

## Supplementary Material for

# Design of pyrazolo-pyrimidines as 11 $\beta$ -HSD1 inhibitors through optimisation of molecular electrostatic potential

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### CNS penetration model protocol

Animals were sourced from the AstraZeneca breeding colony and were kept on a regular chow diet prior to and throughout the study. All animals were handled and housed in accordance with relevant laws and AstraZeneca ethics committee rules. Compounds were dosed orally in a HPMC/Tween vehicle at 30 mg/kg to male Hans Wistar rats (n=2 per compound). Animals were euthanized at 1 hour post dose and terminal blood (100 $\mu$ L blood + 100 $\mu$ L of water) and brain samples were collected from each animal. Samples were frozen at -20°C for storage, prior to analysis.

Blood samples (50 $\mu$ L) were protein precipitated with ice cold acetonitrile (Sigma-Aldrich) containing a generic internal standard taken from the AstraZeneca compound library. The samples were then mixed and centrifuged at 4500g for 10 mins. 50 $\mu$ L of the resulting supernatant was removed and diluted with 300 $\mu$ L of water prior to injection (50 $\mu$ L) onto the LC-MS/MS system.

Brain samples were homogenized after addition of water (4 x dilution by weight) using a FisherBrand 500 homogenizer (ThermoFisher Scientific, Loughborough, UK). 50 $\mu$ L of the resulting homogenate was then protein precipitated with ice cold acetonitrile (Sigma-Aldrich) containing a generic internal standard taken from the AstraZeneca compound library. The samples were then mixed and centrifuged at 4500g for 10 mins. 50 $\mu$ L of the resulting supernatant was removed and diluted with 300 $\mu$ L of water prior to injection (50 $\mu$ L) onto the LC-MS/MS system.

Blood and brain samples were quantified against freshly prepared calibration curves with standards at 1, 5, 10, 50, 100, 500, 1000, 2000, 5000, and 10000 ng/mL. Quality Controls were run before and after the unknown samples at concentrations of 10, 100 and 1000 ng/mL. Calibration standards and quality controls were all prepared in control blood or brain homogenate and were extracted in exactly the same way as the unknown samples above.

Samples were analysed on a TSQ Quantum Vantage mass spectrometer (ThermoFisher Scientific, Hemel Hempstead, UK). Parent and product masses were auto-tuned into the mass spectrometer using QuickQuan™ (version 2.4) and Xcalibur (version 2.1) software (ThermoFisher Scientific, San Jose, California, USA). The capillary temperature was 270°C, the vaporizer temperature was 300°C, the sheath gas and auxiliary gas flows were set at 60 and 20 arbitrary units respectively.

A Surveyor MS Pump Plus HPLC pump, (Thermo Fisher Scientific, Hemel Hempstead, UK) and a CTC Analytics HTS PAL autosampler (Presearch Ltd, Basingstoke, UK) were used to introduce the samples to the mass spectrometer. Chromatography was performed on a Max-RP (50mm x 2.1mm ID, 5 $\mu$ ) HPLC column (Phenomenex, Macclesfield, UK) with mobile phase consisting of eluent (A) water containing 0.1% formic acid and (B) methanol containing 0.1% formic acid. The linear gradient used was (T=minutes): at T=0.0, 95%A:5%B, T=3.0, 5%A:95%B, T=4.0, 5%A:95%B, T=4.1, 95%A:5%B, T=5.0, 95%A:5%B. The flow rate used was 750  $\mu$ L/min.

Subsequent to analysis the data was quantified using QuickCalc™ software (Gubb Inc, Alpharetta, Georgia, USA) by back calculation against the relevant calibration curve. A 1/x weighting was applied to the calibration curves which were constructed using a quadratic fit. Relevant dilution factors were applied to the unknown samples (2-fold for blood samples, 4-fold for brain samples) and a brain/blood ratio was calculated for each animal, this ratio was used to benchmark the compounds.

## **Physicochemical Measurement Protocols**

LogD<sub>7.4</sub> 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<sub>7.4</sub>:**

LogD<sub>7.4</sub> 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<sub>7.4</sub>.

### **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.

## **Biological Measurement Protocols**

### **Measurement of 11 $\beta$ -HSD1 enzyme potency:**

The conversion of cortisone to the active steroid cortisol by 11 $\beta$ -HSD1 oxo-reductase can be measured using a cortisol competitive homogeneous time resolved fluorescence assay (HTRF) assay (CisBio International, R&D, Administration and Europe Office, In Vitro Technologies - HTRF® / Bioassays BP 84175, 30204 Bagnols/Cèze Cedex, France). The evaluation of compounds was carried out using a baculovirus expressed N terminal 6-His tagged full length human, rat, dog or cynomolgous monkey 11 $\beta$ -HSD1 enzyme. The enzyme was purified from a detergent solubilised cell lysate, using a copper chelate column. Inhibitors of 11 $\beta$ -HSD1 reduce the conversion of cortisone to cortisol, which is identified by an increase in signal, in the above assay. The assay incubation was carried out in black 384 well plates (Matrix, Hudson NH, USA). consisting of cortisone (Sigma, Poole, Dorset, UK, 160 nM), glucose-6-phosphate (Roche Diagnostics, 1 mM), NADPH (Roche Diagnostics, 100  $\mu$ M), glucose-6-phosphate dehydrogenase (Roche Diagnostics, 12.5  $\mu$ g/mL), EDTA (Sigma, Poole, Dorset, UK, 1 mM), assay buffer (K<sub>2</sub>HPO<sub>4</sub>/KH<sub>2</sub>PO<sub>4</sub>, 100 mM) pH 7.5, recombinant 11 $\beta$ -HSD1 (1.5  $\mu$ g/mL) plus test compound in a total volume of 20  $\mu$ L. The assay plates were incubated for 25 minutes at 37 °C and the reaction stopped by the addition of 10  $\mu$ L of 0.5 mM glycerolacetic acid (Sigma) plus cortisol-XL665. 10  $\mu$ L of anti-cortisol Cryptate was then added and the plates and incubated for 2h at room temperature. Fluorescence at 665 nm and 620 nm was measured and the 665 nm:620 nm ratio calculated using an Envision plate reader. This data was then used to calculate IC<sub>50</sub> values for each compound (Origin 7.5, Microcal software, Northampton MA, USA).

### **Measurement of *in vitro* median intrinsic clearance in rat hepatocytes:**

The metabolic stability of the test compound is measured in Han Wistar rat hepatocytes using the CAT shaker 96-well shaking block. Hepatocyte cells are prepared from fresh or cryopreserved tissue. Compound at 1  $\mu$ M is incubated with hepatocytes at 10<sup>6</sup> cells/mL in 250  $\mu$ L volume. Cell viability checked to be >90%. Marker compounds: diclofenac, diazepam, dextromethorphan, phenacetin, verapamil and naloxone, which are metabolised by specific human P450 isoforms or UGTs, are added to every plate in order to determine that each plate gives a consistent performance. Incubations are performed for 5, 10, 20 and 30 minutes. After this time a 25  $\mu$ L aliquot of each incubation is quenched with 150  $\mu$ L water and then further diluted to twice this total volume. The sample is then centrifuged

to pellet the precipitate. The supernatant of each sample is analysed by LC-MS/MS (ammonium acetate gradient) to quantify the proportion of parent compound remaining. The parent concentration as a function of time is used to determine a half-life ( $T_{1/2}$ ) and thus an intrinsic clearance for each compound, see equation 1.

$$Cl_{int} (\mu\text{L}/\text{min}/10^6 \text{ cells}) = (\ln(2)/T_{1/2} (\text{min})) * 1000/(\text{cell conc (million cells/mL)}) \quad [1]$$

## **Property Prediction Protocols**

### **PSA:**

PSA as referred to in this manuscript is synonymous with TPSA as described in: Ertl, P.; Rohde, B.; Selzer, P., Fast Calculation of Molecular Polar Surface Area as a Sum of Fragment-Based Contributions and Its Application to the Prediction of Drug Transport Properties, *J. Med. Chem.*, 2000, **43**, 3714-3717.

## **Compound Synthesis Methods**

There follows synthetic schemes for compounds in this paper, unless stated otherwise:

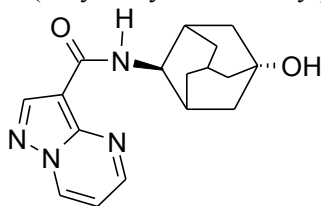
- i. Temperatures are given in degrees Celsius ( $^{\circ}\text{C}$ ); operations were carried out at room temperature (RT), that is, at a temperature in the range of 18-25 $^{\circ}\text{C}$  and under an atmosphere of an inert gas such as argon or nitrogen;
- ii. Evaporation of solvent was carried out using a rotary evaporator under reduced pressure (600-4000 Pa; 4.5-30 mmHg) with a bath temperature of up to 60 $^{\circ}\text{C}$ ;
- iii. Chromatography means flash chromatography on silica gel (FCC);
- iv. In general, the course of reactions was followed by TLC or LCMS (HPLC coupled to mass spectroscopy) and reaction times are given for illustration only;
- v. Yields are given for illustration only and are not necessarily those which can be obtained by diligent process development; preparations were repeated if more material was required;
- vi. Where given, NMR data ( $^1\text{H}$ ) is in the form of delta values for major diagnostic protons, given in parts per million (ppm) relative to tetramethylsilane (TMS), determined at 300 or 400 MHz (unless otherwise stated) using perdeuterio dimethyl sulfoxide ( $\text{DMSO}-d_6$ ) as solvent, unless otherwise stated; peak multiplicities are shown thus: s, singlet; d, doublet; dd, doublet of doublets; dt, doublet of triplets; t, triplet; q, quartet; qn, quintet; m, multiplet; br, broad; protons attached to oxygen or nitrogen may give rise to very broad peaks which are not reported;
- vii. Solvent ratios are given in volume : volume (v/v) terms;
- viii. Mass spectra (MS) were run with an electron energy of 70 electron volts in the chemical ionisation (CI) mode using a direct exposure probe; where indicated ionisation was effected by electron impact (EI), fast atom bombardment (FAB) or electrospray (ESP); values for m/z are given; generally, only ions which indicate the parent mass are reported;
- ix. The purity of compounds submitted for screening was >95% as determined by UV analysis of liquid chromatography-mass spectroscopy (LC-MS) chromatograms at 254 nm and substantiated using the TAC (Total Absorption Chromatogram). Further support for the purity statement was provided using the MS TIC (Total Ion Current) trace in ESI +ve and -ve ion modes;
- x. Purifications by preparative HPLC typically used decreasingly polar mixtures of water (containing an additive such as 0.1% aq. formic acid or 0.5% aq.  $\text{NH}_3$ ) and MeCN as eluents and typically used a Waters XBridge Prep C18 OBD column (5 $\mu$  silica) or similar;
- xi. The following abbreviations may be used below:

Ac	acetyl
aq.	Aqueous

DBU	1,8-Diazabicycloundec-7-ene
DCM	dichloromethane
DIAD	(E)-diisopropyl diazene-1,2-dicarboxylate
DIPEA	diisopropylethylamine
DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethylsulfoxide
Et	ethyl
FCC	flash column chromatography
h	hour(s)
HATU	2-(3H-[1,2,3]Triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V)
HPLC	high performance liquid chromatography
Me	methyl
min	minute(s)
Ph	phenyl
RT	room temperature
sat.	Saturated
THF	tetrahydrofuran

### Compound 2

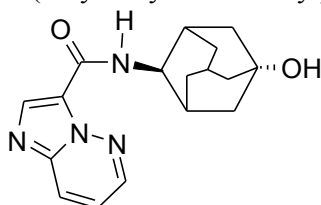
*N*-(5-hydroxy-2-adamantyl)pyrazolo[1,5-a]pyrimidine-3-carboxamide



*N,N*-Diisopropylethylamine (0.536 mL, 3.00 mmol) was added to pyrazolo[1,5-a]pyrimidine-3-carboxylic acid (163 mg, 1.00 mmol), *trans* 4-aminoadamantan-1-ol hydrochloride (204 mg, 1.00 mmol) and *N,N,N',N'*-tetramethyl-*O*-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate (457 mg, 1.20 mmol) in *N,N*-dimethylformamide (4 mL). The resulting mixture was stirred at ambient temperature for 17 hours. The reaction mixture was diluted with ethyl acetate (25 mL), and washed with water (10 mL). The organic layer was evaporated to afford a crude product, which was purified by preparative HPLC to afford the title compound **2** (169 mg, 54%) as a solid; <sup>1</sup>H NMR (400 MHz, DMSO) δ 1.50 (2H, d), 1.63 – 1.72 (4H, m), 1.74 – 1.9 (4H, m), 2.03 – 2.13 (3H, m), 4.05 – 4.12 (1H, m), 4.47 (1H, s), 7.27 (1H, dd), 8.28 (1H, d), 8.56 (1H, s), 8.85 (1H, dd), 9.32 (1H, dd); <sup>13</sup>C NMR (176 MHz, DMSO) δ 29.06, 30.41, 33.62, 44.26, 45.17, 51.48, 65.35, 105.13, 109.75, 137.55, 145.18, 145.60, 152.47, 160.09; HRMS (ESI) calc. for C<sub>17</sub>H<sub>20</sub>O<sub>2</sub>N<sub>4</sub>(MH<sup>+</sup>) 313.16590, found 313.16583.

### Compound 3

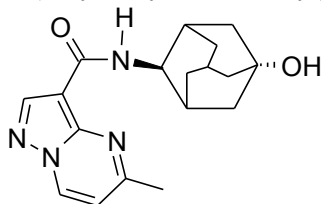
*N*-(5-hydroxy-2-adamantyl)imidazo[2,1-f]pyridazine-3-carboxamide



Made according to the procedure for **compound 2** from imidazo[1,2-b]pyridazine-3-carboxylic acid in 44% yield; <sup>1</sup>H NMR (400 MHz, DMSO) 1.45 - 1.55 (2H, m), 1.62 - 1.73 (4H, m), 1.73 - 1.90 (4H, m), 2.04 - 2.14 (3H, m), 4.12 (1H, d), 4.47 (1H, s), 7.46 (1H, dd), 8.30 (1H, s), 8.36 (1H, dd), 8.80 - 8.93 (2H, m); *m/z* (ES<sup>+</sup>) (M+H)<sup>+</sup> = 313.

**Compound 4**

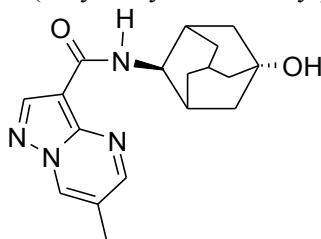
*N*-(5-hydroxy-2-adamantyl)-5-methyl-pyrazolo[1,5-*a*]pyrimidine-3-carboxamide



HATU (644 mg, 1.69 mmol) was added to 5-methylpyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (250 mg, 1.41 mmol), *trans* 4-aminoadamantan-1-ol hydrochloride (287 mg, 1.41 mmol) and DIPEA (0.737 mL, 4.23 mmol) in DMF (10 mL) at RT and the reaction mixture was stirred at RT overnight. The resulting precipitate was isolated by filtration, washed with water and then dried *in vacuo* to afford the title compound (224 mg, 49%) as a white solid; <sup>1</sup>H NMR (400 MHz) 1.50 (2H, d), 1.67 (4H, d), 1.77 (2H, d), 1.87 (2H, d), 2.07 (3H, d), 2.64 (3H, s), 4.06 (1H, d), 4.45 (1H, s), 7.16 (1H, d), 8.42-8.49 (2H, m), 9.15 (1H, d); *m/z* MH<sup>+</sup>=327.

**Compound 5**

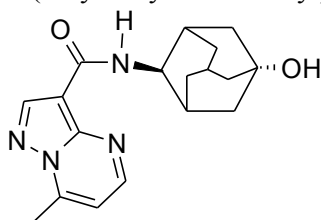
*N*-(5-hydroxy-2-adamantyl)-6-methyl-pyrazolo[1,5-*a*]pyrimidine-3-carboxamide



HATU (1.38 g, 3.62 mmol) was added to 6-methylpyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (534 mg, 3.01 mmol), *trans* 4-aminoadamantan-1-ol hydrochloride (614 mg, 3.01 mmol) and DIPEA (1.58 mL, 9.04 mmol) in DMF (15 mL) and the reaction mixture was stirred at 50 °C for 17 h. The reaction mixture was then diluted with water and the resulting precipitate was collected by filtration, washed with water and dried *in vacuo* to afford the title compound (819 mg, 83%) as a solid; <sup>1</sup>H NMR (400 MHz) 1.48 (2H, d), 1.61-1.72 (4H, m), 1.72-1.89 (4H, m), 1.97-2.13 (3H, m), 2.37 (3H, s), 4.02-4.08 (1H, m), 4.45 (1H, s), 8.25 (1H, d), 8.46 (1H, s), 8.76 (1H, d), 9.16 (1H, dd); *m/z* MH<sup>+</sup>=327.

**Compound 6**

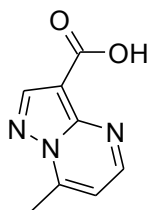
*N*-(5-hydroxy-2-adamantyl)-7-methyl-pyrazolo[1,5-*a*]pyrimidine-3-carboxamide



*trans* 4-aminoadamantan-1-ol hydrochloride (230 mg, 1.13 mmol) was added to **Intermediate 12** (200 mg, 1.13 mmol), HATU (515 mg, 1.35 mmol) and DIPEA (0.59 mL, 3.39 mmol) in DMF (3 mL) and the reaction mixture was stirred at RT for 17 h. The reaction mixture was diluted with water and the resulting precipitate was collected by filtration, washed with water and dried *in vacuo* at 60 °C to afford the title compound (270 mg, 73%) as a white solid; <sup>1</sup>H NMR (400 MHz) 1.48 (2H, d), 1.67 (4H, d), 1.80 (4H, dd), 2.07 (3H, d), 2.80 (3H, s), 4.02-4.12 (1H, m), 4.45 (1H, s), 7.22 (1H, dd), 8.37 (1H, d), 8.57 (1H, s), 8.73 (1H, d); *m/z* MH<sup>+</sup>=327.

**Intermediate 12**

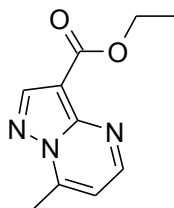
7-Methylpyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid



2 M aq. NaOH (3.47 mL, 6.93 mmol) was added to **Intermediate 13** (431 mg, 2.10 mmol) in ethanol (15 mL) and the reaction mixture was stirred at RT for 17 h. The reaction mixture was concentrated *in vacuo*, diluted with water then acidified with 2 M aq. HCl. The resulting precipitate was collected by filtration and dried *in vacuo* to afford the title compound (329 mg, 88%) as a solid;  $^1\text{H}$  NMR (400 MHz) 2.83 (3H, d), 7.22 (1H, dd), 8.60 (1H, s), 8.70 (1H, d), OH not observed;  $m/z$   $\text{MH}^+$ =178.

