CHAPTER 4

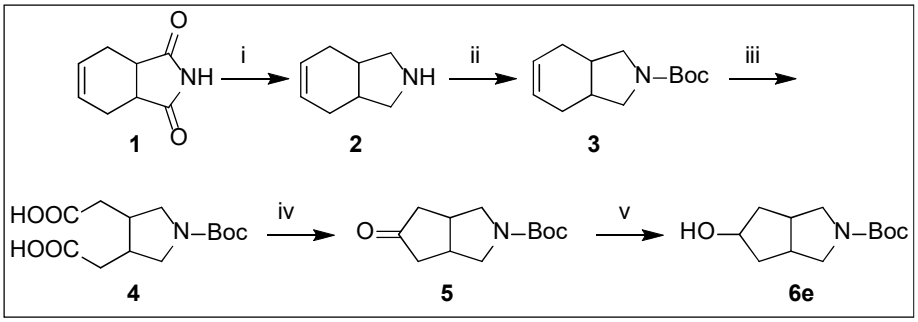
4. Experimental

4.1. Chemistry

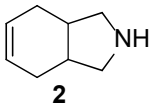
All the solvents and reagents were purchased from commercial sources, used without further purification, or prepared according to published procedures. Reactions were monitored using thin-layer silica gel chromatography (TLC) using 0.25 mm silica gel 60F plates from Merck. Plates were visualised by ultraviolet irradiation at 254 nM or/and staining with ninhydrin and potassium permanganate. Products were purified by flash chromatography on a Combi flash instrument using Redisep® columns. The melting points were recorded on a scientific melting point apparatus and are uncorrected. NMR spectra were recorded at 400 MHz for 1H and 100 MHz for 13C on a 400 UltraSheild spectrometer (Bruker, Germany). Spectra were taken in the indicated solvent at ambient temperature. Chemical shifts (δ) are given in parts per million (ppm), with tetramethyl silane as an internal standard. Multiplicities are recorded as follows: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, bs = broad singlet, m = multiplet. Coupling constants are reported as J values in Hertz (Hz). The purities of all target compounds were determined to be above 95% by ultra-performance liquid chromatography (UPLC). UPLC conditions were as follows: YMC-Triart C18 column at room temperature, 100 × 2.0 mm, 1.9 μM, mobile phase: 0.05 % TFA in water: ACN (gradient), 12 min. run; flow rate, 0.4 mL/min; UV detection λ = 220 nM. Mass spectra were recorded on the Perkin-Elmer Sciex API 3000. Elemental analyses were carried out using a Perkin-Elmer 2400 CHN analyzer. ESI-Q-TOF-MS

measurements were performed on a Xevo G2 QToF (Waters, USA) mass spectrometer. HRMS was recorded on a Bruker-Daltonics Micro-TOF-Q II mass spectrometer. Differential scanning calorimetry (DSC) was recorded on the TA Q2O auto instrument (Waters, USA). X-ray diffraction (XRD) analysis was performed on a Bruker D8 Advance instrument (Germany).

4.1.1. Preparation of tert-butyl 5-hydroxyhexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate (Intermediate 6e).



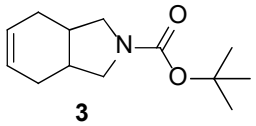
Step I: 2,3,3a,4,7,7a-hexahydro-1H-isoindole **(2)**.



Tetrahydrofuran (2500 mL) was cooled to 0 to -5 ºC prior to the addition of lithium aluminium hydride (50.2 g, 1323 mmol). Subsequently, tetrahydrophthalimide (100 g, 662 mmol) was gradually added to the reaction mixture while maintaining the temperature between 0 and 5 °C. The reaction mixture was warmed to room temperature and stirred for an hour after it had been refluxed for 16 hours at 67 °C. The reaction mixture was cooled to ambient temperature, then to 0 °C. The reaction mixture was carefully quenched with the dropwise addition of 10% aqueous potassium hydroxide (200 mL) while keeping the temperature below 5 °C. The resulting slurry was passed through a Hyflow bed, and residuals were washed with tetrahydrofuran (2 x 200 mL). The combined filtrate was dried over sodium sulphate, filtered, and

concentrated *in vacuo* to afford 2,3,3a,4,7,7a-hexahydro-1H-isoindole (80 g, 649 mmol, 98% yield). The obtained product was used for the next reaction without further purification. **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm 1.86-1.94 (m, 2H), 2.17-2.19 (m, 2H), 2.22-2.29 (m, 2H), 2.67-2.72 (m, 2H), 3.00-3.07 (m, 2H), 5.70 (s, 2H). **ESI-MS** (m/z): 123.65 $[M+H]^+$.

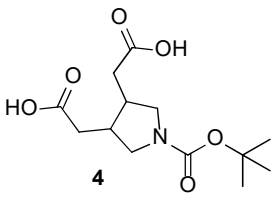
Step II: Tert-butyl 1,3,3a,4,7,7a-hexahydro-2H-isoindole-2-carboxylate **(3)**.



3

2,3,3a,4,7,7a-hexahydro-1H-isoindole (78 g, 633 mmol) was dissolved in tetrahydrofuran (1560 mL), cooled to 0 °C. Boc anhydride (0.184 L, 791 mmol) was added dropwise while the temperature was maintained between 0 and 5 °C. The reaction mixture was warmed to room temperature and stirred at room temperature for 16 hours. The reaction mixture was concentrated and dried *in vacuo*. The obtained residue was dissolved in n-Hexane (3000 mL) and water (5000 mL), and it was stirred at room temperature for 15 minutes. The organic layer was separated, dried over sodium sulphate, filtered, concentrated, and dried *in vacuo* to afford tert-butyl 1,3,3a,4,7,7a-hexahydro-2H-isoindole-2-carboxylate (120 g, 537 mmol, 85% yield) as a brown oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm 1.46 (s, 9H), 1.86-2.03 (m, 2H), 2.18-2.30 (m, 4H), 3.04-3.07 (m, 1H), 3.13-3.17 (m, 1H), 3.35-3.42 (m, 2H), 5.62 (s, 2H). **ESI-MS** (m/z): 223.95 $[M+H]^+$.

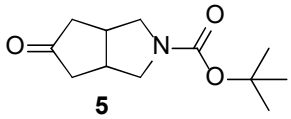
Step III: 2,2'-(1-(tert-butoxycarbonyl)pyrrolidine-3,4-diyl)diacetic acid **(4)**.



4

Tert-butyl 1,3,3a,4,7,7a-hexahydro-2H-isoindole-2-carboxylate (60 g, 269 mmol) was dissolved in ethyl acetate (1440 mL) and cooled to 0 °C. Ruthenium (IV) oxide (0.715 g, 5.37 mmol) was added, followed by a dropwise addition of sodium periodate aqueous solution (4600 mL, 2152 mmol). The temperature was maintained at 0 to 5 °C during addition. The reaction mixture was stirred at 0 to 5 °C for 1 hour. The reaction mixture was filtered through a Hyflow bed, and the residual solid was washed with ethyl acetate. From the combined filtrate, an organic layer was separated. The water layer was extracted with ethyl acetate (2 x 500 mL), and the combined ethyl acetate layer was washed with brine (1 x 500 mL). The organic layer was treated with activated charcoal (30 g), stirred for a hour at room temperature, filtered through Hyflow bed, and the residual charcoal was washed with ethyl acetate. The combined filtrate was concentrated and dried *in vacuo* to give 2,2'-(1-(tert-butoxycarbonyl)pyrrolidine-3,4-diyl)diacetic acid as a white solid (60 g, 209 mmol, 78% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 1.42 (s, 9H), 1.43-1.45 (m, 2H), 1.98-2.28 (m, 2H), 2.28-2.33 (m, 2H), 2.97-3.03 (m, 2H), 3.33-3.40 (m, 2H), 12.2 (bs, 2H). **ESI-MS** (m/z): 286.25 [M-H].

