40 (m, 1H), 1.50-1.65 (m, 2H), 1.88-1.93 (m, 1H), 2.14 (d, 3H), 3.46 (m, 1H), 3.85 (s, 3H), 3.90-4.00 (m, 1H), 7.03 (dd, 1H), 7.33 (m, 2H), 7.41 (dd, 2H), 7.48 (t, 1H), 7.58 (m, 2H), 9.11 (s, 1H).

3-(6-((1-Isopropylpiperidin-3-yl)methyl)-7-oxo-5-o-tolyl-6,7-dihydro-2H-pyrazolo[4,3-d]pyrimidin-2-yl)benzonitrile (31)
Procedure as for **26**
3-(6-((1-isoPropylpiperidin-3-yl)methyl)-7-oxo-5-o-tolyl-6,7-dihydro-2H-pyrazolo[4,3-d]pyrimidin-2-yl)benzonitrile (32 mg, 23%)
m/z (ES+) $[M+H]^+$ = 467;
HRMS calculated for $C_{28}H_{31}ON_6$ $[M+H]^+$ 467.25539, found 467.25507;
^{1}H NMR (400 MHz, DMSO-d_6) δ 0.63 - 0.76 (m, 1H), 0.76 - 0.87 (m, 6H), 1.36 (m, 3H), 1.51 - 1.72 (m, 2H), 1.92 (t, 1H), 2.16 (d, 3H), 2.21 - 2.44 (m, 1H), 2.5 - 2.59 (m, 2H), 3.42 (s, 1H), 3.98 (dd, 1H), 7.27 - 7.39 (m, 2H), 7.42 (dd, 2H), 7.82 (t, 1H), 7.95 (dd, 1H), 8.41 (ddd, 1H), 8.53 - 8.58 (m, 1H), 9.21 (s, 1H).

4-[6-{[1-(2-Methoxyethyl)piperidin-3-yl]methyl}-5-(2-methylpyridin-3-yl)-7-oxo-6,7-dihydro-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl]benzonitrile (32)
1,3,4,6,7,8-Hexahydro-2H- pyrimido [1,2-A] pyrimidine, polymer bound (58.0 mg, 0.25 mmol) was added in one portion to 4-(5-(2-methylpyridin-3-yl)-7-oxo-6-(piperidin-3-ylmethyl)-6,7-dihydro-2H-pyrazolo[4,3-d]pyrimidin-2-yl)benzonitrile (36 mg, 0.08 mmol), and 2-bromoethylmethyl ether (6.50 mg, 0.110 mmol) in DCM (1 mL) and MeCN (0.5 mL) at room temperature. The resulting suspension was shaken at 20 °C for 20 h. The reaction was stopped and filtered. The polymer was washed with 5 mL of DCM. The solvent was evaporated under reduced pressure and the crude product was dissolved in DMSO (1 mL) and purified using Fractionlynx I, (Xbridge Prep C18 5μm OBD 19x150 mm column), with 5 to 95% acetonitrile in 0.2% ammonia at pH 10. Freeze drying of the purest fractions yielded the title compound (18.6 mg, 45%).
m/z (ES+) $[M+H]^+$ = 484;
HRMS calculated for $C_{27}H_{30}O_2N_7$ $[M+H]^+$ 484.24555, found 484.24524;

^{1}H NMR (600 MHz, DMSO-d_6) δ 0.75 (dd, 1H), 1.13 - 1.55 (m, 4H), 1.56 - 1.70 (m, 2H), 1.76 - 1.92(m, 1H), 2.28 - 2.36 (m, 2H), 2.37 (s, 3H), 2.61 (dd, 1H), 3.14 (d, 3H), 3.25 - 3.35 (m, 2H), 3.88 - 3.97 (m, 1H), 7.4 - 7.46 (m, 1H), 7.96 (td, 1H), 8.09 - 8.13 (d, 2H), 8.25 - 8.31 (d, 2H), 8.64 (dd, 1H), 9.29 (s, 1H).

4-[5-(2-Methylpyridin-3-yl)-6-{[1-(oxetan-3-yl)piperidin-3-yl]methyl}-7-oxo-6,7-dihydro-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl]benzonitrile (33)

Sodium triacetoxyhydroborate (24.9 mg, 0.12 mmol) was added in one portion to 4-(5-(2-methylpyridin-3-yl)-7-oxo-6-(piperidin-3-ylmethyl)-6,7-dihydro-2H-pyrazolo[4,3-d]pyrimidin-2-yl)benzonitrile (25 mg, 0.06 mmol) and oxetan-3-one (8.47 mg, 0.12 mmol) in DCE (1 mL) at rt under air. Acetic acid (3.36 µL, 0.06 mmol) was then added and the resulting solution was stirred at rt for 50 h. The reaction mixture was diluted with DCM (5 mL), and washed with 2M NaOH (1 mL). The organic layer was collected through a phase separator and evaporated to afford crude product. It was dissolved in DMSO (1 mL) and purified using Fractionlynx I, (Xbridge Prep C18 5µm OBD 19x150 mm column), with 5 to 95% acetonitrile in 0.2% ammonia at pH 10. Freeze drying of the purest fractions yielded the title compound (16.9 mg, 60%)
m/z (ES+) $[M+H]^+$ = 482;
HRMS calculated for $C_{27}H_{28}O_2N_7$ $[M+H]^+$ 482.22990, found 482.22980;
^{1}H NMR (600 MHz, DMSO-d_6) δ 0.66 - 0.87 (m, 1H), 1.15 - 1.46 (m, 4H), 1.52 - 1.70 (m, 2H), 2.08 - 2.15 (m, 1H), 2.22 - 2.3 (m, 1H), 2.34 (d, 4H), 3.87 - 3.96 (m, 1H), 4.25 (t, 2H), 4.39 (t, 2H), 7.39 (dd, 1H), 7.93 (d, 1H), 8.06 - 8.1 (d, 2H), 8.24 (d, 2H), 8.60 (d, 1H), 9.26 (s, 1H).

4-(5-(2-Methylpyridin-3-yl)-7-oxo-6-(piperidin-3-ylmethyl)-6,7-dihydro-2H-pyrazolo[4,3-d]pyrimidin-2-yl)benzonitrile (13, Ar = 4-CN-Ph)

tert-Butyl 3-((N'-(1-(4-cyanophenyl)-3-(methoxycarbonyl)-1H-pyrazol-4-yl)-2-methylnicotinimidamido)methyl)piperidine-1-carboxylate (4.37 g, 7.83 mmol) and potassium carbonate (3.24 g, 23.5 mmol) were suspended in DMF (20 mL) and the reaction was heated to 130 °C for 30 min in an oil bath and cooled to room temperature. The reaction mixture was evaporated to dryness and redissolved in EtOAc (50 mL), and washed sequentially with water (10 mL) and saturated brine (10 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude Boc protected intermediate. It was then purified by preparative HPLC on a Kromasil C8 column (10 µm 250 x 50 ID mm) using a gradient of 25-70% MeCN in H_2O/MeCN/FA 95/5/0.2 buffer over 25 min with a flow of 100 mL/min. The compounds were detected by UV at 254 nm. Fractions containing the desired compound were evaporated to dryness to afford the desired pure Boc protected intermediate (1.9 g). The piperidine was deprotected by stirring the product with 4N HCl in dioxane (10 mL) overnight. The reaction mixture was evaporated to dryness and redissolved in EtOAc (50 mL), and washed sequentially with 2M NaOH (15 mL), water (5 mL), and saturated brine (10 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to provide product (830 mg). Unsoluble solid remained in the aqueous. It was re-extracted with DCM and this time everything was soluble. The organic layer was dried over $MgSO_4$, filtered and evaporated to afford a second batch of product (776 mg). The two batches were combined and the title compound (1.606 g, 48%) was obtained as a white solid.
m/z (ES+) $[M+H]^+$ = 426.

tert-Butyl 3-((N'-(1-(4-cyanophenyl)-3-(methoxycarbonyl)-1H-pyrazol-4-yl)-2-methylnicotinimidamido)methyl)piperidine-1-carboxylate

Phosphorus pentachloride (3.26 g, 15.7 mmol) was added portionwise to methyl 1-(4-cyanophenyl)-4-(2-methylnicotinamido)-1H-pyrazole-3-carboxylate (2.83 g, 7.83 mmol) in toluene (30 mL) at 20 °C and under air. The resulting suspension was stirred at 110 °C for 6 h. The reaction mixture was evaporated to dryness and redissolved in DCE (30 mL). A solution of tert-butyl 3-(aminomethyl)piperidine-1-carboxylate (2.85 g, 13.3 mmol) in DCE (7 mL) was added. The reaction mixture went from a yellow suspension to a clear yellow solution. N-ethyl-N-isopropylpropan-2-amine (8.18 mL, 47.0 mmol) was added and the reaction stirred at room temperature overnight. The reaction mixture was diluted with DCM (200 mL), and washed sequentially with saturated $NaHCO_3$ (2 x 25 mL), water (20 mL), and saturated brine (20 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product. It was used immediately without further purification (48% yield over the two steps).
m/z (ES+) $[M+H]^+$ = 558.

Methyl 1-(4-cyanophenyl)-4-(2-methylnicotinamido)-1H-pyrazole-3-carboxylate
N-Ethyl-N-isopropylpropan-2-amine (5.17 mL, 29.7 mmol) was added in one portion to methyl 4-amino-1-(4-cyanophenyl)-1H-pyrazole-3-carboxylate (2.40 g, 9.89 mmol), 2-methylnicotinic acid (1.63 g, 11.9 mmol) and 2-(1H-benzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethylisouronium tetrafluoroborate (4.13 g, 12.8 mmol) in DCM (50 mL) at rt and under air. The resulting suspension was stirred at rt for 48 h. The reaction mixture was evaporated to dryness, redissolved in EtOAc (100 mL), and washed sequentially with saturated $NaHCO_3$ (2 x 15 mL), 1M citric acid (15 mL), and saturated brine (25 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford a brown oily product. The collected aqueous phases contained a beige insoluble solid which had been at the interface with the organic phase during the work up. They were filtered and a LCMS of the solid was run, confirming this was product. The aqueous was concentrated and more product slowly precipitated. The title compound (2.83 g, 79%) was recovered by filtration as a beige solid.
m/z (ES+) $[M+H]^+$ = 362;
^{1}H NMR (400 MHz, DMSO-d_6) δ 2.64 (s, 3H), 3.89 (s, 3H), 7.40 (dd, 1H), 7.96 (d, 1H), 8.01 (d, 2H), 8.17 (d, 2H), 8.60 (d, 1H), 9.14 (s, 1H), 9.95 (s, 1H).

Methyl 4-amino-1-(4-cyanophenyl)-1H-pyrazole-3-carboxylate (11, Ar = 4-CN-Ph)
c.HCl (10 drops) were added dropwise to methyl 1-(4-cyanophenyl)-4-nitro-1H-pyrazole-3-carboxylate (3.2 g, 11.76 mmol) and iron (3.28 g, 58.8 mmol) in water (50 mL) and EtOH (50 mL) at rt. The resulting suspension was placed in a preheated oil bath at 100 °C and stirred at that temperature for 25 min. The iron was filtered off and the reaction was evaporated to dryness and redissolved in EtOAc (500 mL), filtered again and washed sequentially with saturated $NaHCO_3$ (25 mL), water (20 mL), and saturated brine (20 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product. It was triturated in EtOH (2 x 30 mL) and filtered. A dark brown coloured liquid and a beige solid were obtained which were both confirmed to be product (2.4 g, 84%).
m/z (ES+) $[M+H]^+$ = 243;
^{1}H NMR (400.13 MHz, DMSO-d_6) δ 3.83 (3H, s), 4.99 (2H, s), 7.57 - 8.01(5H, m).

Methyl 1-(4-cyanophenyl)-4-nitro-1H-pyrazole-3-carboxylate (10, Ar = 4-CN-Ph)
Pyridine (4.27 mL, 50.3 mmol) was added in one portion to methyl 4-nitro-1H-pyrazole-3-carboxylate (4.3 g, 25.1 mmol), 4-cyanophenylboronic acid (7.39 g, 50.3 mmol) and copper(II) acetate (6.85 g, 37.7 mmol) in DCM (49.2 mL) at rt under air. The resulting suspension was stirred at rt for 96 h. The reaction mixture was dry loaded on silica and directly purified by flash chromatography. The crude product was purified by flash silica

chromatography eluting with 20% EtOAc in heptane. Pure fractions were evaporated to dryness to afford methyl 1-(4-cyanophenyl)-4-nitro-1H-pyrazole-3-carboxylate (3.22 g, 47%).
m/z (ES-) $[M-H]^-$ = 271;
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.94 (3H, s), 8.00 - 8.25 (4H, m), 9.87 (1H, s)

(*S*)-6-((1-Isopropylpiperidin-3-yl)methyl)-2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (34)
2-Iodopropane (65.8 µL, 0.66 mmol) was added to (*S*)-2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-6-(piperidin-3-ylmethyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (258 mg, 0.60 mmol) and potassium carbonate (166 mg, 1.20 mmol) in dioxane (2.93 L) at rt under nitrogen. The resulting suspension was stirred at 80 °C for 6 h. Further 2-iodopropane (65.8 µL, 0.66 mmol) was added and the reaction was stirred for 16 h then cooled to rt. The reaction mixture was diluted with EtOAc (25 mL), and washed sequentially with water (15 mL) and saturated brine (10 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 1-5% 7M NH_3 / MeOH in DCM. Product containing fractions were evaporated to dryness to afford crude product. The crude product was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5µ silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 0.5% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford the title compound (124 mg, 44%) as a yellow solid.
m/z (ES+) $[M+H]^+$ = 473;
HRMS. Theoretical for $C_{27}H_{33}N_6O_2$ $[M+H]^+$ = 473.2660. Found, 473.2660.
^{1}H NMR (700 MHz, DMSO-d_6, 69 °C) δ 0.70 - 0.90 (7H, m), 1.18 - 1.42 (2H, m), 1.44 - 1.51 (1H, m), 1.59 - 1.81 (2H, m), 2.01 (1H, t), 2.31 - 2.46 (4H, m), 2.54 - 2.59 (1H, m), 3.48 - 3.68 (1H, m), 3.90 (3H, s), 3.91 - 4.03 (1H, m), 7.05 - 7.08 (1H, m), 7.37 - 7.42 (1H, m), 7.51 (1H, t), 7.59 - 7.62 (2H, m), 7.89 - 7.91 (1H, m), 8.61 - 8.64 (1H, m), 9.07 (1H, s).