### Intermediate 13

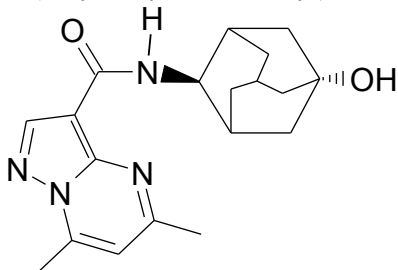
Ethyl 7-methylpyrazolo[1,5-a]pyrimidine-3-carboxylate



Ethyl 5-amino-1H-pyrazole-4-carboxylate (637 mg, 4.11 mmol) and *trans*-4-(dimethylamino)-3-buten-2-one (0.573 mL, 4.93 mmol) were suspended in 70% aq. EtOH (12 mL) and conc. aq. HCl (0.4 mL) in a microwave tube. The reaction mixture was heated at 140°C for 30 min in a microwave reactor and then cooled to RT. The reaction mixture was concentrated *in vacuo*, diluted with EtOAc and washed sequentially with sat. aq.  $\text{NaHCO}_3$  and sat. brine. The organic layer was dried over  $\text{MgSO}_4$ , filtered, concentrated *in vacuo* and purified by preparative HPLC to afford the title compound (431 mg, 51%);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) 1.43 (3H, t), 2.85 (3H, d), 4.45 (2H, q), 6.88 (1H, dd), 8.60 (1H, s), 8.67 (1H, d);  $m/z$   $\text{MH}^+$ =206.

### Compound 7

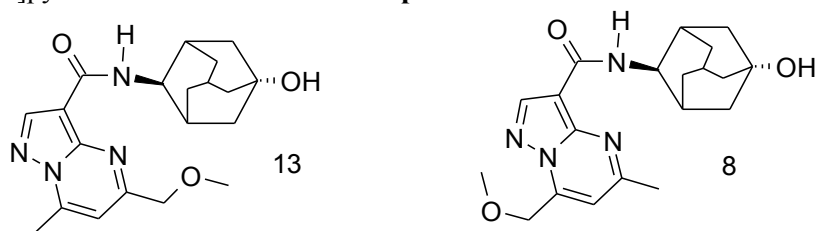
*N*-(5-hydroxy-2-adamantyl)-5,7-dimethyl-pyrazolo[1,5-a]pyrimidine-3-carboxamide



HATU (456 mg, 1.20 mmol) was added to 5,7-dimethylpyrazolo[1,5-a]pyrimidine-3-carboxylic acid (191 mg, 1.00 mmol), *trans* 4-aminoadamantan-1-ol hydrochloride (204 mg, 1.00 mmol) and DIPEA (0.523 mL, 3.00 mmol) in DMF (2 mL) and the reaction mixture was stirred at RT overnight. Water was added to the reaction mixture and the resulting precipitate was collected by filtration. The solid was washed with water and dried *in vacuo* to afford the title compound (205 mg, 60%) as a solid;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) 1.39 (1H, s), 1.57-1.62 (2H, m), 1.77-1.85 (4H, m), 1.99 (4H, t), 2.19-2.29 (3H, m), 2.64 (3H, s), 2.80 (3H, s), 4.22-4.37 (1H, m), 6.71 (1H, d), 8.58-8.65 (2H, m);  $m/z$   $\text{MH}^+$ =341.

**Compound 8 & 13**

*N*-(5-hydroxy-2-adamantyl)-5-(methoxymethyl)-7-methyl-pyrazolo[1,5-*a*]pyrimidine-3-carboxamide **compound 13** and *N*-(5-hydroxy-2-adamantyl)-7-(methoxymethyl)-5-methyl-pyrazolo[1,5-*a*]pyrimidine-3-carboxamide **compound 8**



HATU (464 mg, 1.22 mmol) was added in one portion to a stirred solution of **intermediate 20** [5-(methoxymethyl)-7-methylpyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid compound with 7-(methoxymethyl)-5-methylpyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid] (225 mg, 1.02 mmol), *trans* 4-aminoadamantan-1-ol hydrochloride (207 mg, 1.02 mmol) and DIPEA (0.709 mL, 4.07 mmol) in DMF (5 mL) and the resulting solution was stirred at 20 °C for 17 hours. The reaction mixture was diluted with water (25 mL) and the reaction extracted with EtOAc (3 x 25 mL). The organic layers were combined and evaporated. The crude product was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5  $\mu$  silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 0.5% NH<sub>3</sub>) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford *N*-(5-hydroxy-2-adamantyl)-5-(methoxymethyl)-7-methyl-pyrazolo[1,5-*a*]pyrimidine-3-carboxamide **compound 13** (154 mg, 41%) and *N*-(5-hydroxy-2-adamantyl)-7-(methoxymethyl)-5-methyl-pyrazolo[1,5-*a*]pyrimidine-3-carboxamide **compound 8** (96 mg, 26%) as white solids.

**compound 13**

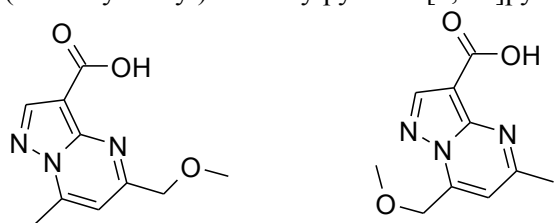
<sup>1</sup>H NMR (400 MHz, DMSO) 1.49 (2H, d), 1.64 - 2.12 (11H, m), 2.80 (3H, s), 3.42 (3H, s), 4.07 (1H, d), 4.44 (1H, s), 4.63 (2H, s), 7.22 (1H, s), 8.50 (1H, d), 8.54 (1H, s); *m/z* (ES<sup>+</sup>) (M+H)<sup>+</sup> = 371.

**compound 8**

<sup>1</sup>H NMR (400 MHz, DMSO) 1.51 (2H, d), 1.72 (6H, dd), 1.87 (2H, d), 2.07 (3H, d), 2.64 - 2.79 (3H, s), 3.51 (3H, s), 4.07 (1H, s), 4.44 (1H, s), 4.96 (2H, s), 7.17 (1H, s), 8.48 (1H, s), 8.52 (1H, d); *m/z* (ES<sup>+</sup>) (M+H)<sup>+</sup> = 371.

**Intermediate 20**

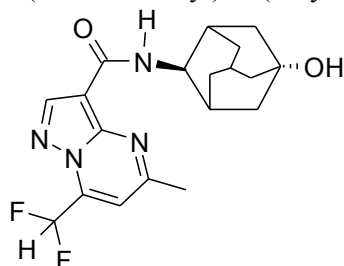
Mixture of 5-(methoxymethyl)-7-methylpyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid and 7-(methoxymethyl)-5-methylpyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid



5-amino-1H-pyrazole-4-carboxylic acid (1.77 g, 13.93 mmol) was slurried in ethanol (5 mL) and acetic acid (15 mL), whilst stirring the mixture, 1-methoxypentane-2,4-dione (1.81 g, 13.93 mmol) was added. The resulting slurry was stirred at 80 °C for 3 hours with a solution forming after a few minutes of heating. The mixture was cooled to room temperature and evaporated to dryness. The residue was triturated with EtOH (30 mL) and the solid filtered and washed with ethanol (15 mL) to give an inseparable mixture of 5-(methoxymethyl)-7-methylpyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid and 7-(methoxymethyl)-5-methylpyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (1.60 g, 26%). *m/z* (ES<sup>+</sup>) (M+Na)<sup>+</sup> = 244.

**Compound 9**

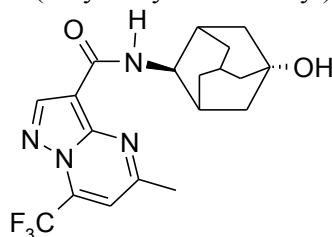
7-(difluoromethyl)-*N*-(5-hydroxy-2-adamantyl)-5-methyl-pyrazolo[1,5-*a*]pyrimidine-3-carboxamide



HATU (448 mg, 1.18 mmol) was added in one portion to a stirred solution of 7-(difluoromethyl)-5-methylpyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (223 mg, 0.98 mmol), *trans* 4-aminoadamantan-1-ol hydrochloride (200 mg, 0.98 mmol) and DIPEA (0.513 mL, 2.95 mmol) in DMF (5 mL) and the resulting solution was stirred at 50 °C for 16 h. The reaction mixture was evaporated to dryness, then redissolved in DCM (20 mL) and washed with saturated brine (10 mL). The organic layer was dried over MgSO<sub>4</sub>, filtered and evaporated to afford crude product that was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5 μ silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 0.1% formic acid) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 7-(difluoromethyl)-*N*-(5-hydroxy-2-adamantyl)-5-methyl-pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (113 mg, 31%) as a white solid; <sup>1</sup>H NMR (400 MHz, DMSO) 1.45 (2H, d), 1.62 (4H, d), 1.71 (2H, d), 1.80 (2H, d), 1.99 (2H, s), 2.05 (1H, s), 2.66 (3H, s), 4.01 (1H, d), 4.40 (1H, s), 7.47 (1H, s), 7.57 (1H, s), 8.34 (1H, d), 8.52 (1H, s); *m/z* (ES<sup>+</sup>), (M+H)<sup>+</sup> = 377.

**Compound 10**

*N*-(5-hydroxy-2-adamantyl)-5-methyl-7-(trifluoromethyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide

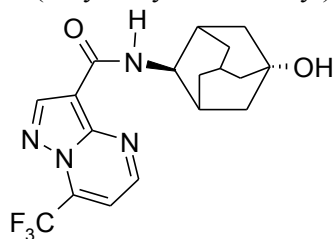


HATU (372 mg, 0.98 mmol) was added to 5-methyl-7-(trifluoromethyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (200 mg, 0.82 mmol), *trans* 4-aminoadamantan-1-ol hydrochloride (199 mg, 0.98 mmol) and DIPEA (0.426 mL, 2.45 mmol) in DMF (10 mL) at 25 °C and the resulting mixture was stirred at RT overnight. The reaction mixture was evaporated to dryness, then redissolved in EtOAc (50 mL), and washed saturated brine (15 mL). The organic layer was dried over MgSO<sub>4</sub>, filtered and evaporated to afford a solid that was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5 μ silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 0.1% formic acid) and MeCN as eluent. Fractions containing the desired compound were evaporated to dryness to afford *N*-(5-hydroxy-2-adamantyl)-5-methyl-7-(trifluoromethyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (163 mg, 51%) as a solid; <sup>1</sup>H NMR (400 MHz, DMSO) 1.47 - 1.56 (2H, m), 1.63 - 1.72 (4H, m), 1.73 - 1.81 (2H, m), 1.81 - 1.91 (2H, m), 2.01 - 2.15 (3H, m), 2.73 (3H, s), 4.05 - 4.11 (1H, m), 4.46 (1H, s), 7.81 (1H, s), 8.36 (1H, d), 8.63 (1H, s). *m/z* (ES<sup>+</sup>) (M+H)<sup>+</sup> = 395.