Step IV: Tert-butyl 5-oxohexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate **(5)**.

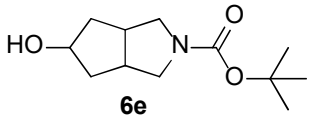


5

2,2'-(1-(tert-butoxycarbonyl)pyrrolidine-3,4-diyl)diacetic acid (50 g, 174 mmol) was added to the acetic anhydride (241 mL, 2550 mmol). The reaction mixture was heated at 135 °C for 45 minutes. Anhydrous sodium acetate (12.13 g, 148 mmol) was gradually added to the reaction mixture at 135 °C and stirred for 30 minutes. The reaction mixture was cooled to ambient temperature, then to 5–10 °C. Methanol (200 mL) was added dropwise, maintaining the temperature between 10 and 15 °C. After completing the

addition, the reaction mixture was poured into the cold water (500 mL). The reaction mixture was basified to 8 pH by the addition of sodium carbonate. Ethyl acetate (750 mL) was added to the reaction mixture and stirred for 15 minutes. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (500 mL). A combined organic layer was dried over sodium sulphate and concentrated under *in vacuo* to yield crude product. The crude product was purified using flash column chromatography using ethyl acetate and n-hexane as gradients. Combined fractions containing pure product were concentrated and dried *in vacuo* to provide pure tert-butyl-5-oxohexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate as a white solid product (25.5 g, 113 mmol, 65.0 % yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 1.47 (s, 9H), 2.14-2.20 (m, 2H), 2.46-2.52 (m, 2H), 2.93-2.94 (m, 2H), 3.17-3.19 (m, 1H), 3.26-3.29 (m, 1H), 3.66-3.67 (m, 2H).

Step V: Tert-butyl 5-hydroxyhexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate (**6e**).

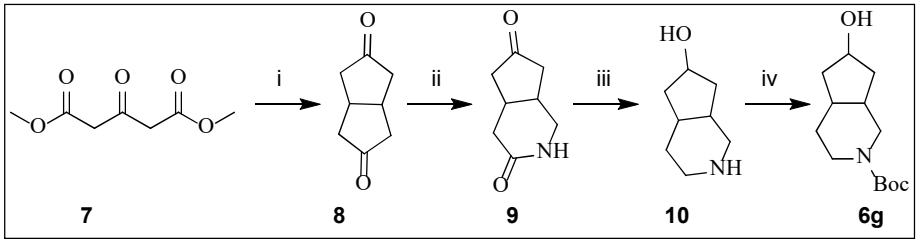


6e

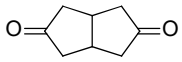
The stirred solution of tert-butyl 5-oxohexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate (20 g, 89 mmol) in methanol (200 mL) was cooled to 0-5 ºC, and sodium borohydride (5.04 g, 133 mmol) was added while the temperature was maintained between 0 and 5 ºC. The reaction mixture was warmed to room temperature and stirred for an hour. The reaction mixture was concentrated *in vacuo*, and the resulting slurry was diluted with ethyl acetate (400 mL) and cold 1N hydrochloric acid $_{(aq)}$ (100 mL). After stirring for 5 minutes, the organic layer was separated, washed with water (100 mL) and brine (100 mL), dried over sodium sulphate, and concentrated under vacuum to get tert-butyl 5-hydroxyhexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate as white solid (19.78 g, 87 mmol, 98% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm 1.46 (s, 9H),

1.48-1.55 (m, 2H), 1.63-1.69 (m, 1H), 2.15-2.22 (m, 2H), 2.59-2.64 (m, 2H), 3.33-3.48 (m, 2H), 3.50-3.53 (m, 2H), 4.29-4.32 (m, 1H). **ESI-MS** (m/z): 228.05 $[M+H]^+$.

4.1.2. Preparation of tert-butyl 6-hydroxyoctahydro-2H-cyclopenta[c]pyridine-2-carboxylate (Intermediate 6g).



Step I: Tetrahydropentalene-2,5(1H,3H)-dione **(8)**.

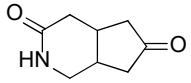


8

Sodium hydroxide (8.86 g, 222 mmol) was dissolved in methanol (140 mL) and cooled at 0 ºC. Dimethyl 3-oxopentanedioate (38.2 g, 219 mmol) was added dropwise over a period of 1 hour. Over the course of the addition, a yellow-white precipitate was deposited. The ice-water bath was then replaced with a heating bath, and the reaction mixture was brought to reflux temperature (~65 °C) over the course of 30 minutes, during which time the precipitate dissolved to afford a pale-yellow solution. After a further 30 minutes at reflux, 40% aqueous solution of oxalaldehyde (12.53 ml, 110 mmol) was added dropwise over a period of 100 minutes, during which time a yellow-orange precipitate was deposited. The heating was then discontinued, and the resultant slurry was allowed to stir overnight. The precipitate was collected by vacuum filtration and washed with methanol (50 mL) to afford the crude disodium enolate of tetramethyl bicyclo[3.3.0]octan-3,7-dione- 2,4,6,8-tetracarboxylate (25.2 g) as a pale yellow crystalline solid. The crystalline solid was then dissolved in a mixture of aqueous hydrochloric acid (99 mL, 99 mmol) and glacial acetic acid (9.79 mL, 171 mmol) and heated to reflux for 3 hours. The reaction mixture was then cooled to room temperature

and extracted with dichloromethane (5 x 25 mL). After removal of the solvent *in vacuo*, the residue was taken up in dichloromethane (150 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 20 mL). The organic phase was dried over anhydrous sodium sulphate and concentrated *in vacuo* to afford a yellow-orange solid. Recrystallization of this material from methanol afforded (3as,6as)-tetrahydropentalene-2,5(1H,3H)-dione as a colourless solid (8 g, 57.9 mmol, 52.8% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm 2.13-2.19 (m, 4H), 2.55-2.63 (m, 4H), 3.01-3.10 (m, 2H).

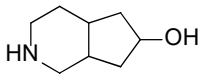
Step II: Hexahydro-3H-cyclopenta[c]pyridine-3,6(4H)-dione **(9)**.



9

Tetrahydropentalene-2,5(1H,3H)-dione (5.06 g, 36.6 mmol) was dissolved in concentrated hydrochloric acid (100 mL, 1152 mmol) and cooled to 0 °C. Sodium azide (3.10 g, 47.6 mmol) was added in portions and stirred at room temperature for 24 hours. After the reaction was completed, dropwise addition of 20% sodium hydroxide aqueous solution was made until the pH of the reaction mixture reached to 10 and was extracted with dichloromethane (3 x 50 mL), the organic phases were combined, washed with saturated brine (50 mL), dried over anhydrous sodium sulphate, filtered, and the filtrate was concentrated under reduced pressure and purified by flash column chromatography to give the title product tetrahydro-1H-cyclopenta[c]pyridine-3,6(2H,4H)-dione as a white solid (2.6 g, 16.97 mmol, 46.3% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm 2.12-2.29 (m, 3H), 2.43-2.50 (m, 1H), 2.51-2.58 (m, 1H), 2.62-2.69 (m, 1H), 2.72-2.74 (m, 1H), 2.79-2.83 (m, 1H), 3.18-3.24 (m, 1H), 3.51-3.56 (m, 1H), 6.32 (s, 1H), **ESI-MS** (m/z): 152.85 $[M]^+$.