(*S*)-2-(3-Methoxyphenyl)-5-(2-methylpyridin-3-yl)-6-(piperidin-3-ylmethyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (13, Ar=3-MeO-Ph)
4M HCl in dioxane (5 mL, 1.22 mmol) was added to (*R*)-tert-butyl 3-((2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-7-oxo-2H-pyrazolo[4,3-d]pyrimidin-6(7H)-yl)methyl)piperidine-1-carboxylate (645 mg, 1.22 mmol) in DCM (1 mL) at rt. The resulting mixture was stirred overnight. The mixture was filtered and the solid material dissolved in MeOH and loaded onto an SCX-2 column, eluting with MeOH followed by 1.4M NH_3 in MeOH. The basic fractions were concentrated to provide a pale orange gum which was triturated from Et_2O to afford the title compound (372 mg, 71%) as a cream solid.
m/z (ES+) $[M+H]^+$ = 431;
^{1}H NMR (400 MHz, DMSO-d_6) δ 1.37 - 1.41 (2H, m), 1.49 - 1.52 (1H, m), 2.03 - 2.08 (1H, m), 2.28 - 2.33 (1H, m), 2.37 (3H, s), 2.60 - 2.68 (1H, m), 2.63 - 2.71 (1H, m), 3.28 (3H, s), 3.35 (1H, s), 3.88 (3H, s), 7.04 - 7.08 (1H, m), 7.41 (1H, d), 7.51 (1H, t), 7.59 - 7.63 (1H, m), 7.60 - 7.65 (1H, m), 7.93 (1H, d), 8.62 - 8.63 (1H, m), 9.16 (1H, s).

(*R*)-tert-Butyl 3-((2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-7-oxo-2H-pyrazolo[4,3-d]pyrimidin-6(7H)-yl)methyl)piperidine-1-carboxylate
(*R*,*E*)-tert-Butyl 3-(((3-(methoxycarbonyl)-1-(3-methoxyphenyl)-1H-pyrazol-4-ylamino)(2-methylpyridin-3-yl)methyleneamino)methyl)piperidine-1-carboxylate (1.54 g, 2.73 mmol) and potassium carbonate (0.435 mL, 7.64 mmol) were heated to 130 °C in DMF (15 mL) for 40 min. The mixture was cooled slightly then concentrated to dryness. The crude residue was

dissolved in EtOAc (75 mL) and sequentially washed with water (50 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated to provide the crude product. The crude product was purified by flash silica chromatography, elution gradient 20-90% EtOAc in isohexane. Pure fractions were evaporated to dryness to afford (R)-tert-butyl 3-((2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-7-oxo-2H-pyrazolo[4,3-d]pyrimidin-6(7H)-yl)methyl)piperidine-1-carboxylate the title compound (645 mg, 44%) as a dark orange gum.
m/z (ES+) $[M+H]^+$ = 531;
^{1}H NMR (400 MHz, DMSO-d_6) δ 1.06 - 1.10 (1H, m), 1.15 (1H, s), 1.30 (8H, s), 1.36 (3H, t), 1.38 (2H, s), 1.45 (1H, d), 1.98 (3H, s), 2.64 - 2.66 (1H, m), 3.68 (2H, d), 3.77 (1H, s), 3.88 (3H, s), 7.05 - 7.08 (1H, m), 7.39 - 7.42 (1H, m), 7.51 (1H, t), 7.60 (1H, d), 7.60 - 7.63 (1H, m), 7.91 - 7.97 (1H, m), 8.63 (1H, d), 9.18 (1H, d)

(*R*,*E*)-tert-Butyl 3-(((3-(methoxycarbonyl)-1-(3-methoxyphenyl)-1H-pyrazol-4-ylamino)(2-methylpyridin-3-yl)methyleneamino)methyl)piperidine-1-carboxylate
Phosphorus pentachloride (1.14 g, 5.46 mmol) was added to a suspension of methyl 1-(3-methoxyphenyl)-4-(2-methylnicotinamido)-1H-pyrazole-3-carboxylate (1.0 g, 2.73 mmol) in toluene (20 mL) at rt. The resulting mixture was heated to reflux for 2 h. The mixture was cooled and concentrated, and the resulting residue dissolved in DCE (20 mL). N-Ethyldiisopropylamine (2.83 mL, 16.4 mmol) and (*R*)-tert-butyl 3-(aminomethyl)piperidine-1-carboxylate (0.994 g, 4.64 mmol) were added and the resulting solution was stirred at rt for 3 d. The reaction mixture was diluted with DCM (100 mL), and washed sequentially with saturated $NaHCO_3$ (50 mL) and water (50 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude title compound that was used without further purification.
m/z (ES+) $[M+H]^+$ = 563.

Methyl 1-(3-methoxyphenyl)-4-(2-methylnicotinamido)-1H-pyrazole-3-carboxylate (12, Ar = 3-MeO-Ph)
N,N-Diisopropylethylamine (10.5 mL, 60.4 mmol) was added to methyl 4-amino-1-(3-methoxyphenyl)-1H-pyrazole-3-carboxylate (2.49 g, 10.1 mmol), 2-methylnicotinic acid (1.72 g, 12.6 mmol) and O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (4.77 g, 12.6 mmol) in DMF (50 mL). The resulting mixture was stirred for 48 h at rt under nitrogen. Water (50 mL) was added the mixture filtered to provide a crude green solid. The solid was washed with water (3 x 20 mL) then Et_2O (2 x 20 mL) and dried to afford the title compound (2.72 g, 74%).
m/z (ES+) $[M+H]^+$ = 367;
^{1}H NMR (400 MHz, DMSO-d_6) δ 2.65 (3H, s), 3.87 - 3.87 (3H, m), 3.89 (3H, s), 6.97 - 7.00 (1H, m), 7.37 - 7.40 (1H, m), 7.45 - 7.51 (2H, m), 7.94 - 7.96 (1H, m), 8.59 - 8.60 (1H, m), 8.99 (1H, s), 9.88 (1H, s).

(*S*)-6-((1-(2-Methoxyethyl)piperidin-3-yl)methyl)-2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (35)
2-Bromoethyl methyl ether (0.079 mL, 0.84 mmol) was added to a stirred suspension of (*S*)-2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-6-(piperidin-3-ylmethyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (362 mg, 0.84 mmol) and potassium carbonate (232 mg, 1.68 mmol) in dioxane (5 mL) under nitrogen. The resulting mixture was stirred at 80 °C for 18 h then cooled to rt. The mixture was concentrated and the resulting residue partitioned between EtOAc (50 mL) and water (30 mL). The aqueous layer was extracted with EtOAc (2 x 30 mL) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated to provide

the crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 10% MeOH in DCM. The product (~90% pure) was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5µ silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 0.5% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford the title compound (153 mg, 37%) as a colourless solid.
m/z (ES+) $[M+H]^+$ = 489;
HRMS (ES+) calculated for $C_{27}H_{33}N_6O_3$ $[M+H]^+$ = 489.26083. Found: 489.26087
^{1}H NMR (400.13 MHz, DMSO-d_6) δ 0.77-0.90 (1H, m), 1.26-1.32 (1H, m), 1.40-1.43 (1H, m), 1.47-1.53 (1H, m), 1.57-1.62 (1H, m), 1.65-1.69 (1H, m), 1.88-1.93 (1H, m), 2.36-2.51 (2H, m), 2.44 (3H, s), 2.60-2.65 (1H, m), 3.20 (3H, d), 3.33-3.39 (3H, m), 3.53-3.59 (1H, m), 3.94 (3H, s), 3.98-4.01 (1H, m), 7.11-7.14 (1H, m), 7.47-7.55 (1H, m), 7.57 (1H, t), 7.66-7.70 (2H, m), 8.01 (1H, m), 8.69-8.70 (1H, m), 9.23 (1H, s)

(*S*)-2-Cyclohexyl-6-((1-(2-methoxyethyl)piperidin-3-yl)methyl)-5-(2-methylpyridin-3-yl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (36)
Potassium carbonate (0.083 mL, 1.46 mmol) was added to (*R*,*E*)-methyl 1-cyclohexyl-4-(N'-((1-(2-methoxyethyl)piperidin-3-yl)methyl)-2-methylnicotinimidamido)-1H-pyrazole-3-carboxylate (363 mg, 0.73 mmol) in DMF (3 mL) at rt. The resulting mixture was stirred at rt overnight before heating to 130 °C for 15 min. The reaction mixture was diluted with EtOAc (50 mL), and washed sequentially with saturated brine (15 mL) and water (15 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 10% MeOH in DCM. Pure fractions were evaporated to dryness to afford the title compound (156 mg, 46%) as an oil which solidified on standing to give a cream solid.
m/z (ES+) $[M+H]^+$ = 465;
HRMS calculated for $C_{26}H_{37}N_6O_2$ ($[M+H]^+$): 465.29725. Found: 465.29694
^{1}H NMR (400 MHz, DMSO-d_6, 30 °C) δ 0.63 - 0.84 (1H, m), 1.14 - 1.32 (3H, m), 1.36 - 1.52 (3H, m), 1.61 (2H, s), 1.69 (1H, d), 1.76 - 1.91 (4H, m), 2.08 (2H, d), 2.32 (3H, s), 2.58 (1H, s), 3.12 (2H, s), 3.13 - 3.18 (4H, m), 3.28 (4H, d), 3.37 (1H, dd), 3.91 (1H, dd), 4.05 (1H, q), 4.42 (1H, ddd), 7.38 (1H, ddd), 7.82 - 7.93 (1H, m), 8.43 (1H, s), 8.60 (1H, dd).

(*R*,*E*)-Methyl 1-cyclohexyl-4-(N'-((1-(2-methoxyethyl)piperidin-3-yl)methyl)-2-methylnicotinimidamido)-1H-pyrazole-3-carboxylate
Carbon tetrachloride (0.141 mL, 1.46 mmol) was added to (*R*)-N-((1-(2-methoxyethyl)piperidin-3-yl)methyl)-2-methylnicotinamide (213 mg, 0.73 mmol), PS-triphenylphosphine (1.48 g, 2.19 mmol) and triethylamine (0.153 mL, 1.10 mmol) in MeCN (3 mL) at rt under nitrogen. The resulting suspension was stirred overnight. A further 2 equivalents of carbon tetrachloride was added as the reaction mixture still contained unreacted starting material. After a further 6 h, methyl 4-amino-1-cyclohexyl-1H-pyrazole-3-carboxylate (163 mg, 0.73 mmol) in MeCN (2 mL) and triethylamine (0.153 mL, 1.10 mmol) were added. The mixture was stirred for 20 min, the filtered and concentrated to provide the crude product which was carried through to the next step without purification.
m/z (ES+) $[M+H]^+$ = 497;

Methyl 4-amino-1-cyclohexyl-1H-pyrazole-3-carboxylate (11, Ar = c-Hex)
Palladium 10% on carbon (24.3 mg, 0.02 mmol) was added to methyl 1-cyclohexyl-4-nitro-1H-pyrazole-3-carboxylate (385 mg, 1.52 mmol) in MeOH (20 mL) under nitrogen. The reaction flask was evacuated and purged with hydrogen three times, and the suspension was then stirred under an atmosphere of hydrogen at rt for 16 h. The reaction mixture was filtered

through celite to give the crude methyl 4-amino-1-cyclohexyl-1H-pyrazole-3-carboxylate (315 mg, 93%) as a purple solid which was used without further purification.
m/z (ES+), $[M+H]^+$ = 224;
^{1}H NMR (400 MHz, DMSO-d_6, 30 °C) δ 1.12 - 1.25 (1H, m), 1.28 - 1.42 (2H, m), 1.57 - 1.70 (3H, m), 1.73 - 1.83 (2H, m), 1.87 - 1.98 (2H, m), 3.74 (3H, s), 3.97 - 4.07 (1H, m), 4.62 (2H, s), 7.16 (1H, s).