**Compound 11**

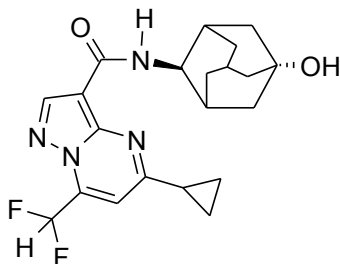
*N*-(5-hydroxy-2-adamantyl)-7-(trifluoromethyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide



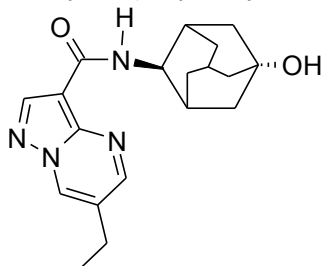
HATU (494 mg, 1.30 mmol) was added to 7-(trifluoromethyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (250 mg, 1.08 mmol), *trans* 4-aminoadamantan-1-ol hydrochloride (264 mg, 1.30 mmol) and DIPEA (0.565 mL, 3.24 mmol) in DMF (10 mL) and the resulting mixture was stirred at RT overnight. The reaction mixture was evaporated to dryness and redissolved in EtOAc (50 mL), and washed with saturated brine (15 mL). The organic layer was dried over MgSO<sub>4</sub>, and evaporated to give a solid. This was dissolved in MeOH:2M NH<sub>4</sub>Cl:MeNO<sub>2</sub> 70:20:10 (7 mL). A precipitate formed on cooling. This was filtered off, washed with water (5 mL) and dried under vacuum at 70 °C to give *N*-(5-hydroxy-2-adamantyl)-7-(trifluoromethyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (290 mg, 71%) as a tan solid; <sup>1</sup>H NMR (400 MHz, DMSO) 1.45 - 1.54 (2H, m), 1.63 - 1.73 (4H, m), 1.74 - 1.89 (4H, m), 2.08 (3H, s), 4.04 - 4.14 (1H, m), 4.46 (1H, s), 7.83 (1H, d), 8.20 (1H, d), 8.73 (1H, s), 9.05 (1H, d); *m/z* (ES<sup>+</sup>) (M+H)<sup>+</sup> = 381.

**Compound 12**

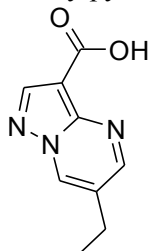
5-cyclopropyl-7-(difluoromethyl)-*N*-(5-hydroxy-2-adamantyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide



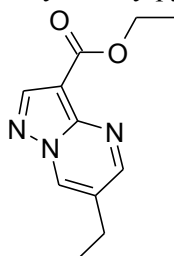
HATU (448 mg, 1.18 mmol) was added in one portion to a stirred solution of 5-cyclopropyl-7-(difluoromethyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (249 mg, 0.98 mmol), *trans* 4-aminoadamantan-1-ol hydrochloride (200 mg, 0.98 mmol) and DIPEA (0.513 mL, 2.95 mmol) in DMF (5 mL). The resulting solution was stirred at 50 °C for 16 h, under nitrogen. The reaction mixture was evaporated to dryness, then redissolved in DCM (20 mL) and washed with saturated brine (10 mL). The organic layer was dried over MgSO<sub>4</sub>, filtered and evaporated to afford crude product that 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% formic acid) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 5-cyclopropyl-7-(difluoromethyl)-*N*-(5-hydroxy-2-adamantyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (241 mg, 61%) as a white solid; <sup>1</sup>H NMR (400 MHz, DMSO) 1.14 (2H, dt), 1.21 - 1.28 (2H, m), 1.46 (2H, d), 1.62 (4H, d), 1.71 (2H, d), 1.76 (2H, t), 1.98 (2H, s), 2.04 (1H, s), 3.11 (1H, d), 4.03 (1H, d), 4.39 (1H, d), 7.54 (1H, s), 7.59 (1H, s), 7.98 (1H, d), 8.48 (1H, s). *m/z* (ES<sup>+</sup>), (M+H)<sup>+</sup> = 403.

**Compound 14**6-Ethyl-*N*-(5-hydroxy-2-adamantyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide

HATU (1.86 g, 4.90 mmol) was added to **Intermediate 9** (780 mg, 4.08 mmol), *trans* 4-aminoadamantan-1-ol hydrochloride (914 mg, 4.49 mmol) and DIPEA (1.42 mL, 8.16 mmol) in DMF (10 mL) and the reaction mixture was stirred at RT for 18 h. The reaction mixture was concentrated *in vacuo*, diluted with water and stirred for 30 min. The resulting precipitate was collected by filtration and washed with water. The solid was purified by crystallisation from EtOAc to afford the title compound (900 mg, 72%) as a white solid; <sup>1</sup>H NMR (400 MHz) 1.28 (3H, t), 1.44-1.52 (2H, m), 1.62-1.70 (4H, m), 1.71-1.80 (2H, m), 1.81-1.89 (2H, m), 2.05-2.14 (3H, m), 2.74 (2H, q), 4.03-4.08 (1H, m), 4.45 (1H, s), 8.24-8.30 (1H, m), 8.48 (1H, s), 8.84 (1H, d), 9.18 (1H, d); m/z MH<sup>+</sup>=341.

**Intermediate 9**6-Ethylpyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid

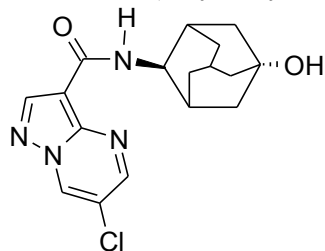
2 M aq. NaOH (8.20 mL, 16.4 mmol) was added to **Intermediate 10** (900 mg, 4.11 mmol) in MeOH (15 mL) and the reaction mixture was stirred at RT for 6 h, then stirred at 50°C for 1 h. The reaction mixture was allowed to cool to RT, then was acidified with 1 M aq. citric acid and partially concentrated *in vacuo*, before it was diluted with water. The resulting precipitate was isolated by filtration, washed with water and dried under vacuum to afford the title compound (782 mg, 99%) as a white solid; <sup>1</sup>H NMR (400 MHz) 1.26 (3H, t), 2.73 (2H, q), 8.50 (1H, s), 8.65 (1H, d), 9.11 (1H, d), 12.30 (1H, s); m/z MH<sup>+</sup>=192.

**Intermediate 10**Ethyl 6-ethylpyrazolo[1,5-*a*]pyrimidine-3-carboxylate

Ethyl 5-amino-1H-pyrazole-4-carboxylate (1.00 g, 6.45 mmol) and acetic acid (3.87 g, 64.5 mmol) were added to 2-(diethoxymethyl)-1,1-diethoxybutane (1.60 g, 6.45 mmol) in ethanol (30 mL) at 70°C. 4 M HCl in 1,4-dioxane (0.25 mL, 1 mmol) was added and the reaction mixture was stirred at 70 °C for 1 h. The mixture was allowed to cool to RT and was concentrated *in vacuo*. The resulting residue was purified by FCC, eluting with 30-100% EtOAc:isohehexane to afford the title compound (980 mg, 70%) as a white solid; <sup>1</sup>H NMR (400 MHz) 1.26 (3H, t), 1.28 (3H, t), 2.74 (2H, q), 4.28 (2H, m), 8.52 (1H, s), 8.80 (1H, d), 9.12 (1H, d); m/z MH<sup>+</sup>=220.

**Compound 15**

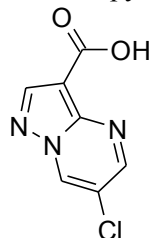
6-Chloro-N-(5-hydroxy-2-adamantyl)pyrazolo[1,5-a]pyrimidine-3-carboxamide



HATU (346 mg, 0.91 mmol) was added to **Intermediate 11** (150 mg, 0.76 mmol), *trans* 4-aminoadamantan-1-ol hydrochloride (155 mg, 0.76 mmol) and DIPEA (0.40 mL, 2.28 mmol) in DMF (2.5 mL) and the reaction mixture was stirred at RT overnight. Water was added to the reaction mixture and the resulting precipitate was collected by filtration, washed with water and dried *in vacuo* to afford the title compound (172 mg, 65%) as a solid; <sup>1</sup>H NMR (400 MHz) 1.48 (2H, d), 1.67 (4H, d), 1.79 (4H, dd), 2.06 (3H, d), 4.01-4.10 (1H, m), 4.45 (1H, s), 8.08 (1H, d), 8.58 (1H, s), 8.94 (1H, d), 9.75 (1H, d); m/z MH<sup>+</sup>=347.

**Intermediate 11**

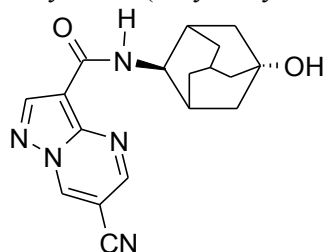
6-Chloropyrazolo[1,5-a]pyrimidine-3-carboxylic acid



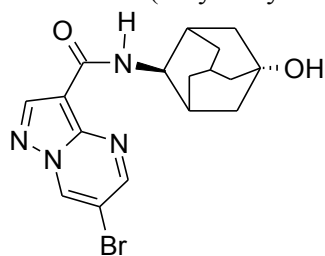
A mixture of ethyl 5-amino-1H-pyrazole-4-carboxylate (500 mg, 3.22 mmol) and 2 M aq. NaOH (4.83 mL, 9.67 mmol) in EtOH (8 mL) was heated at 80 °C overnight. The reaction mixture was concentrated *in vacuo*. EtOH (1 mL), AcOH (3 mL) and 2-chloromalonaldehyde (343 mg, 3.22 mmol) were added to the residue. The mixture was stirred at 80°C for 1 h, and the resulting precipitate was collected by filtration, washed with EtOH and dried *in vacuo* to afford the title compound (256 mg, 40%) as a solid; <sup>1</sup>H NMR (400 MHz) 8.59 (1H, s), 8.84 (1H, d), 9.68 (1H, d), 11.87-13.18 (1H, br s); m/z MH<sup>+</sup>=198.