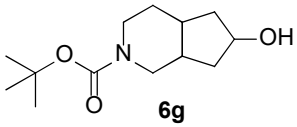
Step III: Octahydro-1H-cyclopenta[c]pyridin-6-ol **(10)**.



10

Hexahydro-3H-cyclopenta[c]pyridine-3,6(4H)-dione (2.5 g, 16.32 mmol) was dissolved in tetrahydrofuran (50 mL) and cooled to 0°C. Lithium aluminium hydride (1.301 g, 34.3 mmol) was added portion wise to the reaction mixture while maintaining the temperature between 0 and 5 °C. The reaction mixture was warmed to room temperature and stirred for an hour after it had been refluxed for 1 hour. The reaction mixture was cooled to ambient temperature and then to 0 °C. The reaction mixture was carefully quenched with the dropwise addition of 10% aqueous potassium hydroxide (5 mL) while keeping the temperature below 5 °C. The resulting slurry was diluted with ethyl acetate (25 mL) and passed through a Hyflow bed, and residuals were twice washed with ethyl acetate (20 mL). The combined filtrate was dried over sodium sulphate, concentrated *in vacuo* to afford octahydro-1H-cyclopenta[c]pyridin-6-ol as thick oil (2.2 g, 15.58 mmol, 95% yield). The obtained product was used for the next reaction without further purification. **ESI-MS** (m/z): 141.60 $[M+H]^+$.

Step IV: Tert-butyl 6-hydroxyoctahydro-2H-cyclopenta[c]pyridine-2-carboxylate (**6g**).

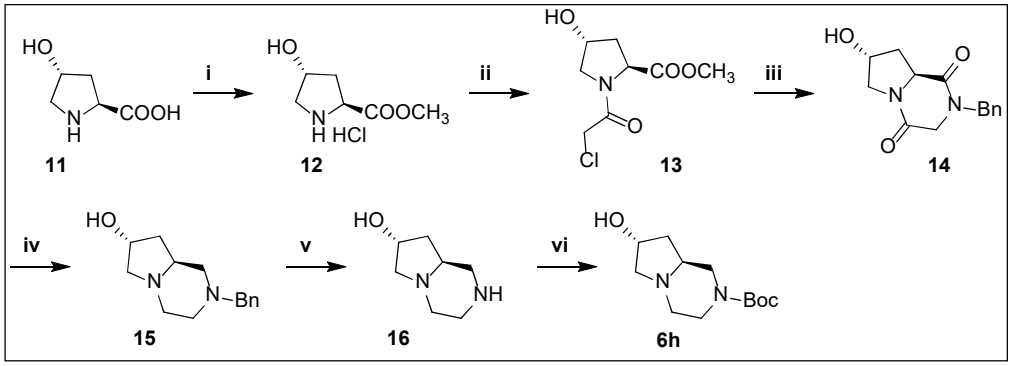


6g

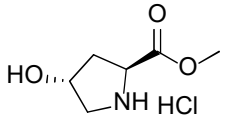
Octahydro-1H-cyclopenta[c]pyridin-6-ol (2 g, 14.16 mmol) was dissolved in acetonitrile (30 mL) and water (10 mL), Sodium carbonate (3 g, 28.3 mmol) was added to it and stirred for five minutes. Boc anhydride (3.95 mL, 17.00 mmol) was dropwise added to the reaction mixture. The reaction mixture was stirred at room temperature for 3 hours. After completion of the reaction, the reaction mixture was concentrated under reduced pressure, the resulting slurry was diluted with cold water, the solids were

precipitated, filtered, washed with water, and dried in vacuum to give tert-butyl 6-hydroxyoctahydro-2H-cyclopenta[c]pyridine-2-carboxylate as a white solid (3.15 g, 13.05 mmol, 92% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm 1.39-1.46 (m, 11H), 1.61-1.68 (m, 4H), 2.01-2.14 (m, 3H), 3.11-3.17 (m, 1H), 3.41-3.43 (m, 2H), 3.56-3.58 (m, 1H), 4.36-4.38 (m, 1H). **ESI-MS** (m/z): 241.90 $[M+H]^+$.

4.1.3. Preparation of tert-butyl (7R,8aS)-7-hydroxyhexahydropyrrolo[1,2-a]pyrazine-2(1H)-carboxylate (Intermediate 6h).



Step I: Methyl (2S,4R)-4-hydroxypyrrolidine-2-carboxylate hydrochloride **(12)**.

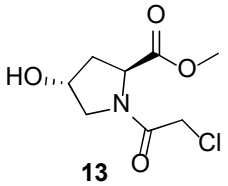


12

To methanol (190 mL) at 0 ºC, acetyl chloride (24.40 mL, 343 mmol) was added dropwise. The reaction mixture was warmed to room temperature and stirred for 30 minutes. (2S,4R)-4-hydroxypyrrolidine-2-carboxylic acid (15 g, 114 mmol) was added to the reaction mixture and heated at reflux for 3 hours. After cooling to ambient temperature, the reaction mixture was concentrated under reduced pressure, diluted with ethyl acetate (100 mL), and stirred in an ice bath for 15 minutes. Filtered, rinsed with ethyl acetate (25 mL), and suction dried to afford (2S,4R)-methyl 4-hydroxypyrrolidine-2-carboxylate hydrochloride as a colourless solid (20.3 g, 112 mmol, 98% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm 2.05-2.12 (m, 1H), 2.16-2.22

(m, 1H), 3.05-3.08 (m, 1H), 3.35-3.37 (m, 1H), 3.75 (s, 3H), 4.41-4.48 (m, 2H), 5.57 (s, 1H), 9.75 (bs, 2H). **ESI-MS** (m/z): 145.50 $[M+H]^+$.

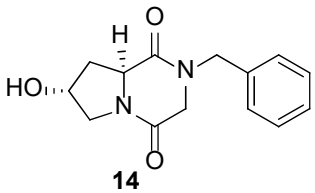
Step II: Methyl (2S,4R)-1-(2-chloroacetyl)-4-hydroxypyrrolidine-2-carboxylate **(13)**.



13

(2S,4R)-methyl 4-hydroxypyrrolidine-2-carboxylate hydrochloride (16.18 g, 89 mmol) was suspended in benzene (485 mL) and cooled to 0 ºC. Chloroacetyl chloride (7.14 mL, 89 mmol) was added dropwise to the reaction mixture while maintaining the reaction temperature at 0 to 5 ºC. After complete addition, the reaction mixture was warmed to room temperature and stirred for 30 minutes, then heated at reflux for 18 hours. After cooling to ambient temperature, the reaction mixture was concentrated under reduced pressure, and the resulting slurry was diluted with ethyl acetate (200 mL) and water (100 mL). The organic phase was separated and washed with water (10 mL) and brine (50 mL), dried over sodium sulphate, concentrated, and dried *in vacuo* to give (2S,4R)-methyl 1-(2-chloroacetyl)-4-hydroxypyrrolidine-2-carboxylate as thick oil (12.2 g, 55.0 mmol, 61.8% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 1.88-1.94 (m, 1H), 2.08-2.14 (m, 1H), 3.38-3.47 (m, 1H), 3.61 (s, 3H), 3.63-3.68 (m, 1H), 4.30-4.36 (m, 4H), 5.23 (s, 1H). **ESI-MS** (m/z): 221.45 $[M]^+$, 223.30 $[M+2]^+$.