Methyl 1-cyclohexyl-4-nitro-1H-pyrazole-3-carboxylate (10, Ar = c-Hex)
(Triphenylphosphoranylidene)acetonitrile (1.76 mg, 5.84 mmol), methyl 4-nitro-1H-pyrazole-3-carboxylate (500 mg, 2.92 mmol) and cyclohexanol (0.304 mL, 2.92 mmol) were heated in toluene (10 mL) at 110 °C for 4 h. The mixture was cooled and concentrated. The crude product was purified by flash silica chromatography, elution gradient 0 to 30% EtOAc/isohexane . Pure fractions were evaporated to dryness to afford methyl 1-cyclohexyl-4-nitro-1H-pyrazole-3-carboxylate (85 mg, 11%) as a colourless oil.
m/z (ES^+), $[M+H]^+$ = 254;
^{1}H NMR (400 MHz, $CDCl_3$, 30 °C) δ 1.18 - 1.35 (1H, m), 1.36 - 1.50 (2H, m), 1.63 - 1.85 (3H, m), 1.85 - 2.04 (2H, m), 2.11 - 2.31 (2H, m), 3.91 - 4.06 (3H, m), 4.07 - 4.24 (1H, m), 8.08 - 8.23 (1H, m).

(*R*)-N-((1-(2-Methoxyethyl)piperidin-3-yl)methyl)-2-methylnicotinamide (17, R6 = $MeO(CH_2)_2$, R5 = 2-Me-3-pyridyl)
2-Bromoethyl methyl ether (0.76 mL, 8.07 mmol) was added to (*S*)-2-methyl-N-(piperidin-3-ylmethyl)nicotinamide (1.98 g, 8.07 mmol) and potassium carbonate (0.551 mL, 9.69 mmol) in dioxane (30 mL). The resulting suspension was stirred at 80 °C for 2.5-3 h then at rt overnight. The mixture was concentrated to dryness, then taken up in DCM and filtered. The filtrate was concentrated and purified by flash silica chromatography, elution gradient 0 to 10% MeOH/NH_3 in DCM. Pure fractions were evaporated to dryness to afford (R)-N-((1-(2-methoxyethyl)piperidin-3-yl)methyl)-2-methylnicotinamide (1.02 g, 43%) as a pale yellow gum.
m/z (ES+) $[M+H]^+$ = 292.

(*S*)-2-Methyl-N-(piperidin-3-ylmethyl)nicotinamide
(*R*)-tert-Butyl 3-((2-methylnicotinamido)methyl)piperidine-1-carboxylate (3.6 g, 10.8 mmol) was stirred in 4M HCl in dioxane (40 mL, 160 mmol) at rt for 3 h. DCM (10 mL) and MeOH (15 mL) were added to aid solubility of the piperidine. The reaction mixture was concentrated to provide a colourless foam. Sat. $NaHCO_3$ (200 mL) was added and the aqueous layer extracted with DCM (3 x 150 mL). The aqueous layer was loaded onto an SCX-2 column and eluted with 1M NH_3/MeOH and the fractions concentrated, filtered and then concentrated to dryness to provide (S)-2-methyl-N-(piperidine-3-ylmethyl)nicotinamide (1.23 g) as a cream crystalline solid. The aqueous extract was concentrated and the inorganics slurried in MeOH and filtered. The filtrate was concentrated to provide a second batch of (S)-2-methyl-N-(piperidin-3-ylmethyl) nicotinamide (1.661 g, 41%) as a cream crystalline solid.
m/z (ES+) $[M+H]^+$ = 234;
^{1}H NMR (400 MHz, DMSO-d_6, 30 °C) δ 0.94 - 1.13 (1H, m), 1.15 - 1.35 (1H, m), 1.56 (2H, dddd), 1.66 - 1.77 (1H, m), 1.89 (1H, s), 2.15 (1H, dd), 2.49 (3H, d), 2.57 - 2.70 (1H, m), 2.80 (1H, t), 2.89 (1H, t), 2.96 - 3.08 (2H, m), 3.26 (1H, s), 3.74 - 3.87 (1H, m), 3.95 (1H, dd), 7.17 - 7.26 (1H, m), 7.60 - 7.72 (1H, m), 8.45 (1H, dt).

2-(3-Methoxyphenyl)-5-(2-methylpyridin-3-yl)-6-(((*3S*)-1-(oxetan-2-ylmethyl)piperidin-3-yl)methyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (37)

(*S*)-2-(3-Methoxyphenyl)-5-(2-methylpyridin-3-yl)-6-(piperidin-3-ylmethyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (127 mg, 0.25 mmol), oxetan-2-ylmethyl 4-methylbenzenesulfonate (61 mg, 0.25 mmol) and potassium carbonate (174 mg, 1.26 mmol) were suspended in MeCN (2 mL) and the reaction was heated to 130 °C for 2 h. The crude product was purified by flash silica chromatography, elution gradient 0 to 30% methanol in DCM. Pure fractions were evaporated to dryness to afford the title compound (50 mg, 40%) as a white solid.
m/z (ES+) $[M+H]^+$ = 501;
HRMS: theoretical for $C_{28}H_{33}N_6O_3$ $[M+H]^+$ = 501.2609; found = 501.2607;
^{1}H NMR (400 MHz, DMSO-d_6, 30°C) δ 0.69 - 0.97 (2H, m), 1.24 - 1.79 (6H, m), 1.86 - 2.03 (1H, m), 2.31 (1H, s), 2.44 (3H, s), 3.57 (2H, s), 3.95 (3H, s), 3.99 (1H, s), 4.25 - 4.42 (1H, m), 4.42 - 4.57 (1H, m), 4.72 - 4.91 (1H, m), 7.13 (1H, dd), 7.42 - 7.51 (1H, m), 7.58 (1H, t), 7.63 - 7.72 (2H, m), 8.00 (1H, t), 8.69 (1H, d), 9.23 (1H, s).

2-(3-Methoxyphenyl)-5-(2-methylpyridin-3-yl)-6-(((*3S*)-1-((tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (38)
2-(Bromomethyl)tetrahydrofuran (103 mg, 0.56 mmol) was added to a stirred suspension of (*S*)-2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-6-(piperidin-3-ylmethyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (258 mg, 0.51 mmol) and potassium carbonate (283 mg, 2.05 mmol) in dioxane (5 mL) at rt under nitrogen. The resulting suspension was heated to 100 °C for 15 min. DMF (3 mL) was added and the reaction heated to 100 °C for a further 12 h. The orange residue was partioned between water (100 mL) and EtOAc (150 mL). The aqueous layer was separated and extracted with EtOAc (3 x 100 mL). The combined organic phases were dried over $MgSO_4$, filtered and concentrated to provide the crude product. The crude product was purified by flash silica chromatography, eluted using increasing amounts of methanol in a 1:1 mixture of EtOAc:DCM up to 25% MeOH. Pure fractions were evaporated to dryness to afford the title compound (105 mg, 40%) as a pale orange oil.
m/z (ES+) $[M+H]^+$ = 515;
HRMS. Theoretical for $C_{29}H_{35}N_6O_3$ $[M+H]^+$ = 515.2765. Found, 515.2763;
^{1}H NMR (400 MHz, DMSO-d_6, 30°C) δ 0.60 - 0.83 (1H, m), 1.09 - 1.47 (5H, m), 1.48 - 1.93 (7H, m), 2.09 - 2.25 (2H, m), 2.47 - 2.56 (1H, m), 2.56 - 2.66 (1H, m), 3.28 - 3.37 (1H, m), 3.36 - 3.63 (3H, m), 3.64 - 3.77 (1H, m), 3.78 - 3.93 (4H, m), 7.00 (1H, dd), 7.35 (1H, dd), 7.45 (1H, t), 7.55 (2H, dd), 7.88 (1H, t), 8.57 (1H, dd), 9.11 (1H, s).

2-(3-Methoxyphenyl)-5-(2-methylpyridin-3-yl)-6-(((*3S*)-1-((tetrahydro-2H-pyran-2-yl)methyl)piperidin-3-yl)methyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (39)
Potassium carbonate (32.1 mg, 0.23 mmol) was added to (*S*)-2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-6-(piperidin-3-ylmethyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (50 mg, 0.12 mmol) and 2-(bromomethyl)tetrahydro-2H-pyran (31.2 mg, 0.174 mmol) in DMF (1 mL) at rt under air. The resulting suspension was stirred at 80 °C for 24 h. The reaction was diluted with EtOAc and filtered and then purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5µ silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 0.5 NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford the title compound (31.7 mg, 52%) as a colourless dry film.
m/z (ES+) $[M+H]^+$ = 529;
^{1}H NMR (400 MHz, DMSO-d_6, 100 °C) δ 0.71 - 0.92 (1H, m), 1.02 - 1.16 (1H, m), 1.18 - 1.55 (8H, m), 1.56 - 1.82 (3H, m), 1.84 - 2.06 (1H, m), 2.08 - 2.20 (1H, m), 2.25 (1H, dt), 2.40 (3H, s), 2.54 (2H, s), 3.24 (2H, s), 3.68 - 3.81 (1H, m), 3.90 (3H, s), 7.01 - 7.09 (1H, m), 7.38 (1H, dd), 7.50 (1H, t), 7.59 (2H, d), 7.88 (1H, d), 8.62 (1H, dd), 8.97 - 9.02 (1H, m).

2-(3-Methoxyphenyl)-5-(2-methylpyridin-3-yl)-6-(((*S*)-1-(((*S*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (40)

To a stirred solution of (*S*)-2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-6-(piperidin-3-ylmethyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (50.0 mg, 0.12 mmol) and (*S*)-(tetrahydrofuran-2-yl)methyl 4-methylbenzenesulfonate (35.7 mg, 0.14 mmol) in DMF (2.0 mL) was added potassium carbonate (32.1 mg, 0.23 mmol) and potassium iodide (28.9 mg, 0.17 mmol) and the mixture heated at 95° C for 3 d. The mixture was cooled to rt and concentrated to provide a residue which was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5µ silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 0.5% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to give the title compound (4.80 mg, 8%).

m/z (ES+) $(M+H)^+$ = 515;

HRMS. Theoretical for $C_{29}H_{35}N_6O_3$ $[M+H]^+$ = 515.2765. Found, 515.2763.

^{1}H NMR (500 MHz, DMSO-d_6) δ 0.64 - 0.82 (m, 1H), 1.15 - 1.48 (m, 6H), 1.51 - 1.91 (m, 6H), 2.17 - 2.29 (m, 2H), 2.37 (s, 3H), 2.6 - 2.73 (m, 1H), 3.44 - 3.55 (m, 1H), 3.56 - 3.65 (m, 1H), 3.7 - 3.84 (m, 1H), 3.85 - 3.97 (m, 4H), 7.04 - 7.08 (m, 1H), 7.38 - 7.42 (m, 1H), 7.51 (t, 1H), 7.58 - 7.64 (m, 2H), 7.91 - 7.96 (m, 1H), 8.62 (dd, 1H), 9.16 (s, 1H).

2-(3-Methoxyphenyl)-5-(2-methylpyridin-3-yl)-6-(((*S*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (41)

To a stirred solution of (*S*)-2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-6-(piperidin-3-ylmethyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (50.0 mg, 0.12 mmol) and (*R*)-(tetrahydrofuran-2-yl)methyl 4-methylbenzenesulfonate (35.7 mg, 0.14 mmol) in DMF (2.0 mL) was added potassium carbonate (32.1 mg, 0.23 mmol) and potassium iodide (28.9 mg, 0.17 mmol) and the mixture heated at 95 °C for 3 d. The mixture was cooled to rt, the DMF evaporated *in vacuo* to a residue which was purified by reverse-phase chromatography to give the title compound (6.30 mg, 11%).

m/z (ES+) $[M+H]^+$ = 515

HRMS calculated for $C_{29}H_{35}N_6O_3$ $[M+H]^+$ 515.27652, found 515.27625;

^{1}H NMR (400 MHz, DMSO-d_6, 100 °C) δ 0.86 (1H, s), 1.24 - 1.54 (5H, m), 1.64 - 1.92 (6H, m), 2 - 2.11 (1H, m), 2.26 - 2.32 (2H, m), 2.43 (3H, s), 2.49 - 2.65 (2H, m), 3.52 - 3.61 (1H, m), 3.66 - 3.75 (1H, m), 3.76 - 3.86 (1H, m), 3.93 (3H, s), 7.06 - 7.12 (1H, m), 7.41 (1H, dd), 7.53 (1H, t), 7.62 (2H, d), 7.91 (1H, d), 8.65 (1H, dd), 9.02 (1H, s).

6-(((*S*)-1-((*S*)-2-Ethoxypropyl)piperidin-3-yl)methyl)-2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (42)

Sodium hydride (7.86 mg, 0.20 mmol) was added to 6-(((*S*)-1-((*S*)-2-hydroxypropyl)piperidin-3-yl)methyl)-2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (80 mg, 0.16 mmol) and iodoethane (0.017 mL, 0.21 mmol) in anhydrous THF (1 mL) at 0 ºC under nitrogen. The resulting solution was stirred at 0 ºC for 10 min and then the reaction mixture was allowed to warm to rt and stirred for 20 min. DMF (1 mL) was added and stirring continued for 48 hrs at rt. The reaction mixture was quenched with saturated NH_4Cl (1 mL), extracted with EtOAc and the organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5µ silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 0.5% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford the title compound (8.00 mg, 9%) as a colourless dry film.

m/z (ES+) $[M+H]^+$ = 517;

HRMS. Theoretical for $C_{29}H_{37}N_6O_3$ $[M+H]^+$ = 517.2920. Found, 517.2922.