**Compound 16**

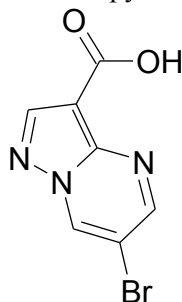
6-Cyano-N-(5-hydroxy-2-adamantyl)pyrazolo[1,5-a]pyrimidine-3-carboxamide



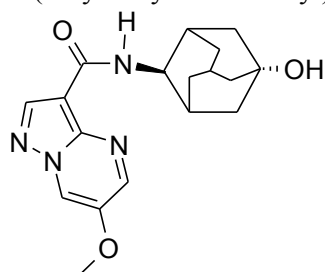
**Intermediate 14** (271 mg, 0.69 mmol), dicyanozinc (65 mg, 0.55 mmol), 9,9-dimethyl-9H-xanthene-4,5-diylbis(diphenylphosphine) (32 mg, 0.06 mmol) and tris(dibenzylideneacetone)dipalladium(0) (25 mg, 0.03 mmol) were suspended in DMF (6 mL) in a microwave tube. The reaction mixture was heated at 130 °C for 10 min in a microwave reactor and cooled to RT. The reaction mixture was diluted with EtOAc and filtered through celite. The filtrate was washed sequentially with water, sat. brine, then dried over MgSO<sub>4</sub>, filtered, concentrated *in vacuo* and purified by FCC, eluting with 0-5% MeOH:DCM to afford the title compound (33 mg, 14%) as a white solid; <sup>1</sup>H NMR (400 MHz) 1.48 (2H, d), 1.67 (4H, d), 1.72-1.87 (4H, m), 2.06 (3H, s), 4.06 (1H, d), 4.46 (1H, s), 8.07 (1H, d), 8.77 (1H, s), 9.12 (1H, d), 10.16 (1H, d); m/z MH<sup>+</sup>=338.

**Intermediate 14**6-Bromo-*N*-(5-hydroxy-2-adamantyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide

HATU (524 mg, 1.38 mmol) was added to **Intermediate 15** (278 mg, 1.15 mmol), *trans* 4-aminoadamantan-1-ol hydrochloride (234 mg, 1.15 mmol) and DIPEA (0.6 mL, 3.45 mmol) in DMF (4 mL) at RT. The reaction mixture was stirred at RT for 3 h. Water was added to the reaction mixture and the resulting precipitate was collected by filtration, washed with water and dried *in vacuo* to afford 6-bromo-*N*-(5-hydroxy-2-adamantyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (321 mg, 71%) as a solid;  $^1\text{H}$  NMR (400 MHz) 1.48 (2H, d), 1.67 (4H, d), 1.79 (4H, dd), 2.06 (3H, d), 4.06 (1H, d), 4.46 (1H, s), 8.08 (1H, d), 8.55 (1H, s), 8.95 (1H, d), 9.79 (1H, d);  $m/z$   $\text{MH}^+$ =391, 393.

**Intermediate 15**6-Bromopyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid

2-Bromomalonaldehyde (475 mg, 3.15 mmol) and 5-amino-1H-pyrazole-4-carboxylic acid (400 mg, 3.15 mmol) in a mixture of ethanol (12 mL) and acetic acid (2 mL) was stirred at 80°C for 1 h. The resulting precipitate was collected by filtration, washed with EtOH and dried *in vacuo* to afford the title compound (284 mg, 37%) as a solid;  $^1\text{H}$  NMR (400 MHz) 8.57 (1H, s), 8.87 (1H, d), 9.73 (1H, d), 12.47 (1H, s);  $m/z$   $\text{MH}^+$ =242, 244.

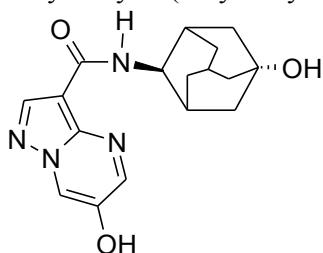
**Compound 17***N*-(5-hydroxy-2-adamantyl)-6-methoxy-pyrazolo[1,5-*a*]pyrimidine-3-carboxamide

**Intermediate 16** (83 mg, 0.25 mmol), potassium carbonate (38 mg, 0.28 mmol), and iodomethane (0.017 mL, 0.28 mmol) were suspended in DMF (3 mL) in a microwave tube. The reaction mixture was heated at 100°C for 30 min in a microwave reactor and cooled to RT. The reaction mixture was concentrated *in vacuo*. The residue was taken up in EtOAc and washed with water. The organic layer was dried over  $\text{MgSO}_4$ , filtered and concentrated *in vacuo* to afford crude product (102 mg). In a separate microwave tube 6-hydroxy-*N*-(5-hydroxy-2-adamantyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (97 mg, 0.30 mmol), potassium carbonate (45 mg, 0.32 mmol) and iodomethane (0.02

mL, 0.32 mmol) were suspended in DMF (3 mL) and the reaction mixture was heated at 100°C for 30 min in a microwave reactor and then cooled to RT. The reaction mixture was concentrated *in vacuo*, taken up in EtOAc and washed with water. The organic layer was dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to afford crude product (87 mg). The two crude products were combined and purified by preparative HPLC to afford the title compound (103 mg, 55% overall); <sup>1</sup>H NMR (400 MHz) 1.43-1.52 (2H, m), 1.62-1.70 (4H, m), 1.72-1.85 (4H, m), 2.01-2.11 (3H, m), 3.91 (3H, s), 4.03-4.09 (1H, m), 4.45 (1H, s), 8.12 (1H, d), 8.42 (1H, s), 8.78 (1H, d), 9.08 (1H, d); m/z MH<sup>+</sup>=343.

### Intermediate 16

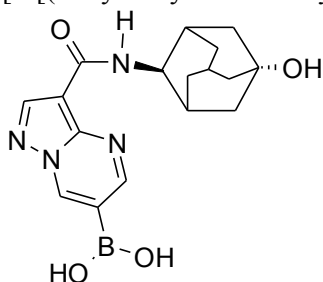
6-Hydroxy-*N*-(5-hydroxy-2-adamantyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide



3-Chloroperoxybenzoic acid (261 mg, 1.17 mmol) was added to **Intermediate 17** (415 mg, 1.17 mmol) in DCM (40 mL) and MeOH (20 mL). The reaction mixture was stirred at RT for 16 h. The reaction mixture was concentrated *in vacuo*, taken up in DCM and washed sequentially with sat. aq. NaHCO<sub>3</sub> and sat. brine, and the organic layer was discarded. The combined aq. layers were acidified with 2 M aq. HCl and extracted with DCM. The organic layer was washed with sat. brine, dried over MgSO<sub>4</sub>, concentrated *in vacuo* and purified by FCC, eluting with 0-10% MeOH:DCM to afford the title compound (56 mg). A precipitate was formed in the aq. phase which was isolated by filtration and dried *in vacuo* to afford additional title compound (97 mg). The aq. layer was concentrated *in vacuo* and purified by FCC, eluting with 0-20% MeOH:DCM to afford additional title compound (27 mg) and the 3 samples were combined to afford the title compound (180 mg, 47%) as a solid; <sup>1</sup>H NMR (400 MHz) 1.47 (2H, d), 1.61-1.70 (4H, m), 1.71-1.86 (4H, m), 1.99-2.12 (3H, m), 4.01-4.08 (1H, m), 4.46 (1H, s), 8.13 (1H, d), 8.36 (1H, s), 8.70 (1H, s), 8.74 (1H, s), 10.81 (1H, s); m/z MH<sup>+</sup>=329.

### Intermediate 17

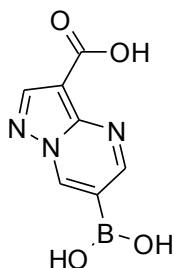
[3-[(5-Hydroxy-2-adamantyl)carbamoyl]pyrazolo[1,5-*a*]pyrimidin-6-yl]boronic acid



HATU (933 mg, 2.45 mmol) was added to **Intermediate 18** (423 mg, 2.04 mmol), *trans* 4-aminoadamantan-1-ol hydrochloride (416 mg, 2.04 mmol) and NEt<sub>3</sub> (1.14 mL, 8.18 mmol) in DMF (10 mL) and the reaction mixture was stirred at RT for 3 h. The reaction mixture was partially concentrated *in vacuo*, then diluted with MeCN. The resulting precipitate was isolated by filtration and dried *in vacuo* to afford the title compound (415 mg, 57%); m/z MH<sup>+</sup>=357.

### Intermediate 18

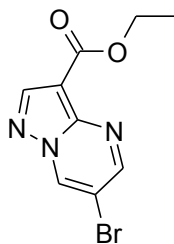
6-Boronopyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid



**Intermediate 19** (1 g, 3.70 mmol), potassium acetate (1.09 g, 11.1 mmol), bis(pinacolato)diboron (1.13 g, 4.44 mmol), and 1,1'-bis(diphenylphosphino)ferrocene-palladium(II)dichloride.DCM (0.212 g, 0.26 mmol) and 1,4-dioxane (20 mL) were combined and the reaction mixture was stirred at 80°C overnight. The reaction mixture was allowed to cool to RT and taken up in EtOAc and 2 M aq. HCl. The organic layer was partially concentrated and was extracted with 2 M aq. NaOH. The aq. extract was acidified with conc. aq. HCl and extracted twice with EtOAc. The combined organic layers were dried over MgSO<sub>4</sub> and concentrated *in vacuo* to afford 3-(ethoxycarbonyl)pyrazolo[1,5-a]pyrimidin-6-ylboronic acid. This material was dissolved in 1 M aq. LiOH (11.1 mL, 11.1 mmol) and the reaction mixture was stirred at 65°C for 40 min, then allowed to cool to RT. 2 M aq. HCl (5.55 mL) was added, and the resulting precipitate was isolated by filtration, washed with water and dried *in vacuo* to afford the title compound (423 mg, 55%); <sup>1</sup>H NMR (400 MHz) 8.56 (1H, s), 8.93 (1H, s), 9.26 (1H, s) OH's not observed; m/z MH<sup>+</sup>=208.