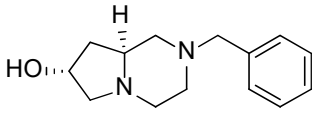
Step III: (7R,8aS)-2-benzyl-7-hydroxyhexahydropyrrolo[1,2-a]pyrazine-1,4-dione **(14)**.



14

(2S,4R)-methyl 1-(2-chloroacetyl)-4-hydroxypyrrolidine-2-carboxylate (11.47g, 51.8 mmol) was added to the mixture of benzylamine (6.78 mL, 62.1 mmol) and triethylamine (9.38 mL, 67.3 mmol). The reaction mixture was heated at 125 ºC for 24 hours. After cooling to ambient temperature, the reaction mixture was concentrated under reduced pressure and purified by flash column chromatography to give (7R,8aS)-2-benzyl-7-hydroxyhexahydropyrrolo[1,2-a]pyrazine-1,4-dione as a white solid (11.88 g, 45.6 mmol, 88% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 1.94-2.01 (m, 1H), 2.10-2.18 (m, 1H), 3.04-3.10 (m, 1H), 3.20-3.29 (m, 1H), 3.54-3.64 (m, 2H), 4.10-4.19 (m, 1H), 4.31-4.36 (m, 1H), 4.47-4.58 (m, 2H), 5.15 (s, 1H), 7.24-7.36 (m, 5H).

Step IV: (7R,8aS)-2-benzyloctahydropyrrolo[1,2-a]pyrazin-7-ol **(15)**.

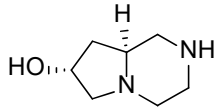


15

(7R,8aS)-2-benzyl-7-hydroxyhexahydropyrrolo[1,2-a]pyrazine-1,4-dione (11.8 g, 45.3 mmol) was dissolved in tetrahydrofuran (236 mL) and cooled to 0°C. Lithium aluminium hydride (8.60 g, 227 mmol) was added portion wise to the reaction mixture while maintaining the temperature between 0 and 5 °C. The reaction mixture was warmed to room temperature and stirred for 15 minutes after it had been refluxed for 18 hour. The reaction mixture was cooled to ambient temperature and then to 0 °C. The reaction mixture was carefully quenched with the dropwise addition of 10% aqueous potassium hydroxide (25 mL) while keeping the temperature below 5 °C. The resulting slurry was diluted with ethyl acetate (118 mL) and passed through a Hyflow bed, and residuals were twice washed with ethyl acetate (60 mL). The combined filtrate was dried over sodium sulphate, concentrated *in vacuo* to afford (7R,8aS)-2-benzyloctahydropyrrolo[1,2-a]pyrazin-7-ol (6.6 g, 28.4 mmol, 62.7% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 1.47-1.50 (m, 2H), 1.65-1.70 (m, 1H), 1.89-1.92 (m, 1H),

1.98-2.26 (m, 3H), 2.65-2.68 (m, 1H), 2.77-2.80 (m, 2H), 3.21-3.28 (m, 1H), 3.46-3.51 (m, 2H), 4.13-4.17 (m, 2H), 4.72 (s, 1H), 7.21-7.32 (m, 5H). **ESI-MS** (m/z): 232.65 $[M+H]^+$.

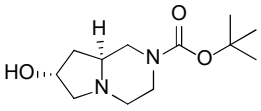
Step V: (7R,8aS)-octahydropyrrolo[1,2-a]pyrazin-7-ol **(16)**.



16

(7R,8aS)-2-benzyloctahydropyrrolo[1,2-a]pyrazin-7-ol (1 g, 4.30 mmol) was dissolved in methanol (10 mL), followed by the addition of 10% Pd/C (0.229 g, 0.215 mmol). The reaction mixture was hydrogenated at 50 psi for 8 hours using the Parr hydrogenation apparatus. After completion of the reaction, the reaction mixture was filtered through a Hyflow bed and washed with methanol (2 x 5 mL). The combined filtrate was concentrated and dried *in vacuo* to provide (7R,8aS)-octahydropyrrolo[1,2-a]pyrazin-7-ol as thick oil (0.580 g, 4.08 mmol, 95% yield). The product was used for the next reaction without further purification. **ESI-MS** (m/z): 142.45 $[M+H]^+$.

Step VI: Tert-butyl (7R,8aS)-7-hydroxyhexahydropyrrolo[1,2-a]pyrazine-2(1H)-carboxylate (**6h**).

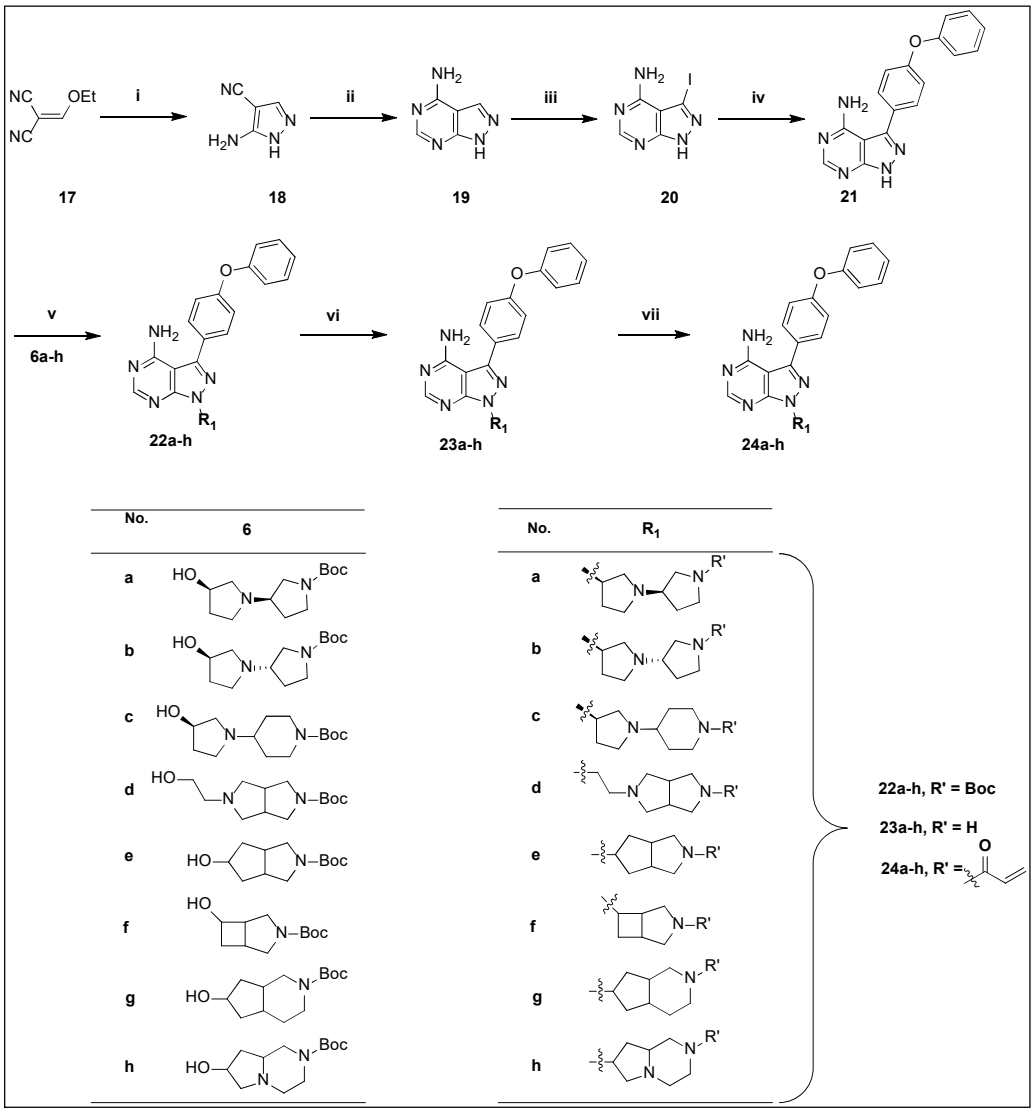


6h

Octahydro-1H-cyclopenta[c]pyridin-6-ol (0.5 g, 3.52 mmol) was dissolved in acetonitrile (7.5 mL) and water (2.5 mL). Sodium carbonate (0.745 g, 7.03 mmol) was added to it and stirred for five minutes. Boc anhydride (0.980 mL, 4.22 mmol) was dropwise added to the reaction mixture. The reaction mixture was stirred at room temperature for 3 hours. After completion of the reaction, the reaction mixture was concentrated under reduced pressure, and the resulting slurry was dissolved in cold water (10 mL) and ethyl acetate (25 mL). The organic phase was separated and washed