6-(((*S*)-1-((*R*)-2-Ethoxypropyl)piperidin-3-yl)methyl)-2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (43)

Sodium hydride (7.86 mg, 0.20 mmol) was added to 6-(((*S*)-1-((*R*)-2-hydroxypropyl)piperidin-3-yl)methyl)-2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (0.08 g, 0.16 mmol) and iodoethane (0.017 mL, 0.21 mmol) in anhydrous DMF (1 mL) at 0 ºC under nitrogen. The resulting solution was stirred at 0 ºC and warmed up to rt for 16 h. The reaction mixture was quenched with saturated ammonium chloride solution (10 mL) and extracted into ethyl acetate (3 x 20 mL), The organic layer was dried over Na_2SO_4, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 10% MeOH in DCM. Product containing fractions were evaporated to dryness to afford a colourless gum 40 mg, 91% pure by LCMS still contained starting material. The product was purified by preparative HPLC (Phenomenex Gemini C18 110A (axia) column, 5µ silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 0.1% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford the title compound (0.015 g, 18%) as a cream foam.

m/z (ES+) $[M+H]^+$ = 517;

HRMS calculated for $C_{29}H_{37}N_6O_3$ $[M+H]^+$ 517.29217, found 517.29169;

^{1}H NMR (400 MHz, $CDCl_3$, 30 °C) 0.75-0.99 (1H, m), 1.05-1.13 (6H, m), 1.20-1.29 (1H, m), 1.30 - 1.47 (2H, m), 1.75-1.83 (2H, br), 1.96 (1H, t), 2.13-2.22 (1H, m), 2.26 - 2.44 (1H, m), 2.49 (4H, d), 2.64-2.75 (1H, m), 3.27 - 3.54 (4H, m), 3.91 (3H, s), 4.20-4.29 (1H, m), 6.98 (1H, dt), 7.26-7.31 (1H, m), 7.33 - 7.45 (2H, m), 7.53 (1H, br), 7.74 (1H, dt), 8.33 (1H, s), 8.66-8.68 (1H, m).

2-(3-Methoxyphenyl)-6-(((*S*)-1-((*S*)-1-methoxypropan-2-yl)piperidin-3-yl)methyl)-5-(2-methylpyridin-3-yl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (44)

(*R*)-1-Methoxypropan-2-yl 4-methylbenzenesulfonate (133 mg, 0.55 mmol) was added in one portion to (*S*)-2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-6-(piperidin-3-ylmethyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (250 mg, 0.50 mmol) and potassium carbonate (0.085 mL, 1.49 mmol) in dioxane (3 mL) at rt. The resulting suspension was stirred at 90 °C for 20 h. A further 1eq (*R*)-1-methyoxypropan-2-yl 4-methylbenzenesulfonate and DMF (5 mL) were added and the mixture transferred to a microwave vial and heated at 100 °C for 1 h and then at 120 °C for 6 h. The reaction mixture was filtered and concentrated, and the crude product purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5µ silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 0.1% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford the title compound (23.6 mg, 9%) as a colourless foam.

m/z (ES+) $[M+H]^+$ = 503;

HRMS calculated for $C_{28}H_{35}O_3N_6$ $[M+H]^+$ 503.27652, found 503.27634;

^{1}H NMR (400 MHz, DMSO-d_6, 30 °C) δ 0.94 - 1.13 (1H, m), 1.15 - 1.35 (1H, m), 1.56 (2H, dddd), 1.66 - 1.77 (1H, m), 1.89 (1H, s), 2.15 (1H, dd), 2.49 (3H, d), 2.57 - 2.70 (1H, m), 2.80 (1H, t), 2.89 (1H, t), 2.96 - 3.08 (2H, m), 3.26 (1H, s), 3.74 - 3.87 (1H, m), 3.95 (1H, dd), 7.17 - 7.26 (1H, m), 7.60 - 7.72 (1H, m), 8.45 (1H, dt).

(*R*)-1-Methoxypropan-2-yl 4-methylbenzenesulfonate

p-Toluenesulfonyl chloride (2.33 g, 12.21 mmol) was added in one portion to (*R*)-1-methoxypropan-2-ol (1.09 mL, 11.1 mmol) and triethylamine (3.09 mL, 22.2 mmol) in DCM (10 mL) at rt under nitrogen. The resulting solution was stirred at rt overnight. The reaction

mixture was diluted with DCM (50 mL), and washed sequentially with saturated NH_4Cl (50 mL) and saturated brine (20 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 10% MeOH in DCM, then again in 100% DCM. Pure fractions were evaporated to dryness to afford the title compound as a colourless oil (0.953 g, 35%).
m/z (ES+) [M+H]+ = 245;
^{1}H NMR (400 MHz, DMSO-*d*$_6$, 30°C) δ 1.14 (6H, d), 2.41 (3H, s), 3.13 (3H, s), 3.25 - 3.38 (2H, m), 4.66 (1H, pd), 7.46 (2H, d), 7.73 - 7.81 (2H, m).

(*S*)-2-(3-Methoxyphenyl)-5-(2-methylpyridin-3-yl)-6-((1-(oxazol-5-ylmethyl)piperidin-3-yl)methyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (45)
Sodium triacetoxyhydroborate (29.5 mg, 0.14 mmol) was added in one portion to oxazole-5-carbaldehyde (13.5 mg, 0.14 mmol) and (*S*)-2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-6-(piperidin-3-ylmethyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (40 mg, 0.09 mmol) in DCE (1 mL) at rt under nitrogen. The resulting mixture was stirred at rt for 16 h. The reaction was diluted with MeOH (2 mL) and evaporated to dryness to give crude product. This was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5µ silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 0.5% ammonia) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford the title compound (15.9 mg, 34%) as a solid film.
m/z (ES+) $[M+H]^+$ = 512;
HRMS calculated for $C_{28}H_{30}N_7O_3$ $[M+H]^+$ 512.24046, found 512.24036;

(*S*)-6-((1-(Furan-2-ylmethyl)piperidin-3-yl)methyl)-2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (46)
Sodium triacetoxyhydroborate (29.5 mg, 0.14 mmol) was added in one portion to furan-2-carbaldehyde (13.39 mg, 0.14 mmol) and (*S*)-2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-6-(piperidin-3-ylmethyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (40 mg, 0.09 mmol) in DCE (1 mL) at rt under nitrogen. The resulting mixture was stirred at rt for 16 h. The reaction was diluted with MeOH (1 mL) and evaporated to dryness to give crude product. The crude product was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5µ silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 0.1% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford the title compound (31.0 mg, 65%) as a colourless dry film.
m/z (ES+) $[M+H]^+$ = 511;
HRMS calculated for $C_{29}H_{31}N_6O_3$ $[M+H]^+$ 511.24522, found 511.24518;
^{1}H NMR (400 MHz, DMSO-*d*$_6$, 100 °C) δ 0.80 (1H, s), 1.19 - 1.53 (4H, m), 1.71 (2H, s), 1.90 - 1.99 (1H, m), 2.29 - 2.63 (5H, m), 3.40 (2H, s), 3.90 (3H, s), 6.13 (1H, s), 6.31 (1H, s), 7.06 (1H, d), 7.33 - 7.39 (1H, m), 7.43 - 7.53 (2H, m), 7.56 - 7.62 (2H, m), 7.86 (1H, d), 8.58 - 8.63 (1H, m), 8.99 (1H, s)

(*S*)-6-((1-Isobutylpiperidin-3-yl)methyl)-2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (47)
Potassium carbonate (25.7 mg, 0.19 mmol) was added to (*S*)-2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-6-(piperidin-3-ylmethyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (40 mg, 0.09 mmol) and 1-bromo-2-methylpropane (0.019 mL, 0.18 mmol) in DMF (1 mL) at rt under nitrogen. The resulting suspension was stirred at 100 °C for 18 h. The reaction mixture was filtered through a HPLC filter and evaporated to dryness, and the residue was purified by

preparative HPLC (Waters XBridge Prep C18 OBD column, 5µ silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 0.5% ammonia) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford the title compound (18.3 mg, 40%) as a solid.
m/z (ES+) $[M+H]^+$ = 487;
HRMS calculated for $C_{28}H_{35}N_6O_2$ $[M+H]^+$ 487.28160, found 487.28128;
^{1}H NMR (400.13 MHz, DMSO-d_6) δ 0.75 (6H, dd), 0.85 (1H, m), 1.32 (3H, m), 1.47 (1H, m), 1.52 (3H, m), 1.85 (1H, m), 1.93 (2H, d), 2.34 (1H, m), 2.40 (3H, s), 3.65 (1H, m), 3.80 (3H, s), 3.82 (1H, m), 7.05 (1H, m), 7.35 (1H, m), 7.50 (1H, m), 7.60 (2H, m), 7.88 (1H, m), 8.62 (1H, m), 9.00 (1H, s).

(*S*)-6-((1-(Cyclobutylmethyl)piperidin-3-yl)methyl)-2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (48)
Potassium carbonate (32.1 mg, 0.23 mmol) was added to (*S*)-2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-6-(piperidin-3-ylmethyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (50 mg, 0.12 mmol) and bromomethylcyclobutane (17 mg, 0.12 mmol) in DMF (1 mL) at rt under air. The resulting suspension was stirred at 80 °C for 16 h. The reaction was diluted with EtOAc and filtered and then purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5µ silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 0.5 NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford the title compound (38.0 mg, 66%).
m/z (ES+) $[M+H]^+$ = 499;
HRMS calculated for $C_{29}H_{35}O_2N_6$ $[M+H]^+$ 499.28160, found 499.28134;
^{1}H NMR (400 MHz, DMSO-d_6, 100 °C) δ 0.84 (1H, s), 1.18 - 1.99 (13H, m), 2.22 (2H, d), 2.28 - 2.38 (2H, m), 2.40 (3H, s), 3.90 (3H, d), 7.06 (1H, d), 7.38 (1H, dd), 7.50 (1H, t), 7.59 (2H, d), 7.87 (1H, d), 8.62 (1H, d), 9.00 (1H, s).

(*S*)-6-((1-(Cyclopentylmethyl)piperidin-3-yl)methyl)-2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (49)
Potassium carbonate (48.1 mg, 0.35 mmol) was added to (*S*)-2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-6-(piperidin-3-ylmethyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (40 mg, 0.09 mmol) and (iodomethyl)cyclopentane (36.5 mg, 0.174 mmol) in dioxane (1 mL) at rt under nitrogen. The resulting suspension was stirred at 80 °C for 17 h. Reaction purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5µ silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 0.5%NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford the title compound (20.9 mg, 23%) as a solid.
m/z (ES+) $[M+H]^+$ = 513;
HRMS calculated for $C_{30}H_{37}N_6O_2$ $[M+H]^+$ 513.29725, found 513.29694;
^{1}H NMR (400 MHz, DMSO-d_6, 100 °C) δ 0.85 (1H, s), 1.11 (2H, s), 1.26-1.30 (3H, m), 1.40-1.51 (4H, m), 1.52-1.72 (4H, m), 1.87-1.95 (2H, m), 2.07 (2H, d), 2.40 (4H, s), 2.90-2.99 (2H, m) 3.64 (1H, s), 3.90 (3H, s), 7.06 (1H, d), 7.38 (1H, d), 7.49 (1H, t), 7.59 (2H, d), 7.87 (1H, d), 8.61 (1H, d), 8.99 (1H, s).

(*S*)-6-((1-(Cyclohexylmethyl)piperidin-3-yl)methyl)-2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (50)
Potassium carbonate (32.1 mg, 0.23 mmol) was added to (*S*)-2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-6-(piperidin-3-ylmethyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (50 mg, 0.12 mmol) and (bromomethyl)cyclohexane (30.8 mg, 0.174 mmol) in DMF (1 mL) at rt under air. The resulting suspension was stirred at 80 °C for 16 h. The reaction was diluted

with EtOAc and filtered and then purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5µ silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 0.5 NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford the title compound (42.0 mg, 69%).
m/z (ES+) $[M+H]^+$ = 527;
HRMS calculated for $C_{31}H_{38}O_2N_6$ $[M+H]^+$ 527.31290, found 527.31268;
^{1}H NMR (400 MHz, DMSO-d_6, 100°C) δ 0.69 - 0.94 (4H, m), 1.01 - 1.77 (17H, m), 1.87 (1H, td), 1.96 (2H, d), 2.40 (3H, s), 3.90 (3H, s), 7.06 (1H, ddd), 7.37 (1H, dd), 7.50 (1H, dd), 7.55 - 7.63 (2H, m), 7.87 (1H, dd), 8.62 (1H, dd), 9.00 (1H, s).

2-(3-Methoxyphenyl)-5-(2-methylpyridin-3-yl)-6-(((*3S*)-1-((tetrahydrofuran-3-yl)methyl)piperidin-3-yl)methyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (51)
To a stirred solution of (*S*)-2-(3-methoxyphenyl)-5-(2-methylpyridin-3-yl)-6-(piperidin-3-ylmethyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (50.0 mg, 0.12 mmol) and (tetrahydrofuran-3-yl)methyl 4-methylbenzenesulfonate (35.7 mg, 0.14 mmol) in DMF (2.0 mL) was added potassium carbonate (32.1 mg, 0.23 mmol) and potassium iodide (28.9 mg, 0.17 mmol) and the mixture heated at 95° C for 3 d. The mixture was cooled to rt and the DMF evaporated *in vacuo* to a residue which was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5µ silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 0.5% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to give the title compound (9.30 mg, 16%).
m/z (ES+) $(M+H)^+$ = 515;
HRMS. Theoretical for $C_{29}H_{35}N_6O_3$ $[M+H]^+$ = 515.2765. Found, 515.2763.