### Intermediate 19

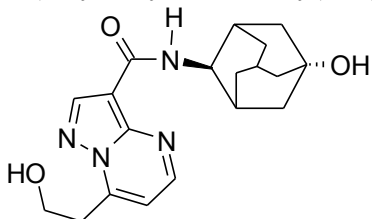
Ethyl 6-bromopyrazolo[1,5-a]pyrimidine-3-carboxylate



Ethyl 5-amino-1H-pyrazole-4-carboxylate (6.17 g, 39.8 mmol) and acetic acid (30 mL) were added to 2-bromomalonaldehyde (6 g, 39.8 mmol) in ethanol (90 mL) at 70°C and the reaction mixture was stirred at 70°C for 1 h, then allowed to cool to RT. The reaction mixture was concentrated *in vacuo* and DCM (1 L) and 1 M aq. NaOH (180 mL) was added. The resulting mixture was filtered and the liquid phases were separated. The aq. layer was extracted with DCM and the combined organic layers dried over MgSO<sub>4</sub>, filtered, concentrated *in vacuo* and purified by FCC, eluting with 10-50% EtOAc:isohexane to afford title compound (5.58 g, 52%); <sup>1</sup>H NMR (400 MHz) 1.30 (3H, t), 4.30 (2H, q), 8.61 (1H, s), 8.90 (1H, d), 9.75 (1H, d).

### Compound 18

*N*-(5-hydroxy-2-adamantyl)-6-(2-hydroxyethyl)pyrazolo[1,5-a]pyrimidine-3-carboxamide

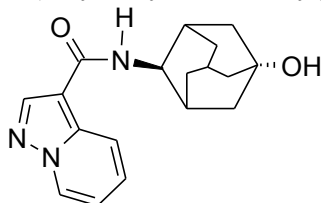


HATU (430 mg, 1.13 mmol) was added to 6-(2-hydroxyethyl)pyrazolo[1,5-a]pyrimidine-3-carboxylic acid hydrate (212 mg, 0.94 mmol), 4-aminoadamantan-1-ol hydrochloride (230 mg, 1.13 mmol) and DIPEA (0.492 mL, 2.82 mmol) in DMF (10 mL) and the resulting mixture was stirred at RT overnight. The reaction mixture was evaporated to dryness. EtOAc (50 mL), and water (15 mL) were added. A white precipitate was filtered off. This was dried under vacuum to give *N*-(5-hydroxy-2-adamantyl)-6-(2-hydroxyethyl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (330 mg, 98 %) as a solid.

$^1\text{H}$  NMR (400 MHz, DMSO) 1.44 - 1.53 (2H, m), 1.62 - 1.72 (4H, m), 1.72 - 1.88 (4H, m), 2.02 - 2.13 (3H, m), 2.84 (2H, t), 3.70 (2H, q), 4.03 - 4.10 (1H, m), 4.45 (1H, s), 4.75 (1H, t), 8.27 (1H, d), 8.49 (1H, s), 8.79 (1H, d), 9.14 (1H, d);  $m/z$  ( $\text{ES}^+$ ) ( $\text{M}+\text{H}$ ) $^+$  = 357.

#### Compound 19

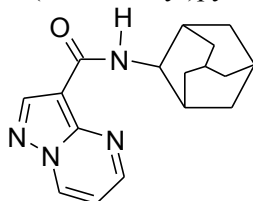
*N*-(5-hydroxy-2-adamantyl)pyrazolo[1,5-*a*]pyridine-3-carboxamide



Made according to the procedure for **compound 2** from pyrazolo[1,5-*a*]pyridine-3-carboxylic acid in 75% yield;  $^1\text{H}$  NMR (400 MHz, DMSO) 1.28 - 1.41 (2H, m), 1.56 - 1.78 (6H, m), 1.96 - 2.14 (5H, m), 3.94 - 4.05 (1H, m), 4.39 (1H, s), 6.98 - 7.07 (1H, m), 7.38 - 7.48 (1H, m), 7.52 (1H, d), 8.16 (1H, d), 8.69 - 8.80 (2H, m);  $m/z$  ( $\text{ES}^+$ ) ( $\text{M}+\text{H}$ ) $^+$  = 312.

#### Compound 20

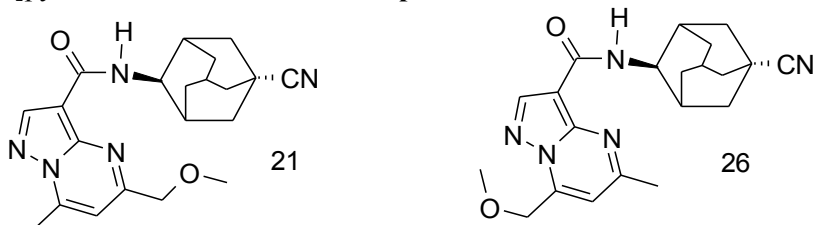
*N*-(2-adamantyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide



HATU (456 mg, 1.20 mmol) was added in one portion to a stirred solution of pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (163 mg, 1.00 mmol), adamantan-2-amine hydrochloride (188 mg, 1.00 mmol) and DIPEA (0.523 mL, 3.00 mmol) in DMF (3 mL). The resulting solution was stirred at RT overnight. Water (10 mL) was added to the reaction which produced a precipitate. The precipitate was collected by filtration, washed with water (20 mL) and dried under vacuum to afford *N*-(2-adamantyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (196 mg, 66%) as a cream solid;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) 1.70 (2H, d), 1.80 (2H, s), 1.88 - 1.96 (6H, m), 2.04 (4H, d), 4.35 (1H, d), 6.97 (1H, dd), 8.39 (1H, d), 8.60 - 8.73 (2H, m), 8.77 (1H, dd);  $m/z$  ( $\text{ES}^+$ ) ( $\text{M}+\text{H}$ ) $^+$  = 297.

#### Compound 21 & 26

*N*-(5-cyano-2-adamantyl)-5-(methoxymethyl)-7-methyl-pyrazolo[1,5-*a*]pyrimidine-3-carboxamide **compound 21** and *N*-(5-cyano-2-adamantyl)-7-(methoxymethyl)-5-methyl-pyrazolo[1,5-*a*]pyrimidine-3-carboxamide **compound 26**



HATU (472 mg, 1.24 mmol) was added in one portion to a stirred solution of **intermediate 20** a mixture of [5-(methoxymethyl)-7-methylpyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid and 7-(methoxymethyl)-5-methylpyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid] (229 mg, 1.04 mmol), 4-aminoadamantane-1-carbonitrile (182 mg, 1.04 mmol) and DIPEA (0.721 mL, 4.14 mmol) in DMF (5 mL). The resulting solution was stirred at 50 °C for 17 hours. The reaction mixture was allowed to cool to RT, then diluted with water (25 mL) and the resulting precipitate was collected by filtration. The crude product was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5  $\mu$  silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 0.5%

NH<sub>3</sub>) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford *N*-(5-cyano-2-adamantyl)-5-(methoxymethyl)-7-methyl-pyrazolo[1,5-a]pyrimidine-3-carboxamide **compound 21** (85 mg, 22%) and *N*-(5-cyano-2-adamantyl)-7-(methoxymethyl)-5-methyl-pyrazolo[1,5-a]pyrimidine-3-carboxamide **compound 26** (35.0 mg, 9%) as white solids.

**compound 21**

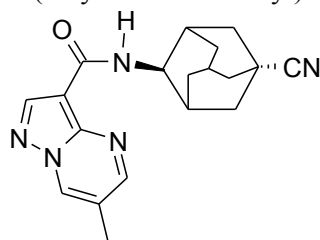
<sup>1</sup>H NMR (400 MHz, DMSO) 1.66 (2H, d), 1.86 - 2.25 (11H, m), 2.80 (3H, s), 3.43 (3H, s), 4.17 (1H, d), 4.63 (2H, s), 7.23 (1H, s), 8.42 - 8.70 (2H, m); m/z (ES<sup>+</sup>) (M+H)<sup>+</sup> = 380.

**compound 26**

<sup>1</sup>H NMR (400 MHz, DMSO) 1.58 (2H, d), 1.77 - 2.14 (11H, m), 2.51 (3H, s), 3.41 (3H, s), 4.02 - 4.08 (1H, m), 4.86 (2H, s), 7.07 (1H, s), 8.39 (1H, s), 8.47 (1H, d); m/z (ES<sup>+</sup>) (M+H)<sup>+</sup> = 380.

**Compound 22**

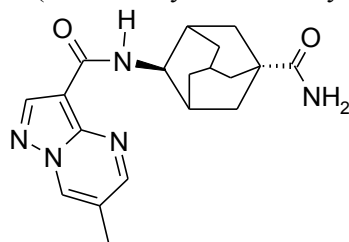
*N*-(5-cyano-2-adamantyl)-6-methyl-pyrazolo[1,5-a]pyrimidine-3-carboxamide



DBU (0.261 mL, 1.75 mmol) was added to **Intermediate 4** (206 mg, 0.58 mmol) in DCM (10 mL) and the reaction mixture was stirred at RT for 10 min. Ethyl phosphorodichloridate (0.138 mL, 1.17 mmol) was added and the reaction mixture was stirred at RT for 1 h. Additional ethyl phosphorodichloridate (0.040 mL) was added and the solution was stirred at RT for a further 4 h. The reaction mixture was quenched with water and extracted three times with DCM. The combined organic layers washed with sat. brine, concentrated *in vacuo* and purified by FCC, eluting with 0-4% MeOH:DCM to afford the title compound (139 mg, 71%) as a white solid; <sup>1</sup>H NMR (400 MHz) 1.72 (2H, d), 1.97 (2H, d), 2.02-2.14 (5H, m), 2.15-2.29 (4H, m), 2.44 (3H, s), 4.16-4.30 (1H, m), 8.37 (1H, d), 8.55 (1H, s), 8.83 (1H, d), 9.24 (1H, dd); m/z MH<sup>+</sup>=336.