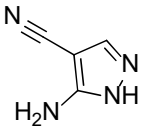
with water (10 mL) and brine (10 mL), dried over sodium sulphate, concentrated, and dried *in vacuo* to give a crude product, which was purified by flash column chromatography to afford tert-butyl (7R,8aS)-7-hydroxyhexahydropyrrolo[1,2-a]pyrazine-2(1H)-carboxylate as a thick oil (0.79 g, 3.26 mmol, 93% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm 1.47-1.50 (m, 11H), 1.65-1.70 (m, 1H), 1.89-1.92 (m, 1H), 2.01-2.07 (m, 1H), 2.13-2.26 (m, 2H), 2.65-2.68 (m, 1H), 2.77-2.80 (m, 1H), 3.21-3.28 (m, 1H), 3.42-3.51 (m, 2H), 4.13-4.17 (m, 1H), 4.71-4.72 (m, 1H). **ESI-MS** (m/z): 242.10 $[M+H]^+$.

4.1.4. Preparation of compounds 24a-h of Series 1



Step I: 5-amino-1H-pyrazole-4-carbonitrile **(18)**.

Commercially available 2-(ethoxymethylene)malononitrile (50 g, 409 mmol) was



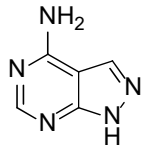
18

added portion wise to hydrazine monohydrate (32.2 mL, 655 mmol) at 25 ºC. The reaction mixture was refluxed for 3 hours. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The resulting solid was suspended in cold water (50 mL), stirred for 30 minutes, and refrigerated at 4

ºC overnight. The solid product thus obtained was filtered and washed with cold water (2 x 30 mL). The product was dried in an oven at 50 ºC for 4 hours. 5-amino-1H-pyrazole-4-carbonitrile (30.5 g, 282 mmol, 69% yield) was obtained as a light yellow-orange solid. **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 6.18 (bs, 2H), 7.67 (s, 1H), 12.04 (s, 1H).

Step II: 1H-pyrazolo[3,4-d]pyrimidin-4-amine **(19)**.

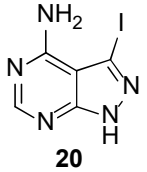
5-amino-1H-pyrazole-4-carbonitrile (26.5 g, 245 mmol) was added to formamide (78



19

mL, 1961 mmol) at 25 ºC. The reaction mixture was refluxed at 180 °C for 6 hours. The reaction mixture was cooled to room temperature, and water (200 mL) was added to it. It was stirred for 1 hour. The solid product was filtered and washed with water (2 x 100 mL). The solid product was suction dried, and after it was suspended in ethanol (90 mL), stirred for 30 min and filtered, it was then dried in vacuum to afford 1H-pyrazolo[3,4-d]pyrimidin-4-amine as a light yellow solid (28.7 g, 212 mmol, 87% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 7.66 (bs, 2H), 8.06 (s, 1H), 8.10 (s, 1H), 13.30 (s, 1H). **ESI-MS (**m/z): 135.65 $[M+H]^+$.

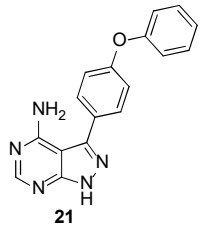
Step III: 3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine **(20)**.



20

1H-pyrazolo[3,4-d]pyrimidin-4-amine (33.2 g, 246 mmol) was dissolved in N,N-dimethylformamide (140 mL) at room temperature, and N-iodosuccinimide (66.3 g, 295 mmol) was added. The resulting mixture was heated at 85–90 °C for 18 hours. The reaction mixture was cooled to room temperature before it was diluted with water (1000 mL). After 20 minutes of stirring, aqueous sodium thiosulfate solution (10% w/v, 250 mL) was added. After 30 minutes of stirring, the mixture was filtered and washed with water (2 x 200 mL). The resultant solid product was washed with cold ethanol (2 x 100 mL) and dried *in vacuo* at 60 °C for 4 hours to afford 3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine as yellow solid product (54.4 g, 208 mmol, 85% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 8.14 (s, 1H), 13.78 (s, 1H). **ESI-MS** (m/z): 261.65 $[M+H]^+$.

Step IV: 3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine **(21)**.



21

3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine (2.7 g, 10.34 mmol) was dissolved in dry N,N-dimethylformamide (67.5 mL). 4-phenoxyphenyl boronic acid (3.32 g, 15.52 mmol), bis(triphenylphosphine)palladium(II) chloride (0.726 g, 1.03 mmol), and an aqueous potassium bicarbonate solution (25.9 mL, 51.17 mmol) was added to it. The reaction mixture was purged with nitrogen for 10 minutes. The reaction mixture was heated at 90 °C for 3 hours under a nitrogen atmosphere. The reaction mixture was cooled to room temperature, filtered, diluted with cold water (350 mL), and stirred for 30 minutes. The resulting solid was filtered, washed with water (25 mL), and dried *in vacuo* to obtain the crude product. The crude product was purified by flash column

chromatography to get pure 3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine as an off-white solid (2.32 g, 7.65 mmol, 74% Yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 7.07-7.10 (m, 2H), 7.16-7.20 (s, 3H), 7.40-7.46 (m, 2H), 7.63-7.70 (m, 2H), 8.24 (s, 1H). **ESI-MS** (m/z): 303.75 $[M+H]^+$.

Step V: General procedure for the preparation of compound **22a-h.**

To a solution of various substituted alcohol **6a–h** (1 mmol) in dry tetrahydrofuran (10 mL), triphenylphosphine (0.525 g, 2 mmol), and 3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (0.303g, 1 mmol) were added. The reaction mixture was stirred for 15 minutes and then cooled to 0 ºC, and diisopropyl diazodicarboxylate (0.389 mL, 2 mmol) was added to it. The mixture was stirred at room temperature for 18 hours. The reaction mixture was diluted with water and extracted with ethyl acetate (3 x 50 mL), and the combined organic phase was washed with water (50 mL) and brine (50 mL), dried over sodium sulphate, and concentrated *in vacuo*. The obtained crude product was purified by flash column chromatography to get desired product (**22a–h)**.