2-(3-Methoxyphenyl)-5-methyl-6-(((*S*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (52)
Methyl 4-amino-1-(3-methoxyphenyl)-1H-pyrazole-3-carboxylate (150 mg, 0.61 mmol) and 1,1-dimethoxy-N,N-dimethylethanamine (0.296 mL, 1.82 mmol) were dissolved in MeOH (2.5 mL) and sealed into a microwave tube. The reaction was heated to 150 for 30 min in the microwave reactor and cooled to rt. The resulting mixture was evaporated to dryness and the residue was azeotroped with toluene (3 x 20 mL) to afford a yellow oil. This oil and ((*R*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methanamine (120 mg, 0.61 mmol) were dissolved in MeOH (2.5 mL) and sealed into a microwave tube. The reaction was heated to 140 °C for 80 min in the microwave reactor and cooled to rt. The crude product was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5µ silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 1% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford the title compound (75 mg, 28%) as a cream solid.
m/z (ES+), $[M+H]^+$ = 438;
HRMS. Theoretical for $C_{24}H_{32}N_5O_3$ $[M+H]^+$ = 438.2500. Found, 438.2496;
^{1}H NMR (400 MHz, DMSO-d_6, 30 °C) δ 1.04 - 1.16 (1H, m), 1.34 - 1.47 (2H, m), 1.52 - 1.66 (2H, m), 1.66 - 1.80 (2H, m), 1.81 - 2.04 (3H, m), 2.11 (1H, t), 2.31 (2H, d), 2.58 (3H, s), 2.61 - 2.74 (2H, m), 3.54 (1H, q), 3.68 (1H, q), 3.82 - 3.90 (4H, m), 3.97 (2H, d), 7.03 (1H, dd), 7.48 (1H, t), 7.54 - 7.61 (2H, m), 9.01 (1H, s).

5-Isobutyl-2-(3-methoxyphenyl)-6-(((*S*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (53)
Methyl 1-(3-methoxyphenyl)-4-((*Z*)-3-methyl-N'-(((*S*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)butanimidamido)-1H-pyrazole-3-carboxylate (578 mg, 1.13 mmol) and potassium carbonate (468 mg, 3.39 mmol) were suspended in DMF (15 mL) and

the reaction was stirred at 100 °C overnight. The reaction mixture was evaporated to dryness and redissolved in water (25 mL) and EtOAc (25 mL). The aqueous layer was re-extracted with EtOAc (25 mL) and the combined organics washed with brine. The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5µ silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 0.5% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford the title compound (38 mg, 7%) as a colourless dry film.
m/z (ES+) $[M+H]^+$ = 480;
HRMS calculated for $C_{27}H_{38}N_5O_3$ $[M+H]^+$ 480.29692, found 480.29660;
^{1}H NMR (400 MHz, DMSO-d_6, 30 °C) δ 1.06 (6H, dd), 1.16 (1H, d), 1.47 (2H, dd), 1.80 (7H, d), 2.38 (4H, d), 2.75 (4H, d), 3.60 (1H, td), 3.69 - 3.78 (1H, m), 3.93 (4H, m), 4.05 (2H, s), 7.10 (1H, ddd), 7.55 (1H, t), 7.6 - 7.69 (2H, m), 9.15 (1H, s).

Methyl 1-(3-methoxyphenyl)-4-((*Z*)-3-methyl-1-(((*R*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methylamino)butylideneamino)-1H-pyrazole-3-carboxylate
3-Methyl-N-(((*R*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)butanamide (320 mg, 1.13 mmol), PS-triphenylphosphine (1133 mg, 3.40 mmol) and triethylamine (0.237 mL, 1.70 mmol) were dissolved in MeCN (10 mL) under nitrogen. Perchloromethane (0.437 mL, 4.53 mmol) was added and the resulting mixture was stirred at rt for 16 h. Methyl 4-amino-1-(3-methoxyphenyl)-1H-pyrazole-3-carboxylate (280 mg, 1.13 mmol) was added and the resulting solution stirred at rt for 1 hr. The reaction mixture was filtered and the PS-PPh3 washed with DCM. The volatiles were removed to give the crude title compound as a dark brown tar which was used without further purification.

3-Methyl-N-(((*R*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)butanamide (17, R6 = (R)-THF-CH_2, R5 = iBu)
3-Methylbutanoyl chloride (0.203 mL, 1.66 mmol) was added dropwise to a stirred solution of ((*R*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methanamine (300 mg, 1.51 mmol) and triethylamine (0.316 mL, 2.27 mmol) in DCM (5 mL) at 5 ºC. The resulting solution was stirred at rt for 1 h. The reaction mixture was washed sequentially with water (2 x 20 mL), 2M HCl (20 mL) and sat. $NaHCO_3$ (20 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 8% (1% NH_3 in MeOH) in DCM. Pure fractions were evaporated to dryness to afford the title compound (345 mg, 81%) as a cream waxy solid.
m/z (ES+), $[M+H]^+$ = 295;
^{1}H NMR (400 MHz, DMSO-d_6, 30 °C) δ 0.78 - 0.92 (7H, m), 1.29 - 2.01 (13H, m), 2.29 (2H, d), 2.68 (1H, d), 2.76 - 2.95 (3H, m), 3.52 - 3.6 (1H, m), 3.65 - 3.74 (1H, m), 3.82 - 3.91 (1H, m), 7.63 - 7.74 (1H, m).

5-(2-Methylpyridin-3-yl)-2-phenyl-6-(((*S*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (54)
(*R*)-(Tetrahydrofuran-2-yl)methyl 4-methylbenzenesulfonate (75 mg, 0.29 mmol) was added dropwise to (*S*)-5-(2-methylpyridin-3-yl)-2-phenyl-6-(piperidin-3-ylmethyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (107 mg, 0.27 mmol) and potassium carbonate (92 mg, 0.67 mmol) in DMF (2 mL). The resulting suspension was stirred at rt for 60 h. Then heated to 120 °C for 2 h in the microwave. The reaction mixture was diluted with EtOAc (50 mL) and water and washed sequentially with sat. NH_4Cl (50 mL), water (50 mL), and sat. brine (50 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product. The

crude product was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5µ silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 0.1% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford the title compound (57.0 mg, 44%) as a colourless dry film.
m/z (ES+) $[M+H]^+$ = 485;
HRMS. Theoretical for $C_{28}H_{33}N_6O_2$ $[M+H]^+$ = 485.26595. Found, 485.26578.
^{1}H NMR (400 MHz, DMSO-d_6, 100°C) δ 0.73 (1H, s), 1.13 - 1.45 (4H, m), 1.52 - 1.81 (4H, m), 1.88 - 1.99 (1H, m), 2.13 - 2.2 (2H, m), 2.31 (3H, s), 2.42 - 2.49 (2H, m), 3.44 (2H, q), 3.51 - 3.63 (2H, m), 3.63 - 3.75 (1H, m), 3.81 (1H, s), 7.28 (1H, dd), 7.39 (1H, t), 7.51 (2H, t), 7.79 (1H, d), 7.85 - 7.99 (2H, m), 8.52 (1H, dd), 8.88 (1H, s).

(*S*)-5-(2-Methylpyridin-3-yl)-2-phenyl-6-(piperidin-3-ylmethyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (13, Ar = Ph)
A suspension of (*R*)-tert-butyl 3-((5-(2-methylpyridin-3-yl)-7-oxo-2-phenyl-2H-pyrazolo[4,3-d]pyrimidin-6(7H)-yl)methyl)piperidine-1-carboxylate (1.47 g, 2.94 mmol) in HCl (4M in dioxane) (50 mL, 1.65 mol) was stirred at rt for 4 h. The solvent was evaporated in vacuo to yield a white solid. This material was dissolved in MeOH, loaded onto an SCX2 ion exchange column and washed with MeOH. The product was eluted from the column using 1% NH_3 / MeOH and the solvent evaporated to yield the title compound (1.14 g, 97%) as a yellow solid.
m/z (ES+) $[M+H]^+$ = 401;
^{1}H NMR (400.13 MHz, DMSO-d_6) δ 0.80 - 0.93 (1H, m), 1.06 - 1.19 (1H, m), 1.28 - 1.52 (3H, m), 1.93 - 2.07 (2H, m), 2.24 - 2.33 (1H, m), 2.38 (3H, s), 2.65 - 2.70 (1H, m), 3.40 - 3.54 (1H, m), 3.84 - 3.96 (1H, m), 7.39 - 7.42 (1H, m), 7.47 - 7.51 (1H, m), 7.59 - 7.63 (2H, m), 7.92 - 7.95 (1H, m), 8.03 - 8.05 (2H, m), 8.62 - 8.63 (1H, m), 9.14 (1H, s)

(*R*)-tert-Butyl 3-((5-(2-methylpyridin-3-yl)-7-oxo-2-phenyl-2H-pyrazolo[4,3-d]pyrimidin-6(7H)-yl)methyl)piperidine-1-carboxylate
(*R,Z*)-tert-Butyl 3-((N'-(3-(methoxycarbonyl)-1-phenyl-1H-pyrazol-4-yl)-2-methylnicotinimidamido)methyl)piperidine-1-carboxylate (2.85 g, 5.35 mmol) and potassium carbonate (2.22 g, 16.1 mmol) were suspended in DMF (30 mL) and the reaction was heated to 130 °C for 45 min then cooled to rt. The reaction mixture was evaporated to near dryness, redissolved in EtOAc (150 mL), and washed sequentially with sat. $NaHCO_3$ (100 mL) and sat. brine (100 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 10% MeOH in EtOAc. Pure fractions were evaporated to dryness to afford the title compound (1.53 g, 57%) as a yellow solid.
m/z (ES-) $[M-H]^-$ = 499;
^{1}H NMR (400.13 MHz, DMSO-d_6) δ 0.95 - 1.46 (14H, m), 2.31 - 2.45 (4H, m), 2.61 - 2.67 (1H, m), 3.46 - 3.94 (4H, m), 7.39 - 7.42 (1H, m), 7.49 (1H, t), 7.59 - 7.63 (2H, m), 7.91 - 7.97 (1H, m), 8.03 - 8.06 (2H, m), 8.63 (1H, d), 9.16 (1H, d)

(*R,Z*)-tert-Butyl 3-((N'-(3-(methoxycarbonyl)-1-phenyl-1H-pyrazol-4-yl)-2-methylnicotinimidamido)methyl)piperidine-1-carboxylate
Phosphorus pentachloride (1.86 g, 8.92 mmol) was added portionwise to methyl 4-(2-methylnicotinamido)-1-phenyl-1H-pyrazole-3-carboxylate (1.5 g, 4.46 mmol) in toluene (50 mL) at rt and under nitrogen. The resulting suspension was stirred at 110 °C for 4 h to give a yellow suspension. The reaction mixture was evaporated to dryness and redissolved in DCE (50 mL). This was added to a solution of (*R*)-tert-butyl 3-(aminomethyl)piperidine-1-

carboxylate (1.44 g, 6.69 mmol) and N-ethyl-N-isopropylpropan-2-amine (4.66 mL, 26.8 mmol) in DCE (50 mL). The reaction mixture went from a yellow suspension to a yellow solution when stirred at rt for 20 min. The reaction mixture was diluted with DCM (100 mL), and washed sequentially with sat. $NaHCO_3$ (200 mL), water (200 mL) and brine (100 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude title compound (2.85 g, 120%) which was used without further purification.
m/z (ES+) $[M+H]^+$ = 533.

Methyl 4-(2-methylnicotinamido)-1-phenyl-1H-pyrazole-3-carboxylate (12, Ar = Ph)
2-(1H-Benzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (4.36 g, 11.5 mmol) was added to a stirred solution of methyl 4-amino-1-phenyl-1H-pyrazole-3-carboxylate (2.5 g, 11.51 mmol) and 2-methylnicotinic acid (1.58 g, 11.5 mmol) in DMF (100 mL). The reaction was stirred at rt for 1 h then treated with N,N-dimethylethanamine (2.49 mL, 23.0 mmol). The resulting reaction was stirred at rt for 16 h then evaporated to dryness. The resulting residue was partitioned between EtOAc (300 mL) and sat $NaHCO_3$ (100 mL). The layers were separated and the organic layer was washed with water (2 x 100 mL) and brine (100 mL) then dried ($MgSO_4$), filtered and evaporated to yield crude product. The crude product was purified by flash silica chromatography, elution gradient 30 to 40% EtOAc in DCM. Pure fractions were evaporated to dryness to afford methyl 4-(2-methylnicotinamido)-1-phenyl-1H-pyrazole-3-carboxylate (2.75 g, 71%) as a white solid.
m/z (ES+) $[M+H]^+$ = 337;
^{1}H NMR (400.13 MHz, DMSO-d_6) δ 2.65 (3H, s), 3.89 (3H, s), 7.37 - 7.44 (2H, m), 7.53 - 7.58 (2H, m), 7.92 - 7.97 (3H, m), 8.59 - 8.60 (1H, m), 8.98 (1H, s), 9.88 (1H, s).