**Intermediate 4**

*N*-(5-carbamoyl-2-adamantyl)-6-methyl-pyrazolo[1,5-a]pyrimidine-3-carboxamide

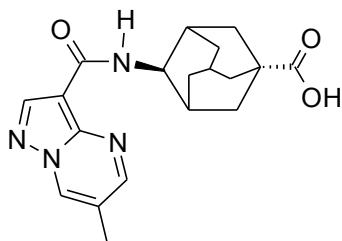


DIPEA (0.476 mL, 2.73 mmol) was added to **Intermediate 5** (322 mg, 0.91 mmol) and HATU (691 mg, 1.82 mmol) in DMF (10 mL) and the reaction mixture was stirred at RT for 30 min. Ammonium chloride (97 mg, 1.82 mmol) was added and the reaction mixture was stirred at RT for 17 h. The reaction mixture was diluted with water and the resulting precipitate was collected by filtration, washed with water and dried *in vacuo* at 50 °C to afford the title compound (219 mg, 68%) as a white solid; <sup>1</sup>H NMR (400 MHz) 1.58 (2H, d), 1.75-2.06 (11H, m), 2.37 (3H, s), 4.03-4.16 (1H, m), 6.71 (1H, s), 7.00 (1H, s), 8.32 (1H, d), 8.47 (1H, s), 8.77 (1H, d), 9.17 (1H, dd); m/z MH<sup>+</sup>=354.

**Intermediate 5**

4-[(6-Methylpyrazolo[1,5-a]pyrimidine-3-carbonyl)amino]adamantane-1-carboxylic acid

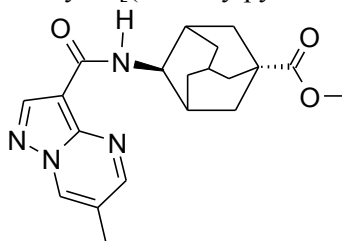




2 M aq. NaOH (1.62 mL, 3.23 mmol) was added to **Intermediate 6** (361 mg, 0.98 mmol) in MeOH (20 mL) and the reaction mixture was stirred at 65°C for 6 h and then allowed to cool to RT overnight. The reaction mixture was concentrated *in vacuo*, diluted with water and acidified with 2 M aq. HCl. The resulting precipitate was collected by filtration, washed with water and dried *in vacuo* at 50 °C to afford the title compound (327 mg, 94%) as a white solid; <sup>1</sup>H NMR (400 MHz) 1.60 (2H, d), 1.78-2.07 (11H, m), 2.37 (3H, s), 4.05-4.13 (1H, m), 8.32 (1H, d), 8.47 (1H, s), 8.77 (1H, d), 9.17 (1H, s), 12.09 (1H, s); m/z MH<sup>+</sup>=355.

### Intermediate 6

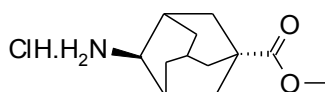
Methyl 4-[(6-methylpyrazolo[1,5-a]pyrimidine-3-carbonyl)amino]adamantane-1-carboxylate



HATU (509 mg, 1.34 mmol) was added to 6-methylpyrazolo[1,5-a]pyrimidine-3-carboxylic acid (198 mg, 1.11 mmol), **Intermediate 7** (274 mg, 1.11 mmol) and DIPEA (0.583 mL, 3.34 mmol) in DMF (10 mL) and the reaction mixture was stirred at RT for 17 h. The reaction mixture was diluted with water and the resulting precipitate was collected by filtration, washed with water and dried *in vacuo* to afford the title compound (376 mg, 92%) as a solid; <sup>1</sup>H NMR (400 MHz) 1.61 (2H, d), 1.80-2.07 (11H, m), 2.37 (3H, s), 3.60 (3H, s), 4.10 (1H, d), 8.31 (1H, d), 8.47 (1H, s), 8.76 (1H, t), 9.17 (1H, dd); m/z MH<sup>+</sup>=369.

### Intermediate 7

Methyl 4-aminoadamantane-1-carboxylate hydrochloride



4 M HCl in 1,4-dioxane (64.0 mL, 256 mmol) was added to **Intermediate 8** (10.0 g, 51.2 mmol, mixture of *cis/trans* isomers) and the reaction mixture was stirred at RT for 18 h. The reaction mixture was concentrated *in vacuo* and then triturated with Et<sub>2</sub>O to afford the *trans* title compound (9.10 g, 72%) as a white solid; <sup>1</sup>H NMR (400 MHz) 1.50-1.53 (2H, m), 1.81-1.82 (2H, m), 1.92 (5H, s), 1.99 (2H, d), 2.07 (2H, s), 3.35-3.30 (1H, m), 3.60 (3H, s), 8.18 (3H, s).

### Intermediate 8

4-Aminoadamantane-1-carboxylic acid

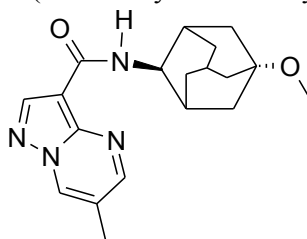


4-Oxoadamantane-1-carboxylic acid (10.0 g, 51.5 mmol), 5% Pd/C (1.10 g, 10.3 mmol) in 7 M ammonia in MeOH (250 mL) was stirred under an atmosphere of hydrogen for 18 h. The reaction mixture was diluted with water and filtered through celite. The reaction mixture was concentrated *in vacuo* and the residue was triturated with acetonitrile to afford 4-aminoadamantane-1-carboxylic acid

as an uncharacterised mixture of *cis* and *trans* isomers (8.63 g, 86%) which was used without further purification.

### Compound 23

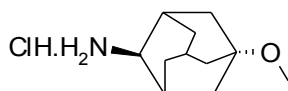
*N*-(5-methoxy-2-adamantyl)-6-methyl-pyrazolo[1,5-a]pyrimidine-3-carboxamide



HATU (773 mg, 2.03 mmol) was added to **Intermediate 1** (369 mg, 1.69 mmol), 6-methylpyrazolo[1,5-a]pyrimidine-3-carboxylic acid (300 mg, 1.69 mmol) and DIPEA (0.887 mL, 5.08 mmol) in DMF (10 mL) and the reaction mixture was stirred at RT for 16 h. The reaction mixture was concentrated *in vacuo*. The residue was redissolved in EtOAc and washed with sat. brine. The organic layer was dried over MgSO<sub>4</sub>, filtered, concentrated *in vacuo* and triturated with 7:2:1 DMSO:MeCN:water to afford the title compound (175 mg, 30%); <sup>1</sup>H NMR (400 MHz) 1.46-1.57 (2H, m), 1.68-1.79 (4H, m), 1.79-1.90 (4H, m), 2.07-2.17 (3H, m), 2.37 (3H, s), 3.13 (3H, s), 4.05 - 4.13 (1H, m), 8.26 (1H, d), 8.47 (1H, s), 8.77 (1H, d), 9.17 (1H, d); *m/z* MH<sup>+</sup>=341.

### Intermediate 1

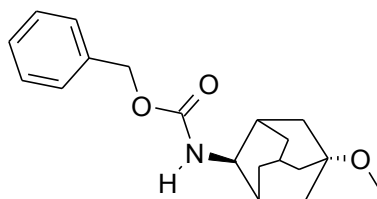
5-Methoxyadamantan-2-amine hydrochloride



**Intermediate 2** (10.7 g, 33.9 mmol) and 5% Pd/C (1.0 g) in ethanol (100 mL) were stirred under hydrogen at RT for 18 h. The reaction mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was taken up in Et<sub>2</sub>O and acidified with 4 M aq. HCl. The resulting precipitate was isolated by filtration, washed with Et<sub>2</sub>O to afford the title compound (6.5g, 88%) as a solid; <sup>1</sup>H NMR (400 MHz) 1.35-1.41 (2H, m), 1.54-1.78 (6H, m), 1.90-1.96 (2H, m), 2.05-2.09 (1H, m), 2.13-2.18 (2H, m), 3.10 (3H, s), 3.21-3.27(1H, m), 8.21 (3H, s).

### Intermediate 2

Benzyl N-(5-methoxy-2-adamantyl)carbamate



Benzyl chloroformate (10.5 mL, 73.4 mmol) was added dropwise to the **Intermediate 3** (*cis/trans* mixture, 13.3 g, 73.4 mmol) and NEt<sub>3</sub> (20.5 mL, 147 mmol) in anhydrous THF (25 mL) at 5°C. The reaction mixture was allowed to warm to RT and stirred at RT for 2 h. The reaction mixture was concentrated *in vacuo*. the residue was dissolved in EtOAc and was washed sequentially with water and sat. brine. The organic layer was dried over MgSO<sub>4</sub>, filtered, concentrated *in vacuo* and purified by FCC, eluting with 20-60% EtOAc:isohexane to afford the *cis* and *trans* isomers, where the *trans* title compound is the major isomer (10.5g, 45%); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 1.42-1.50 (2H, m), 1.63-1.71 (2H, m), 1.72-1.80 (4H, m), 1.81-1.91 (2H, m), 2.10-2.18 (3H, m), 3.22 (3H, s), 3.73-3.82(1H, m), 5.09 (2H, s), 7.28-7.39 (6H, m).