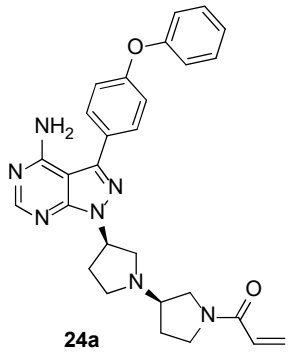
Step VI: General procedure for the preparation of compound **23a-h.**

22a–h (0.5 mmol) was dissolved in dichloromethane (5 mL) and cooled to 0 ºC prior to the dropwise addition of trifluoroacetic acid (0.564 mL, 7.32 mmol). The reaction mixture was stirred at room temperature for 3 hours. After the reaction was finished, it was concentrated under reduced pressure. The resulting mixture was diluted with water (10 mL), basified with saturated sodium bicarbonate (pH 8), and an aqueous layer was extracted with dichloromethane (3 x 10 mL). The combined organic phase was washed with brine (15 mL), dried over sodium sulphate, concentrated, and dried *in vacuo* to get product (**23a–h)**. The obtained products **23a–h** was used without further purification in the following step.

Step VII: General procedure for the preparation of compound **24a-h**.

Triethyl amine (0.203 mL, 1.45 mmol) was added to the dichloromethane (10 mL) solution of the **23a-h** (0.485 mmol) and cooled to 0 ºC. Acryloyl chloride (0.05 g, 0.558 mmol) was added to the reaction mixture dropwise and stirred for 2 hours. After completion of the reaction, the reaction mixture was washed with water (10 mL) and brine (10 mL), dried over sodium sulphate, concentrated, and dried *in vacuo*. The obtained crude product was purified using preparative HPLC to get pure final products (**24a-h)**.

4.1.4.1. 1-((3R,3'R)-3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-[1,3'-bipyrrolidin]-1'-yl)prop-2-en-1-one (24a).



24a

Following the procedure described in Section 4.1.4, **24a** was synthesised from **23a** and isolated as a white solid with a 48% yield.

Purity by UPLC: 95.30%

Melting point: 175-177 ºC

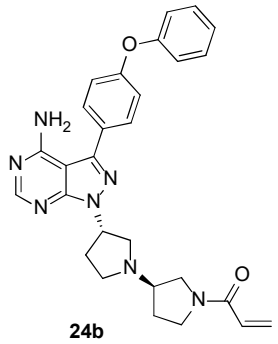
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.68-1.91 (m, 1H), 1.98-2.09 (m, 1H), 2.29-2.32 (m, 2H), 2.78-2.87 (m, 4H), 3.20-3.28 (m, 1H), 3.35-3.37 (m, 1H), 3.50-3.78 (m, 3H), 5.39-3.41 (m, 1H), 5.63-5.66 (m, 1H), 6.12-6.14 (m, 1H), 6.55-6.57 (m, 1H), 7.10-7.16 (m, 5H), 7.40-7.44 (m, 2H), 7.66 (d, J = 8.4 Hz, 2H), 8.24 (s, 1H).

^{13}C NMR (100 MHz, DMSO-*d*6) δ ppm 22.49, 34.83, 50.85, 50.98, 53.78, 57.08, 59.20, 62.64, 97.85, 119.43, 119.45, 124.25, 127.74, 127.97, 128.40, 128.82, 130.55, 130.59, 143.80, 154.42, 156.14, 156.78, 157.58, 158.67, 165.02.

Elemental (CHNS) analysis: Calculated for $C_{28}H_{29}N_7O_2$: C, 67.86; H, 5.90; N, 19.78; found: C, 67.79; H, 5.93; N, 19.81;

ESI-MS (m/z): 496.25 $[M+H]^+$

4.1.4.2. 1-((3S,3'R)-3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-[1,3'-bipyrrolidin]-1'-yl)prop-2-en-1-one (24b).



24b

Following the procedure described in Section 4.1.4, **24b** was synthesised from **23b** and isolated as a white solid with a 45% yield.

Purity by UPLC: 95.24%

Melting point: 171-173 ^oC

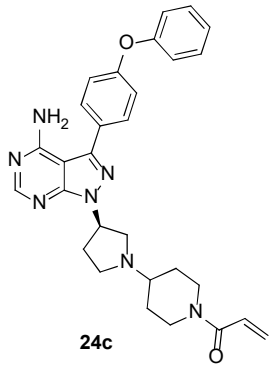
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.71-1.90 (m, 1H), 1.98-2.12 (m, 1H), 2.29-2.32 (m, 2H), 2.78-2.90 (m, 4H), 3.17-3.26 (m, 1H), 3.35-3.38 (m, 1H), 3.50-3.78 (m, 3H), 5.39-3.41 (m, 1H), 5.64-5.65 (m, 1H), 6.08-6.13 (m,1H), 6.65-6.60(m, 1H), 7.10-7.19 (m, 5H), 7.41-7.44 (m, 2H), 7.66 (d, J = 8.0 Hz, 2H), 8.24 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 22.40, 34.83, 50.85, 50.98, 53.78, 57.11, 59.16, 62.64, 97.85, 119.43, 119.45, 124.25, 127.74, 127.97, 128.40, 128.82, 130.55, 130.59, 143.80, 154.42, 156.14, 156.78, 157.57, 158.67, 165.16.

Elemental (CHNS) analysis: Calculated for $C_{28}H_{29}N_7O_2$: C, 67.86; H, 5.90; N, 19.78; found: C, 67.78; H, 5.90; N, 19.75;

ESI-MS (m/z): 496.20 $[M+H]^+$

4.1.4.3. (R)-1-(4-(3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)pyrrolidin-1-yl)piperidin-1-yl)prop-2-en-1-one (24c).



24c

Following the procedure described in Section 4.1.4, **24c** was synthesised from **23c** and isolated as a white solid with a 56% yield.

Purity by UPLC: 96.25%

Melting point: 165-167 ^oC

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.27-1.31 (m, 4H), 1.39-1.71 (m, 1H),1.86-1.97 (m, 1H), 2.29-2.32 (m, 2H), 2.80-2.89 (m, 3H),3.10-3.25 (m, 2H), 3.89-4.01 (m, 1H), 4.12-4.18 (m, 1H), 5.39-3.41 (m, 1H), 5.62-5.65 (m, 1H), 6.05 (dd, J =16.4, 2.4 Hz,

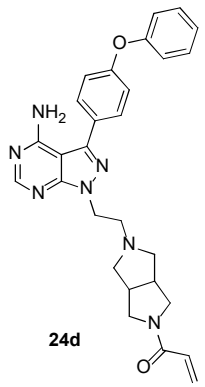
1H), 6.73-6.80 (m, 1H), 7.10-7.20 (m, 5H), 7.41-7.45 (m, 2H), 7.66 (d, *J* = 8.8 Hz, 2H), 8.24 (s, 1H).

^{13}C NMR (100 MHz, DMSO-*d*6) δ ppm 24.25, 28.40, 28.46, 39.36, 46.99, 47.36, 53.20, 63.78, 97.85, 119.41, 119.45, 124.25, 127.73, 127.97, 128.36, 128.82, 130.55, 130.59, 143.85, 154.42, 156.14, 156.78, 157.58, 158.65, 165.00.

Elemental (CHNS) analysis: Calculated for $C_{29}H_{31}N_7O_2$: C, 68.35; H, 6.13; N, 19.24; found: C, 68.23; H, 6.15; N, 19.29;

ESI-MS (m/z): 510.45 $[M+H]^+$

4.1.4.4. 1-(5-(2-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)hexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl)prop-2-en-1-one (24d).



24d

Following the procedure described in Section 4.1.4, **24d** was synthesised from **23d** and isolated as a white solid with a 56% yield.