2-(4-Methoxyphenyl)-5-(2-methylpyridin-3-yl)-6-(((*S*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (55)
Methyl 1-(4-methoxyphenyl)-4-((*Z*)-2-methyl-N'-(((*S*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)nicotinimidamido)-1H-pyrazole-3-carboxylate (258 mg, 0.47 mmol) and potassium carbonate (195 mg, 1.42 mmol) were suspended in DMF (3 mL) and the reaction was stirred at 100 °C overnight. The crude product was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5µ silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 0.5% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford the title compound (99 mg, 40%) as a colourless dry film.
m/z (ES+) $[M+H]^+$ = 515;
HRMS. Theoretical for $C_{29}H_{35}N_6O_3$ $[M+H]^+$ = 515.2766. Found, 515.2765;
^{1}H NMR (400 MHz, DMSO-d_6, 100 °C) δ 0.74 - 0.91 (1H, m), 1.20 - 1.50 (4H, m), 1.63 - 1.89 (5H, m), 1.97 - 2.06 (1H, m), 2.24 - 2.28 (2H, m), 2.39 (3H, s), 2.51 - 2.60 (3H, m), 3.50 - 3.57 (1H, m), 3.63 - 3.71 (1H, m), 3.72 - 3.84 (2H, m), 3.86 (3H, s), 7.14 (2H, d), 7.37 (1H, dd), 7.87 (1H, d), 7.92 (2H, d), 8.61 (1H, dd), 8.86 (1H, s).

Methyl 1-(4-methoxyphenyl)-4-((*Z*)-2-methyl-N'-(((*S*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)nicotinimidamido)-1H-pyrazole-3-carboxylate
2-Methyl-N-(((*R*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)nicotinamide (144 mg, 0.45 mmol), PS-triphenylphosphine (454 mg, 1.36 mmol) and triethylamine (0.095 mL, 0.68 mmol) were dissolved in MeCN (2 mL) under nitrogen. Perchloromethane (0.131 mL, 1.36 mmol) was added and the resulting mixture was stirred at rt for 16 h. Methyl 4-amino-1-(4-methoxyphenyl)-1H-pyrazole-3-carboxylate (112 mg, 0.45 mmol) in MeCN (2 mL) was added and the resulting solution stirred at rt for 8 hrs. The reaction mixture was

filtered and the PS-PPh_3 washed with DCM. The volatiles were removed to give the crude title compound (248 mg, 100%) as a tan solid which was used without further purification.

Methyl 1-(3-ethoxyphenyl)-4-((*Z*)-2-methyl-N'-(((*S*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)nicotinimidamido)-1H-pyrazole-3-carboxylate (56)
Methyl 1-(3-ethoxyphenyl)-4-((*Z*)-2-methyl-N'-(((*S*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)nicotinimidamido)-1H-pyrazole-3-carboxylate (2.87 g, 5.11 mmol) and potassium carbonate (2.12 g, 15.3 mmol) were suspended in DMF (50 mL) and the reaction was stirred at 100 °C overnight. The reaction mixture was evaporated to dryness and redissolved in water (25 mL) and EtOAc. The aqueous layer was re-extracted with EtOAc and the combined organic extracts washed with brine. The organic layer was evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 10% MeOH in DCM. Pure fractions were evaporated to dryness to afford the title compound (1.35 g, 50%) as a beige dry film.
m/z (ES+) $[M+H]^+$ = 529;
HRMS calculated for $C_{30}H_{37}N_6O_3$ $[M+H]^+$ 529.29217, found 529.29175;
^{1}H NMR (400 MHz, DMSO-d_6, 30 °C) δ 0.64 - 0.87 (1H, m), 1.13 - 1.55 (6H, m), 1.55 - 1.97 (5H, m), 2.21 (2H, s), 2.37 (3H, s), 2.43 - 2.63 (4H, m), 3.41 - 3.99 (5H, m), 4.15 (2H, q), 7.01 - 7.07 (1H, m), 7.36 - 7.44 (1H, m), 7.49 (1H, t), 7.56 - 7.64 (2H, m), 7.9 - 7.98 (1H, m), 8.6 - 8.65 (1H, m), 9.15 (1H, s).

Methyl 1-(3-ethoxyphenyl)-4-((*Z*)-(2-methylpyridin-3-yl)(((*R*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methylamino)methyleneamino)-1H-pyrazole-3-carboxylate
2-Methyl-N-(((*R*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)nicotinamide (1.62 g, 5.11 mmol), PS-triphenylphosphine (5.11 g, 15.3 mmol) and triethylamine (1.07 mL, 7.67 mmol) were dissolved in MeCN (30 mL) under nitrogen. Perchloromethane (1.97 mL, 20.4 mmol) was added and the resulting mixture was stirred at rt for 16 h. Methyl 4-amino-1-(3-ethoxyphenyl)-1H-pyrazole-3-carboxylate (1.67 g, 6.39 mmol) was added and the resulting solution stirred at rt for 1 hr. The reaction mixture was filtered and the PS-PPh_3 washed with DCM. The volatiles were removed to give the crude title compound (2.87 g, 100%) as a dark brown tar which was used without further purification.
m/z (ES+), $[M+H]^+$ = 561.

2-Methyl-N-(((*R*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)nicotinamide (19)
O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (318 mg, 0.84 mmol) was added to a suspension of ((*R*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methanamine, 2-methylnicotinic acid (115 mg, 0.84 mmol) and N,N-diisopropylethylamine (0.322 mL, 1.84 mmol) in DMF (5 mL) at rt and stirred for 72 h. Water (5 mL) was added and the organic layer separated and washed sequentially with sat. $NaHCO_3$ (15 mL) and brine (15 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated to provide crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 10% 7M NH_3/MeOH in DCM. Pure fractions were evaporated to dryness to afford the title compound (227 mg, 85%) as a white solid.
m/z (ES+), $[M+H]^+$ = 318;

((*R*)-1-(((*R*)-Tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methanamine
tert-Butyl ((*R*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methylcarbamate (250 mg, 0.84 mmol) was stirred in DCM (5.00 mL) and TFA (0.5 mL) at rt for 4 h. The crude

product was purified by ion exchange chromatography, using an SCX column. The desired product was eluted from the column using 7M NH_3/MeOH and pure fractions were evaporated to dryness to afford the title compound (166 mg, 100%) as a colourless gum.
m/z (ES+), $[M+H]^+$ = 199;
^{1}H NMR (400 MHz, DMSO-d_6, 30 °C) δ 0.73 - 0.87 (1H, m), 1.31 - 2.00 (13H, m), 2.26 - 2.31 (2H, m), 2.34 - 2.40 (1H, m), 2.64 - 2.75 (1H, m), 2.84 - 3.00 (1H, m), 3.51 - 3.61 (1H, m), 3.65 - 3.74 (1H, m), 3.82 - 3.94 (1H, m).

tert-Butyl ((*R*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methylcarbamate
(*S*)-tert-Butyl piperidin-3-ylmethylcarbamate hydrochloride (1.07 g, 4.26 mmol), (*R*)-(tetrahydrofuran-2-yl)methyl 4-methylbenzenesulfonate (1.2 g, 4.68 mmol), potassium iodide (0.848 g, 5.11 mmol) and potassium carbonate (1.18 g, 8.51 mmol) were suspended in MeCN (10 ml) and sealed into a microwave tube. The reaction was heated to 120 °C for 30 min in the microwave reactor and cooled to rt. The reaction mixture was filtered then evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 8% MeOH(7N NH_3) in DCM. Pure fractions were evaporated to dryness to afford the title compound (1.04 g, 82%) as a white solid.
m/z (ES+), $[M+Na]^+$ = 321;
^{1}H NMR (400 MHz, DMSO-d_6, 30 °C) δ 0.52 - 0.67 (1H, m), 1.14 (9H, s), 1.17 - 1.26 (1H, m), 1.28 - 1.80 (8H, m), 2.00 - 2.15 (2H, m), 2.39 - 2.64 (4H, m), 2.94 (1H, d), 3.29 - 3.39 (1H, m), 3.43 - 3.51 (1H, m), 3.58 - 3.71 (1H, m), 6.53 (1H, s).

(*R*)-(Tetrahydrofuran-2-yl)methyl 4-methylbenzenesulfonate
4-Methylbenzene-1-sulfonyl chloride (11.8 g, 61.7 mmol) was added portionwise to (*R*)-(tetrahydrofuran-2-yl)methanol (6 g, 58.8 mmol) and pyridine (9.50 mL, 117 mmol) in DCM (120 mL) cooled to 0 ºC over a period of 5 min under nitrogen. The resulting solution was stirred at rt for 16 h. The DCM was removed under reduced pressure and replaced with diethyl ether (50 mL) the organic phase was washed with 10% aq. $CuSO_4$ solution (2 x 50 mL) and dried over $MgSO_4$. The volatiles were removed to give the crude product which was purified by flash silica chromatography, elution gradient 10 to 80% EtOAc in isohexane. Pure fractions were evaporated to dryness to afford the title compound (10.1 g, 67%) as acolourless oil.
m/z (ES+), $[M+H]^+$ = 257;
^{1}H NMR (400 MHz, DMSO-d_6, 30 °C) δ 1.42 - 1.55 (1H, m), 1.67 - 1.92 (3H, m), 2.42 (3H, s), 3.55 - 3.67 (2H, m), 3.86 - 4.05 (3H, m), 7.48 (2H, d), 7.74 - 7.81 (2H, m).

5-(2-Methylpyridin-3-yl)-2-(3-propoxyphenyl)-6-(((*S*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (57)
Copper(II) acetate (80 mg, 0.44 mmol) was added to 5-(2-methylpyridin-3-yl)-6-(((*S*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (120 mg, 0.29 mmol) and pyridine (0.048 mL, 0.59 mmol) in DCM (2 mL) at rt under air. Air was bubbled through the resulting suspension for 20 h then the mixture was stirred for 70 h. Silica (~25 g) was added and the resulting mixture concentrated to dryness and purified by flash silica chromatography, elution gradient 0 to 20% MeOH in DCM. The fractions containing pure product were combined and evaporated to dryness to afford the title compound (48.0 mg, 30%) as a colourless dry film.
m/z (ES+) $[M+H]^+$ = 544;
HRMS calculated for $C_{31}H_{39}N_6O_3$ $[M+H]^+$ 543.30782, found 543.30743;

^{1}H NMR (400 MHz, DMSO-d_6, 30 °C) δ 0.80 (1H, s), 1.00 (3H, t), 1.14 - 1.97 (13H, m), 2.22 (2H, s), 2.37 (3H, d), 3.43 - 3.98 (6H, m), 4.05 (2H, t), 7.02 - 7.08 (1H, m), 7.36 - 7.44 (1H, m), 7.49 (1H, t), 7.56 - 7.63 (2H, m), 7.9 - 7.98 (1H, m), 8.6 - 8.65 (1H, m), 9.16 (1H, s).

2-(3-Isopropoxyphenyl)-5-(2-methylpyridin-3-yl)-6-(((*S*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (58)

Copper(II) acetate (100 mg, 0.55 mmol) was added to 3-isopropoxyphenylboronic acid (132 mg, 0.73 mmol), 5-(2-methylpyridin-3-yl)-6-(((*S*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (150 mg, 0.37 mmol) and pyridine (0.059 mL, 0.73 mmol) in DCM (2 mL) at rt under air. Air was bubbled through the resulting suspension for 20 h then the mixture was stirred for the weekend. Silica (~25 g) was added and the resulting mixture concentrated to dryness and purified by flash silica chromatography, elution gradient 0 to 20% MeOH in DCM. Pure fractions of the two isomers were kept to one side and the mixed fractions recolumned under the same conditions. The fractions containg pure isomers by TLC were combined and were evaporated to dryness to afford the title compound (47.0 mg, 24%) as a colourless dry film.
m/z (ES+) $[M+H]^+$ = 544;
HRMS calculated for $C_{31}H_{39}N_6O_3$ $[M+H]^+$ 543.30762, found 543.30774;
^{1}H NMR (400 MHz, DMSO-d_6, 100 °C) δ 1.22 - 1.54 (12H, m), 1.6 - 1.89 (6H, m), 2.2 - 2.33 (1H, m), 2.40 (3H, s), 3.17 - 3.23 (1H, m), 3.46 - 4.05 (6H, m), 4.7 - 4.78 (1H, m), 7 - 7.06 (1H, m), 7.34 - 7.4 (1H, m), 7.47 (1H, t), 7.51 - 7.59 (2H, m), 7.88 (1H, d), 8.59 - 8.64 (1H, m), 8.98 (1H, s).