### Intermediate 3

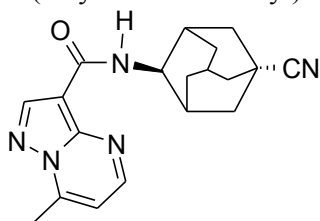
5-Methoxyadamantan-2-amine (*cis/trans* mixture)



5-Hydroxyadamantan-2-one (33.3 g, 200 mmol) was added to 60%NaH in mineral oil (9.60 g, 240 mmol) in DMF (100 mL) at 5°C and the reaction mixture was allowed to warm to RT and stirred for 1 h, then was stirred at 60 °C for 1 h. The reaction mixture was cooled to 0 °C and methyl iodide (16.2 mL, 260 mmol) was added dropwise and the reaction mixture was then stirred at RT for 3 days. The reaction mixture was quenched with sat. aq. NH<sub>4</sub>Cl (10 mL), diluted with EtOAc and washed sequentially with water and sat. brine. The organic layer was dried over MgSO<sub>4</sub>, filtered, concentrated *in vacuo* and purified by FCC, eluting with 20-100% EtOAc:isohexane to afford crude 5-methoxyadamantan-2-one (13.9 g, 38%) as a colourless oil. A portion of the 5-methoxyadamantan-2-one (10 g, 55.5 mmol) was combined with 7 M NH<sub>4</sub> in MeOH (317 mL, 2219 mmol) and 5% Pd/C (23.6 g, 11.1 mmol) and the reaction mixture was stirred under hydrogen at RT for 18 h. The reaction mixture was filtered through celite and the filtrate was concentrated *in vacuo*, taken up in toluene and concentrated *in vacuo* to afford crude 5-methoxyadamantan-2-amine (11.8 g) as a pale yellow oil (mixture of *cis/trans* products, not characterised).

#### Compound 24

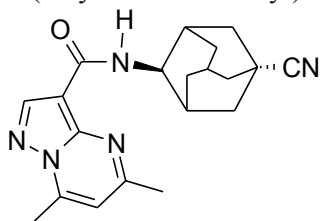
*N*-(5-cyano-2-adamantyl)-7-methyl-pyrazolo[1,5-*a*]pyrimidine-3-carboxamide



HATU (324 mg, 0.85 mmol) was added in one portion to a stirred solution of 7-methylpyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (151 mg, 0.85 mmol), 4-aminoadamantane-1-carbonitrile (150 mg, 0.85 mmol) and *N*-ethyl-*N*-isopropylpropan-2-amine (0.445 mL, 2.55 mmol) in DMF (3.5 mL). The resulting solution was stirred at 50 °C for 6 hours under nitrogen. The reaction mixture was evaporated to dryness, then re-dissolved in DCM (20 mL) and washed with saturated brine (10 mL). The organic layer was dried over MgSO<sub>4</sub>, 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% formic acid) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford *N*-(5-cyano-2-adamantyl)-7-methyl-pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (77 mg, 27%) as a white solid; <sup>1</sup>H NMR (400 MHz, DMSO) 1.66 (2H, d), 1.90 (2H, d), 1.69 - 2.07 (5H, m), 2.08 - 2.22 (4H, m), 2.80 (3H, d), 4.13 - 4.20 (1H, m), 7.23 (1H, dd), 8.43 (1H, d), 8.59 (1H, s), 8.73 (1H, d); *m/z* (ES<sup>+</sup>), (M+H)<sup>+</sup> = 336.

#### Compound 25

*N*-(5-cyano-2-adamantyl)-5,7-dimethyl-pyrazolo[1,5-*a*]pyrimidine-3-carboxamide

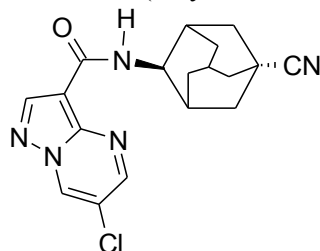


HATU (401 mg, 1.06 mmol) was added in one portion to a stirred solution of 5,7-dimethylpyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (168 mg, 0.88 mmol), 4-aminoadamantane-1-carbonitrile (155 mg, 0.88 mmol) and DIPEA (0.460 mL, 2.64 mmol) in DMF (3.5 mL). The resulting solution was stirred at 50 °C for 17 h. The reaction mixture was evaporated and water added to the residue. This was extracted with EtOAc (2 x 50 mL). The organic layer was dried over MgSO<sub>4</sub>,

filtered and evaporated to afford the crude product as an orange solid which was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5 $\mu$  silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 0.1% formic acid) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford *N*-(5-cyano-2-adamantyl)-5,7-dimethyl-pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (129 mg, 42%) as an orange solid. <sup>1</sup>H NMR (400 MHz, DMSO) 1.68 (2H, d), 1.94 (2H, d), 1.98 - 2.05 (5H, m), 2.07 - 2.21 (4H, m), 2.60 (3H, s), 2.74 (3H, s), 4.16 (1H, d), 7.13 (1H, s), 8.49 (1H, s), 8.62 (1H, d) ; *m/z* (ES<sup>+</sup>) (M+H)<sup>+</sup> = 350.

#### Compound 27

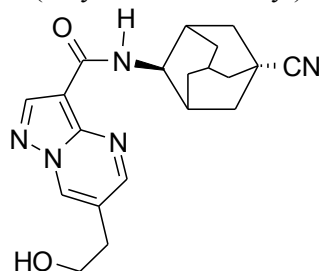
6-chloro-*N*-(5-cyano-2-adamantyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide



HATU (326 mg, 0.86 mmol) was added in one portion to a stirred solution of 6-chloropyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (169 mg, 0.86 mmol), 4-aminoadamantane-1-carbonitrile (151 mg, 0.86 mmol) and DIPEA (0.448 mL, 2.57 mmol) in DMF (3.5 mL) and the resulting solution was stirred at 50 °C for 17 h. The reaction mixture was evaporated to afford a brown oil. This was dissolved in DCM (20 mL), and was washed with brine (10 mL). The organic layer was dried over MgSO<sub>4</sub>, filtered and evaporated to afford the crude product as a brown oil that was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5 $\mu$  silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 0.1% formic acid) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 6-chloro-*N*-(5-cyano-2-adamantyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (90 mg, 30%) as a white solid; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 1.69 (2H, d), 1.99 (2H, d), 2.05 - 2.11 (3H, m), 2.14 - 2.22 (4H, m), 2.26 (2H, d), 4.33 (1H, d), 8.12 (1H, d), 8.63 (1H, d), 8.67 (1H, s), 8.83 (1H, d); *m/z* (ES<sup>+</sup>), (M+H)<sup>+</sup> = 256.

#### Compound 28

*N*-(5-cyano-2-adamantyl)-6-(2-hydroxyethyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide



HATU (518 mg, 1.36 mmol) was added portionwise to 6-(2-hydroxyethyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (235 mg, 1.13 mmol), 4-aminoadamantane-1-carbonitrile (200 mg, 1.13 mmol) and DIPEA (0.593 mL, 3.40 mmol) in DMA (5.81 mL) at room temperature under nitrogen. The resulting solution was stirred at room temperature for 3 hours. The mixture was diluted with water (25 mL) and extracted with ethyl acetate (25 mL). An emulsion formed hence the mixture was filtered. The solid filtered off was predominantly product by LCMS. The organic layer was separated off from the aqueous phase, dried over MgSO<sub>4</sub>, filtered and evaporated give a solid. The two solid samples were combined and purified by flash silica chromatography, elution gradient 0 to 5% MeOH in DCM. Pure fractions were evaporated to dryness to afford a white solid that was recrystallised from ethanol (10 mL) to give *N*-(5-cyano-2-adamantyl)-6-(2-hydroxyethyl)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (120 mg, 29%) as a solid; <sup>1</sup>H NMR (400 MHz, DMSO) 1.66 (2H, d), 1.86 - 2.22 (11H, m), 2.84 (2H,

t), 3.69 (2H, q), 4.16 (1H, d), 4.75 (1H, t), 8.32 (1H, d), 8.50 (1H, s), 8.79 (1H, d), 9.15 (1H, d); m/z (ES<sup>+</sup>) (M+H)<sup>+</sup> = 366.

**Summary statistics of the  $\beta$ HSD-compound 7 complex crystal structure.**

Beam-line	ESRF ID14.4
Wavelength (Å)	1.00003
Detector type	ADSC CCD
Data collection date	1/10/2010
Space group	P3 <sub>1</sub> 2 <sub>1</sub>
Cell constants a; b; c (Å)	107.79 107.79 136.02 90.0 90.0 120.0
Resolution range (Å) <sup>1</sup>	46.67-3.2 (3.6-3.2)
Completeness overall (%) <sup>1</sup>	97.2 (99.5)
Reflections, unique	15064
Multiplicity <sup>1</sup>	3.4 (3.6)
Mean(I)/sd(I) <sup>1</sup>	6.2 (2.0)
R <sub>merge</sub> overall <sup>2</sup>	0.113 (0.507)
R <sub>value</sub> overall (%) <sup>3</sup>	27.5
R <sub>value</sub> free (%) <sup>1</sup>	33.30
Non hydrogen protein atoms	3960
Non hydrogen ligand atoms	146
Solvent molecules	0
Bond lengths (Å)	0.010
Bond angles (°)	1.35
Average B values (Å <sup>2</sup> )	22.56
Φ, Ψ angle distribution for residues <sup>4</sup>	
In favoured regions (%)	88.30
In allowed regions (%)	7.00
In outlier regions (%)	4.7

**1** Values in parentheses refer to the outer resolution shell

**2**  $R_{\text{merge}} = S_{hkl} [(\sum_i |I_i - \langle I \rangle|) / \sum_i I_i]$

**3**  $R_{\text{value}} = S_{hkl} [|F_{\text{obs}}| - |F_{\text{calc}}|] / S_{hkl} |F_{\text{obs}}|$

$R_{\text{free}}$  is the cross-validation R factor computed for the test set of 5% of unique reflections

**4** Ramachandran statistics as defined by Coot