Purity by UPLC: 96.62%

Melting point: 148-150 °C

^{1}H NMR (400 MHz, $CDCL_3$) δ ppm 2.57-2.70 (m, 4H), 2.75-2.77 (m, 1H), 2.85-2.87 (m, 1H), 2.93-2.96 (m, 1H), 3.05-3.08 (m, 1H), 3.24-3.36 (m, 2H), 3.68-3.79 (m, 2H),

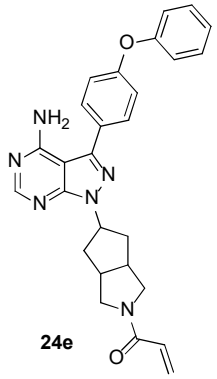
4.50-4.57 (m, 2H), 5.41 (bs, 2H), 5.62 (dd, *J* =8.8, 3.2 Hz, 1H), 6.27-6.37 (m, 2H), 7.07-7.09 (m, 2H), 7.13-7.18 (m, 3H), 7.39 (t, *J* = 8.0 Hz, 2H), 7.66 (d, *J* = 6.4 Hz, 2H), 8.37 (s, 1H).

^{13}C NMR (100 MHz, DMSO-*d*6) δ ppm 37.95, 51.01, 51.20, 53.12, 54.22, 58.67, 97.85, 119.43, 119.46, 124.23, 127.74, 127.97, 128.41, 128.86, 130.50, 130.54, 142.70, 154.18, 156.18, 156.78, 157.58, 158.67, 165.01.

Elemental (CHNS) analysis: Calculated for $C_{28}H_{29}N_7O_2$: C, 67.86; H, 5.90; N, 19.78; found: C, 68.03; H, 5.99; N, 19.73;

ESI-MS (m/z): 496.15 $[M+H]^+$

4.1.4.5. 1-(5-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (24e).



24e

Following the procedure described in Section 4.1.4, **24e** was synthesised from **23e** and isolated as a white solid with a 67% yield.

Purity by UPLC: 95.38%

Melting point: 170-172 ºC

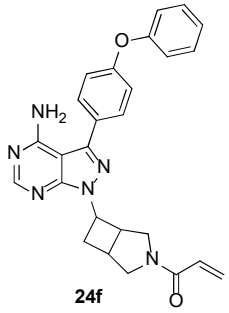
^{1}H NMR (400 MHz, $CDCL_3$) δ ppm 2.11-2.17 (m, 2H), 2.50-2.58 (m, 2H), 3.10-3.21 (m, 2H), 3.45-3.54 (m, 2H), 3.82-3.87 (m, 2H), 5.53-5.59 (m, 3H), 5.62 (dd, *J* = 9.6, 2.8 Hz, 1H), 6.36-6.50 (m, 2H), 7.07-7.09 (m, 2H), 7.13-7.20 (m, 3H), 7.37-7.41 (m, 2H), 7.62-7.66 (m, 2H), 8.36 (s, 1H).

^{13}C NMR (100 MHz, DMSO-*d*6) δ ppm 37.95, 38.19, 42.53, 51.58, 52.08, 58.09, 97.91, 119.08, 119.10, 124.25, 127.62, 127.92, 128.40, 128.78, 130.56, 130.62, 143.80, 154.42, 156.14, 156.78, 157.59, 158.67, 165.06.

Elemental (CHNS) analysis: Calculated for $C_{27}H_{26}N_6O_2$: C, 69.51; H, 5.62; N, 18.01; found: C, 69.55; H, 5.58; N, 18.18;

ESI-MS (m/z): 467.20 $[M+H]^+$

4.1.4.6. 1-(6-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-3-azabicyclo[3.2.0]heptan-3-yl)prop-2-en-1-one (24f).



24f

Following the procedure described in Section 4.1.4, **24f** was synthesised from **23f** and isolated as a white solid with a 34% yield.

Purity by UPLC: 96.54%

Melting point: 143-145 °C

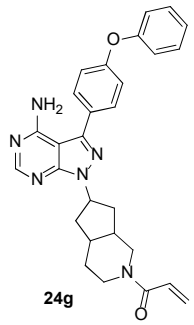
^{1}H NMR (400 MHz, $CDCL_3$) δ ppm 2.08-2.11 (m, 1H), 2.45-2.48 (m, 1H), 3.35-3.50 (m, 2H), 3.73-3.77 (m, 1H), 3.86-3.89 (m, 1H), 4.21-4.27 (m, 1H), 4.42-4.46 (m, 1H), 5.51 (bs, 2H), 5.58 (m, 1H), 5.64 (dd, *J* =10.0, 2.0 Hz, 1H), 6.36-6.50 (m, 2H), 7.08-7.19 (m, 5H), 7.38-7.41 (m, 2H), 7.62-7.66 (m, 2H), 8.36 (s, 1H).

^{13}C NMR (100 MHz, DMSO-*d*6) δ ppm 29.59, 32.77, 38.44, 52.51, 53.20, 59.77, 97.73, 111.59, 119.61, 123.85, 127.57, 127.84, 128.40, 128.82, 130.55, 130.59, 144.55, 154.42, 156.24, 156.82, 157.57, 158.64, 165.00.

Elemental (CHNS) analysis: Calculated for $C_{26}H_{24}N_6O_2$: C, 69.01; H, 5.35; N, 18.57; found: C, 69.12; H, 5.39; N, 18.61;

ESI-MS (m/z): 453.15 $[M+H]^+$

4.1.4.7. 1-(6-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)octahydro-2H-cyclopenta[c]pyridin-2-yl)prop-2-en-1-one (24g).



24g

Following the procedure described in Section 4.1.4, **24g** was synthesised from **23g** and isolated as a white solid with a 63% yield.

Purity by UPLC: 96.31%

Melting point: 158-160 °C

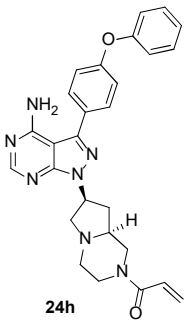
^{1}H NMR (400 MHz , $CDCL_3$) δ ppm 1.59-1.62 (m, 1H),1.79-1.83 (m, 1H), 2.10-2.43 (m, 4H), 2.58-2.60 (m, 1H), 2.73-2.74 (m, 1H), 3.16-3.35 (m, 1H), 3.59-4.13 (m, 3H), 5.47 (bs, 2H), 5.56-5.58 (m, 1H), 5.66-5.72 (m, 1H), 5.32 (dd, *J* =16, 4.8 Hz, 1H), 6.53-6.63 (m, 1H), 7.08-7.10 (m, 2H), 7.13-7.20 (m, 3H), 7.38-7.42 (m, 2H), 7.67 (d, *J* =8.4 Hz, 1H), 8.39 (s, 1H).

^{13}C NMR (100 MHz, DMSO-*d*6) δ ppm 26.88, 37.85, 38.14, 42.58, 42.62, 43.12, 46.36, 58.67, 97.85, 119.42, 119.44, 124.25, 127.71, 127.88, 128.40, 128.83, 130.57, 130.61, 143.74, 154.44, 156.17, 156.78, 157.58, 158.67, 165.04.

Elemental (CHNS) analysis: Calculated for $C_{28}H_{28}N_6O_2$: C, 69.98; H, 5.87; N, 17.49; found: C, 70.06; H, 5.92; N, 17.48;

ESI-MS (m/z): 481.35 $[M+H]^+$

4.1.4.8. 1-((7S,8aS)-7-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl)prop-2-en-1-one (24h).



24h

Following the procedure described in Section 4.1.4, **24h** was synthesised from **23h** and isolated as a light yellow solid with a 60% yield.