5-(2-Methylpyridin-3-yl)-6-(((*3S*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (21)

To a solution of 5-(2-Methylpyridin-3-yl)-2-(tetrahydro-2H-pyran-2-yl)-6-(((*S*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (1.134 g, 2.30 mmol) in MeOH (10 mL) was added 2M HCl (5.76 mL, 11.5 mmol) and the resulting solution stirred overnight at rt. The reaction was neutralised with sat. $NaHCO_3$ and the MeOH removed under reduced pressure. DCM (50 mL) was added and the layers separated. The aqueous layer was re-extracted with DCM and the combined organics dried over $MgSO_4$, filtered and evaporated to afford crude product which was purified by flash silica chromatography, elution gradient 0 to 20% MeOH in DCM. Pure fractions were evaporated to dryness to afford the title compound (0.856 g, 92%) as a colourless dry film.
m/z (ES+), $[M+H]^+$ = 409;
^{1}H NMR (400 MHz, DMSO-d_6, 30°C) δ 0.63 - 0.87 (1H, m), 1.12 - 1.96 (10H, m), 2.14 - 2.26 (2H, m), 2.32 (3H, d), 2.45 - 2.61 (2H, m), 3.41 - 4.01 (5H, m), 7.31 - 7.43 (1H, m), 7.84 - 7.97 (1H, m), 8.14 (1H, s), 8.57 - 8.65 (1H, m), 14.28 (1H, s).

5-(2-Methylpyridin-3-yl)-2-(tetrahydro-2H-pyran-2-yl)-6-(((*S*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (20)

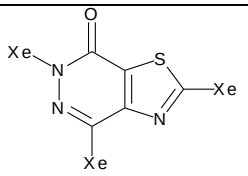
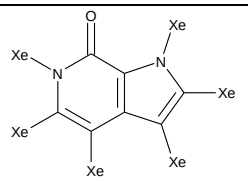
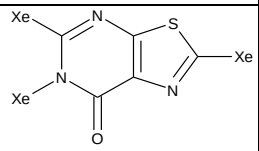
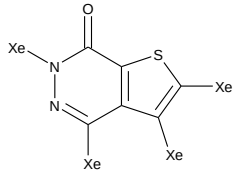
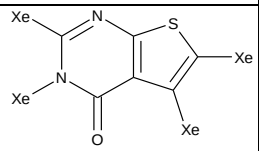
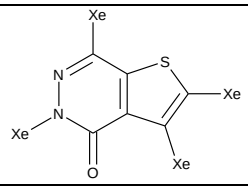
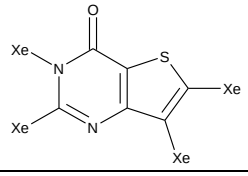
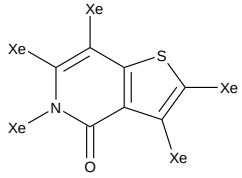
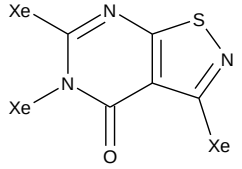
Methyl 4-((*Z*)-2-methyl-N'-(((*S*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)nicotinimidamido)-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole-3-carboxylate (5.17 g, 9.86 mmol) and potassium carbonate (4.08 g, 29.6 mmol) were suspended in DMF (15 mL) and the reaction was stirred at 100 °C overnight. The reaction mixture was evaporated to dryness and redissolved in water (25 mL) and EtOAc (25 mL). The aqueous layer was re-extracted with EtOAc (25 mL) and the combined organic extracts were washed with brine. The organic layer was evaporated to afford crude product which was purified by flash silica chromatography, elution gradient 0 to 10% MeOH in DCM. Pure fractions were evaporated to dryness to afford the title compound (1.13 g, 23%) as a white solid.

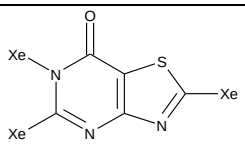
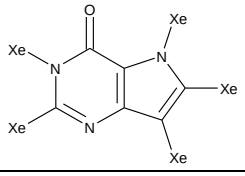
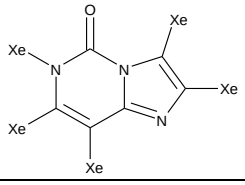
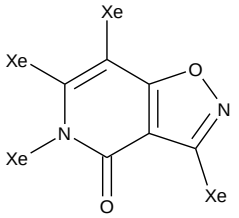
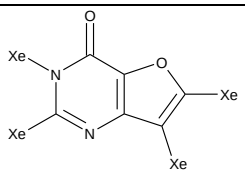
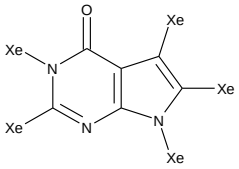
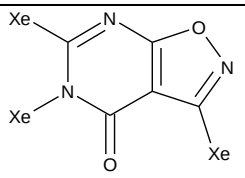
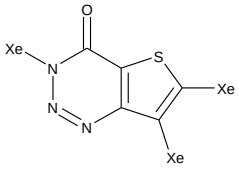
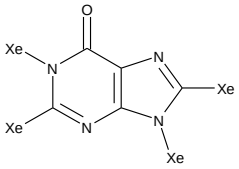
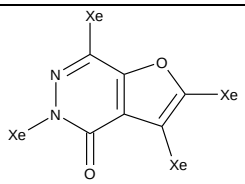
m/z (ES+), $[M+H]^+$ = 493;
^{1}H NMR (400 MHz, DMSO-d_6, 100 °C) δ 0.81 (1H, s), 1.19 - 1.53 (5H, m), 1.56 - 1.89 (9H, m), 1.93 - 2.33 (6H, m), 2.36 (3H, s), 3.41 - 4.05 (7H, m), 5.68 - 5.74 (1H, m), 7.32 - 7.38 (1H, m), 7.84 (1H, d), 8.39 (1H, s), 8.60 (1H, d).

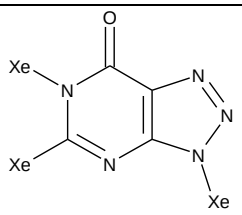
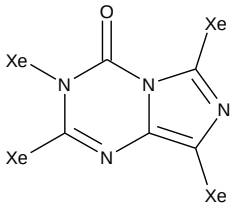
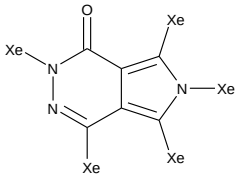
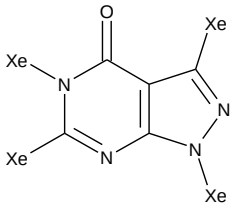
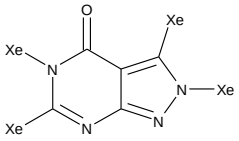
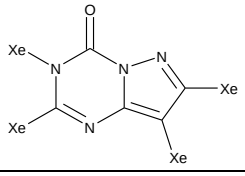
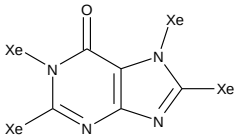
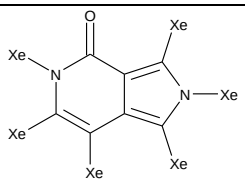
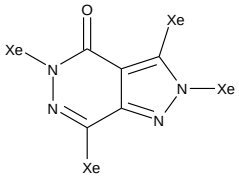
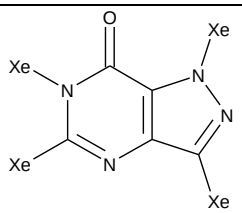
Methyl 4-((*Z*)-2-methyl-N'-(((*S*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)nicotinimidamido)-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole-3-carboxylate
2-Methyl-N-(((*R*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)nicotinamide (3.13 g, 9.86 mmol), PS-triphenylphosphine (13.2 g, 39.4 mmol) and triethylamine (2.06 mL, 14.8 mmol) were dissolved in MeCN (100 mL) under nitrogen. Perchloromethane (7.61 mL, 78.9 mmol) was added and the resulting mixture was stirred at rt for 16 h. Methyl 4-amino-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazole-3-carboxylate (2.22 g, 9.86 mmol) was added and the resulting solution stirred at rt for 1 hr. The reaction mixture was filtered and the PS-PPh_3 washed with DCM. The volatiles were removed to give the crude title compound as a dark brown tar which was used without further purification.
m/z (ES+), $[M+H]^+$ = 525.
^{1}H NMR (400 MHz, DMSO-d_6, 30 °C) δ 0.93 (1H, d), 1.45 (2H, ddd), 1.54 - 2.06 (9H, m), 2.25 - 2.38 (2H, m), 2.43 - 2.56 (2H, m), 2.63 - 2.73 (1H, m), 2.93 (1H, d), 3.05 - 3.16 (2H, m), 3.52 - 3.61 (1H, m), 3.66 - 3.75 (1H, m), 3.83 - 3.94 (1H, m), 7.21 - 7.29 (1H, m), 7.62 - 7.7 (1H, m), 8.33 - 8.42 (1H, m), 8.44 - 8.51 (1H, m).

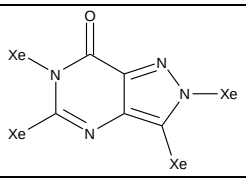
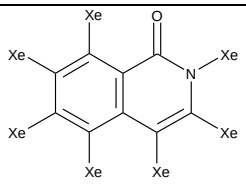
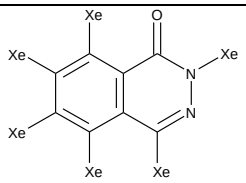
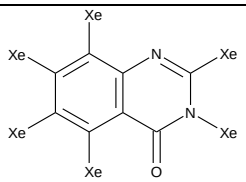
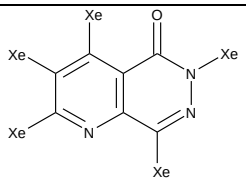
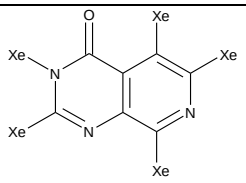
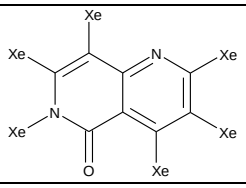
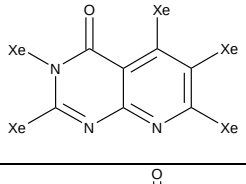
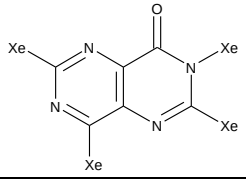
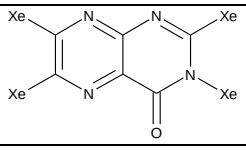
2-(3-(Difluoromethoxy)phenyl)-5-(2-methylpyridin-3-yl)-6-(((*S*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (59)
Copper(II) acetate (168 mg, 0.93 mmol) was added to 3-(difluoromethoxy)phenylboronic acid (232 mg, 1.23 mmol), 5-(2-methylpyridin-3-yl)-6-(((*S*)-1-(((*R*)-tetrahydrofuran-2-yl)methyl)piperidin-3-yl)methyl)-2H-pyrazolo[4,3-d]pyrimidin-7(6H)-one (252 mg, 0.62 mmol) and pyridine (0.100 mL, 1.23 mmol) in DCM (2 mL) at rt under air. Air was bubbled through the resulting suspension for 20 h then the mixture was stirred for 72 h. Silica (~25 g) was added and the resulting mixture concentrated to dryness and purified by flash silica chromatography, elution gradient 0 to 20% MeOH in DCM. The fractions containing pure product by TLC were combined and were evaporated to dryness to afford the title compound (75 mg, 22%) as a colourless dry film.
m/z (ES+) $[M+H]^+$ = 551;
HRMS calculated for $C_{29}H_{33}N_6O_3F_2$ $[M+H]^+$ 551.25767, found 551.25751;
^{1}H NMR (400 MHz, DMSO-d_6, 100 °C) δ 0.83 (1H, s), 1.17 - 1.53 (4H, m), 1.57 - 1.89 (4H, m), 1.95 - 2.15 (1H, m), 2.21 - 2.34 (2H, m), 2.40 (3H, s), 2.43 - 2.65 (3H, m), 3.46 - 4.15 (5H, m), 7.08 - 7.49 (3H, m), 7.65 (1H, t), 7.83 - 7.96 (3H, m), 8.59 - 8.65 (1H, m), 9.05 (1H, s).

Full information on cores from scaffold hop

Core	N	Mean	Std Dev	Median	Quantile 25	Quantile75	Structure	SMILES	Name
AA	2	1.92	0.69	1.92	1.43	2.4		[Xe]n1c(=O)c2c(c(n1)[Xe])nc(s2)[Xe]	thiazolo[4,5-d]pyridazin-7-one
AB	19	1.91	1.17	1.86	0.89	2.36		[Xe]n1c(c(c2c(c(n(c2c1=O)[Xe])[Xe])[Xe])[Xe])[Xe]	pyrrolo[2,3-c]pyridin-7-one
AC	2	1.25	0.25	1.25	1.07	1.42		[Xe]n1c(nc2c(c1=O)nc(s2)[Xe])[Xe]	thiazolo[5,4-d]pyrimidin-7-one
AD	5	1.16	0.46	1.2	0.76	1.55		[Xe]n1c(=O)c2c(c(n1)[Xe])c(c(s2)[Xe])[Xe]	thieno[2,3-d]pyridazin-7-one
AE	23	1.06	0.76	1.11	0.32	1.62		[Xe]n1c(nc2c(c1=O)c(c(s2)[Xe])[Xe])[Xe]	thieno[2,3-d]pyrimidin-4-one
AF	38	0.73	0.66	0.79	0.24	1.3		[Xe]n1c(=O)c2c(c(sc2c(n1)[Xe])[Xe])[Xe]	thieno[2,3-d]pyridazin-4-one
AG	70	0.7	0.85	0.87	0.36	1.13		[Xe]n1c(nc2c(c(sc2c1=O)[Xe])[Xe])[Xe]	thieno[3,2-d]pyrimidin-4-one
AH	23	0.6	1.31	0.81	-0.22	1.47		[Xe]n1c(c(c2c(c1=O)c(c(s2)[Xe])[Xe])[Xe])[Xe]	thieno[3,2-c]pyridin-4-one
AI	5	0.58	0.28	0.54	0.34	0.85		[Xe]n1c(nc2c(c1=O)c(ns2)[Xe])[Xe]	isothiazolo[5,4-d]pyrimidin-4-one

AJ	9	0.4	1.2	0.35	-0.84	1.58		[Xe]n1c(nc2c(c1=O)sc(n2)[Xe])[Xe]	thiazolo[4,5-d]pyrimidin-7-one
AK	4	0.37	0.62	0.4	-0.24	0.96		[Xe]n1c(nc2c(c(n(c2c1=O)[Xe])[Xe])[Xe])[Xe]	pyrrolo[3,2-d]pyrimidin-4-one
AL	14	0.36	0.49	0.17	0.02	0.76		[Xe]n1c(c(c2nc(c(n2c1=O)[Xe])[Xe])[Xe])[Xe]	imidazo[1,2-c]pyrimidin-5-one
AM	14	0.21	0.49	0.38	-0.23	0.5		[Xe]n1c(c(c2c(c1=O)c(no2)[Xe])[Xe])[Xe]	isoxazolo[4,5-c]pyridin-4-one
AN	8	0.09	0.71	-0.15	-0.44	0.54		[Xe]n1c(nc2c(c(oc2c1=O)[Xe])[Xe])[Xe]	furo[3,2-d]pyrimidin-4-one
AO	3	-0.01	1.13	-0.46	-0.85	1.27		[Xe]n1c(nc2c(c1=O)c(c(n2[Xe])[Xe])[Xe])[Xe]	pyrrolo[2,3-d]pyrimidin-4-one
AP	2	-0.04	0.29	-0.04	-0.25	0.16		[Xe]n1c(nc2c(c1=O)c(no2)[Xe])[Xe]	isoxazolo[5,4-d]pyrimidin-4-one
AQ	2	-0.22	0.18	-0.22	-0.35	-0.1		[Xe]n1c(=O)c2c(c(c(s2)[Xe])[Xe])nn1	thieno[3,2-d]triazin-4-one
AR	4	-0.31	0.55	-0.12	-0.9	0.09		[Xe]n1c(nc2c(c1=O)nc(n2[Xe])[Xe])[Xe]	purin-6-one (3-sub)
AS	2	-0.35	0.22	-0.35	-0.5	-0.19		[Xe]n1c(=O)c2c(c(oc2c(n1)[Xe])[Xe])[Xe]	furo[2,3-d]pyridazin-4-one

AT	7	-0.45	0.51	-0.5	-0.63	-0.33		[Xe]n1c(nc2c(c1=O)nnn2[Xe])[Xe]	triazolo[4,5-d]pyrimidin-7-one
AU	2	-0.81	0.11	-0.81	-0.88	-0.73		[Xe]n1c(nc2c(nc(n2c1=O)[Xe])[Xe])[Xe]	imidazo[1,5-a][1,3,5]triazin-4-one
AV	27	-0.82	0.66	-0.82	-1.1	-0.38		[Xe]n1c(=O)c2c(n(c(c2c(n1)[Xe])[Xe])[Xe])[Xe]	pyrrolo[3,4-d]pyridazin-4-one
AW	6	-0.87	0.8	-0.62	-1.2	-0.4		[Xe]n1c(nc2c(c1=O)c(nn2[Xe])[Xe])[Xe]	pyrazolo[3,4-d]pyrimidin-4-one
AX	5	-0.93	1.9	-0.55	-2.64	0.6		[Xe]n1c(nc2c(c1=O)c(n(n2)[Xe])[Xe])[Xe]	pyrazolo[3,4-d]pyrimidin-4-one
AY	4	-1.04	0.19	-1	-1.23	-0.88		[Xe]n1c(nc2c(c(nn2c1=O)[Xe])[Xe])[Xe]	pyrazolo[1,5-a][1,3,5]triazin-4-one
AZ	3	-1.11	0.32	-1.14	-1.42	-0.78		[Xe]n1c(nc2c(c1=O)n(c(n2)[Xe])[Xe])[Xe]	purin-6-one (1-sub)
BA	2	-1.39	0.05	-1.39	-1.42	-1.35		[Xe]n1c(c(c2c(n(c(c2c1=O)[Xe])[Xe])[Xe])[Xe])[Xe]	pyrrolo[3,4-c]pyridin-4-one
BB	2	-1.41	0.08	-1.41	-1.47	-1.35		[Xe]n1c(=O)c2c(n(nc2c(n1)[Xe])[Xe])[Xe]	pyrazolo[3,4-d]pyridazin-4-one
BC	2	-1.61	0.01	-1.61	-1.61	-1.6		[Xe]n1c(nc2c(nn(c2c1=O)[Xe])[Xe])[Xe]	pyrazolo[4,3-d]pyrimidin-7-one (1-sub)

BD	77	-1.77	0.49	-1.86	-2.09	-1.61		[Xe]n1c(nc2c(n(nc2c1=O)[Xe])[Xe])[Xe]	pyrazolo[4,3-d]pyrimidin-7-one (2-sub)
CA	293	1.35	0.79	1.31	0.85	1.82		[Xe]n1c(c(c2c(c(c(c(c2c1=O)[Xe])[Xe])[Xe])[Xe])[Xe])[Xe]	isoquinolin-1-one
CB	375	1.08	0.76	1.04	0.53	1.66		[Xe]n1c(=O)c2c(c(c(c(c2c(n1)[Xe])[Xe])[Xe])[Xe])[Xe]	phthalazin-1-one
CC	817	0.52	1.02	0.49	0.03	1.12		[Xe]n1c(nc2c(c(c(c(c2c1=O)[Xe])[Xe])[Xe])[Xe])[Xe]	quinazolin-4-one
CD	3	0.19	0.11	0.16	0.1	0.31		[Xe]n1c(=O)c2c(c(c(nc2c(n1)[Xe])[Xe])[Xe])[Xe]	pyrido[2,3-d]pyridazin-5-one
CE	5	0.04	0.67	0.05	-0.49	0.57		[Xe]n1c(nc2c(nc(c(c2c1=O)[Xe])[Xe])[Xe])[Xe]	pyrido[3,4-d]pyrimidin-4-one
CF	4	-0.06	1.02	0.35	-1.1	0.55		[Xe]n1c(c(c2c(c1=O)c(c(c(n2)[Xe])[Xe])[Xe])[Xe])[Xe]	1,6-naphthyridin-5-one
CG	10	-0.36	1.16	-0.75	-1.35	0.51		[Xe]n1c(nc2c(c1=O)c(c(c(n2)[Xe])[Xe])[Xe])[Xe]	pyrido[2,3-d]pyrimidin-4-one
CH	9	-0.91	0.8	-1.13	-1.52	-0.48		[Xe]n1c(nc2c(nc(nc2c1=O)[Xe])[Xe])[Xe]	pyrimido[5,4-d]pyrimidin-4-one
CI	3	-1.21	0.87	-0.83	-2.2	-0.6		[Xe]n1c(nc2c(c1=O)nc(c(n2)[Xe])[Xe])[Xe]	pteridin-4-one

Biological Protocols:

^{125}I-ghrelin displacement assay: Isolated plasma membranes from HEK cells stably overexpressing the GHSR-1a receptor (4μg/well) were incubated for 60 min at room temperature with 20 pM [^{125}I]human ghrelin (Perkin Elmer NEX388) in the presence or absence of relevant concentrations of cold competing compounds. Incubations were performed in 100 μL total volume of assay buffer (50 mM Tris-HCl, 5 mM $MgCl_2.6H_2O$, 0.01% BSA, pH7.4) containing 10 μM methyl arachidonyl fluorophosphonate (Sigma M2939). The binding reaction was stopped by rapid filtration over Whatman GF/C filters pre-soaked with 0.5% polyethyeneimine and rinsed three times with ice-cold wash buffer (50 mM Tris-HCl, 5 mM $MgCl_2.6H_2O$, 50 mM NaCl, pH7.4) and the radioactivity bound to the membranes was measured using γ-counter.

Invitrogen Tango functional assay: Tango^{TM} U2OS-GHSR-1a assay cells were plated at 10,000 cells per well in 384-well plate in assay medium (100% Freestyle Medium containing 10 ng/mL doxycycline) in a final volume of 40 μL and incubated overnight at 37 ºC/5% CO_2. Appropriate concentrations of compounds were also included at the time of plating. Following overnight incubation cells were loaded with LiveBLAzer FRET B/G substrate for 2 h at room temperature in the dark and the fluorescence emission values at 460 nm and 530 nm obtained using a FLIPR^{TETRA} at 409 nm excitation. Maximal agonist response was measured relative to MK0677.

Free Feeding in Male GHS-R1a KO and WT Mice: 7 weeks old male mice were group housed and acclimatised to reverse lighting (9.00am off , 9.00pm on) for 2 weeks. Mice were then single housed for 5 d prior to the study to capture baseline food intake and bodyweight data on which randomisation was carried out. On the day of the study the mice were dosed (either with compound or an equivalent volume of vehicle) and given a pre-weighed amount of food sequentially between 8.00am and 9.00am (ie 1-0 h prior to lights out). Food was re-weighed 2 h post-dosing, and food intake in the intervening period was calculated.

80 animals in total were assigned to eight groups:
KO Vehicle Control n=12, KO compound **41** 20 mg/kg PO n=10 per group
WT Vehicle n=12, WT compound **41** 20 mg/kg PO n=10 per group
Compound was formulated in 1% Pluronic F127 (BASF) in de-ionised water the day before the study, at a concentration of 4 mg/mL, and animals dosed with 5 mL/Kg compound suspension. Rat fub was used in free multiple cover calculations.

Procedures for determination of physicochemical properties:

$logD_{7.4}$, plasma-protein binding and solubility measurements were made as described in; Buttar, D.; Colclough, N.; Gerhardt, S.; MacFaul, P. A.; Phillips, S. D.; Plowright, A.; Whittamore, P.; Tam, K.; Maskos, K.; Steinbacher, S.; Steuber, H. A. Combined spectroscopic and crystallographic approach to probing drug–human serum albumin interactions. *Bioorg. Med. Chem.* **2010**, *18*, 7486-7496.

$logD_{7.4}$:

$LogD_{7.4}$ measurements were made using a shake-flask method where the extent of partitioning between pH 7.4 buffer and octanol was measured. Compounds were dissolved in a known volume buffer, and following the addition of a known amount of octanol, the solutions were shaken for 30 min. Following centrifugation, analysis of the aqueous layer was performed by LC–UV to quantify the amount of compound in solution and then compared to analysis of the compound in solution before the addition of octanol to calculate the partitioning coefficient, $D_{7.4}$.

Solubility:

Assessments of aqueous solubility were made after an incubation of 24 h in pH 7.4 phosphate buffer. After centrifugation, analysis of the supernatant liquid was performed by LC–UV to quantify the amount of compound in solution.

Protein binding strength *via* equilibrium dialysis:

Dialysis membranes (Spectra/Por 2, 12–14 kDa molecular weight cut-off, 47 mm diameter, Spectrum Laboratories) were prepared for use by washing with distiled water and subsequent soaking in phosphate buffer (pH 7.4). Membranes were then blotted dry and placed between two 1 mL Teflon dialysis half-cells (Braun ScienceTec, Les Ulis, France). Each half-cell was filled individually with 1 mL of protein solution containing the compound of interest, while the corresponding half-cell was filled with 1 mL of isotonic phosphate buffer. Dialysis units were immersed in a 37 °C temperature-controlled water bath and rotated at 30 rpm for 18–19 h using a Dianorm apparatus (Braun ScienceTec). After this period, samples from both the half-cell containing buffer (protein free) and the half-cell containing protein were submitted for HPLC analysis using an Agilent 1100 series HPLC with a 110 binary pump and a UV diode ray detector. Acquisition and integration were carried out using Chemstation software (Agilent Technologies) version A.06.03 with relevant customised macro software. Integration of the subsequent chromatograms, are used to calculate the concentration of drug in the protein containing solution (Dp) and in the protein-free solutions (Df), which are then used to derive the binding constant for the test compound (K_1) assuming a 1:1 binding model as shown in Eq. 1 where the compound can only bind to a single site on the protein molecule. This is expressed mathematically in Eq. 2 where D and Df are the total and free drug concentrations, respectively, and Pr is the total protein concentration.

$$\mathrm{D} + \mathrm{P} \overset{K_1}{\rightleftharpoons} \mathrm{DP} \qquad \text{Eq. 1}$$

$$D = (D_\mathrm{f} + D_\mathrm{p}) = \frac{K_1 \cdot D_\mathrm{f} \cdot \mathrm{Pr}}{1 + K_1 \cdot D_\mathrm{f}} + D_\mathrm{f} \qquad \text{Eq. 2}$$

pK_a:

pK_a measurements were made using a high throughput pK_a screening assay employing pressure-assisted capillary electrophoresis and mass spectrometry as described in; Wan, H; Holmén, A; Wang, YD; Lindberg, W; Englund, M; Någård, M; Thompson, R., High throughput screening of pK_a values of pharmaceuticals by pressure-assisted capillary electrophoresis and mass spectrometry, *Rapid Communications in Mass Spectrometry, 2003, 1:2639-2648*.