Purity by UPLC: 95.58%

Melting point: 162-164 °C

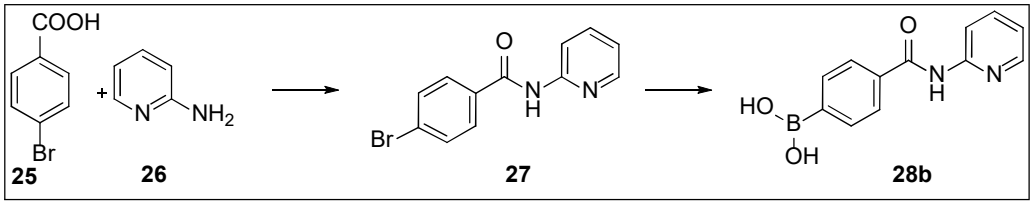
^{1}H NMR (400 MHz , $CDCL_3$) δ ppm 2.26-2.42 (m, 4H), 2.77-3.03 (m, 2H), 3.08-3.10 (m, 1H), 3.19-3.41 (m, 1H), 3.52-3.54 (m, 1H), 3.91-4.09 (m, 1H), 4.63-4.85 (m, 1H), 5.48-55 (m, 3H), 5.72 (d, *J* =10.4 Hz, 1H), 5.72 (d, *J* =16.8 Hz, 1H), 5.32 (dd, *J* =16.4, 10 Hz, 1H), 6.53-6.63 (m, 1H), 7.08-7.10 (m, 2H), 7.13-7.20 (m, 3H), 7.38-7.42 (m, 2H), 7.67 (d, *J* =8.4 Hz, 1H), 8.39 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 26.85, 42.28, 43.36, 45.66, 48.12, 59.21, 60.18, 97.86, 119.41, 119.43, 124.21, 127.66, 127.89, 128.38, 128.81, 130.53, 130.58, 143.80, 154.42, 156.15, 156.82, 157.54, 158.64, 165.01.

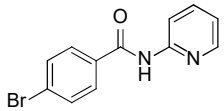
Elemental (CHNS) analysis: Calculated for $C_{27}H_{27}N_7O_2$: C, 67.34; H, 5.65; N, 20.36; 17.49; found: C, 67.36; H, 5.88; N, 20.35;

ESI-MS (m/z): 482.25 $[M+H]^+$

4.1.5. Preparation of (4-(pyridin-2-ylcarbamoyl)phenyl)boronic acid (Intermediate 28b).



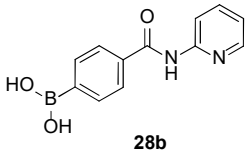
Step I: 4-bromo-N-(pyridin-2-yl)benzamide **(27)**.



4-Bromobenzoic acid (10 g, 49.7 mmol) was dissolved in N,N-Dimethylformamide (100 mL), after which 4-Dimethylaminopyridine was added. (6.08 g, 49.7 mmol). The reaction mixture was cooled to 0 ºC before the addition of 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (14.30 g, 74.6 mmol). Following 30 minutes of stirring at 0 ºC, pyridin-2-amine (4.73 g, 50.2 mmol) was added to the reaction mixture.

The mixture was brought to room temperature and stirred for 18 hours. Once the reaction had completed, the reaction mixture was poured into cold water (600 mL) and stirred for an hour. Precipitated white solids were collected by filtration, washed with water (2 x 100 mL), suction dried, and then dried at 50 ºC to obtain 4-bromo-N-(pyridin-2-yl)benzamide (12 g, 43.3 mmol, 87% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm 7.06-7.09 (m, 1H), 7.63 (dd, *J* =6.8, 1.6 Hz, 2H), 7.75-7.77 (m, 1H), 7.81 (dd, *J* =6.8, 1.6 Hz, 2H), 8.23-8.24 (m, 1H), 8.37 (d, *J* =8.4 Hz, 1H), 8.84 (s, 1H). **ESI-MS** (m/z): 276.95[M] $^+$, 278.85[M+2] $^+$

Step II: (4-(pyridin-2-ylcarbamoyl)phenyl)boronic acid (**28b**)



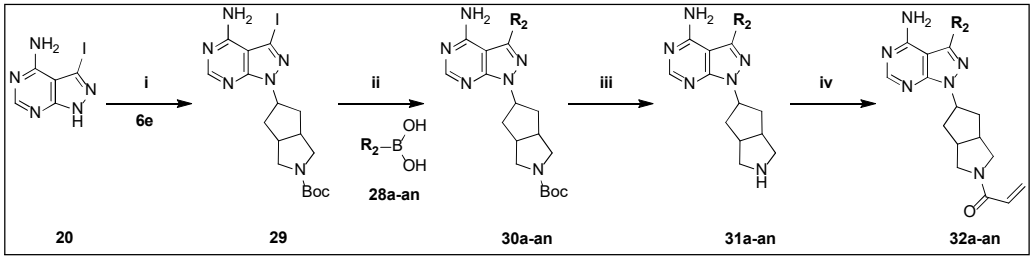
28b

4-Bromo-N-(pyridin-2-yl)benzamide (10 g, 36.1 mmol) was dissolved in tetrahydrofuran (150 mL) under inert conditions. After that, it was cooled to -60 to -65 ºC. 2.5 molar solution of butyllithium in hexane (36.1 mL, 90 mmol) was added dropwise to the reaction mixture, during addition, the reaction temperature was maintained between -60 to -65 ºC. After stirring the reaction mixture at -60 ºC for 1 hour, triisopropyl borate (12.54 mL, 54.1 mmol) was added dropwise. The mixture was brought to room temperature and stirred for 18 hours. After the reaction was finished, the reaction mixture was acidified with 2N hydrochloric acid $_{(aq)}$ to a pH of 3 and stirred for 30 minutes. The reaction mixture was then basified to a pH of 8 with a saturated sodium bicarbonate solution. The reaction mixture was extracted with ethyl acetate (3 x 100 mL), and the combined organic phase was washed with water (100 mL) and brine (100 mL), dried over sodium sulphate, and concentrated *in vacuo*. The resulting slurry was diluted with diisopropyl ether (100 mL) and stirred for 15 minutes. The precipitated

product was collected by filtration, washed with diisopropyl ether (2 x 25 mL), suction dried, and then dried at 50 ºC to provide (4-(pyridin-2-ylcarbamoyl)phenyl)boronic acid as a light yellow solid (6.2 g, 25.6 mmol, 71.0% yield).). **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm 7.17 (s, 1H), 7.84-8.03 (m, 5H), 8.19-8.25 (m, 3H), 8.39 (s, 1H), 10.75 (s, 1H), **ESI-MS** (m/z): 482.25 $[M+H]^+$.

The aforementioned procedures were used to synthesise the boronic acid derivatives **28a-an** from their respective starting materials.

4.1.6. Preparation of compounds 32a-an of Series 2



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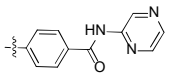
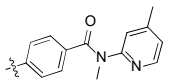
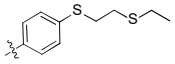
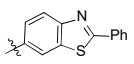
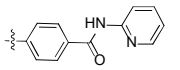
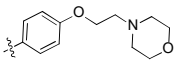
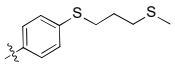
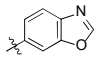
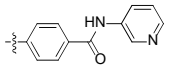
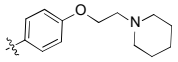
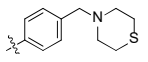
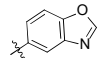
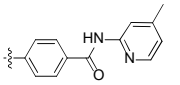
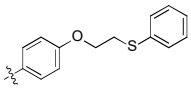
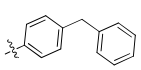
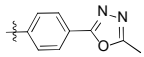
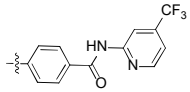
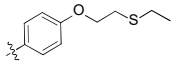
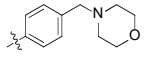
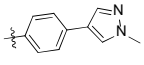
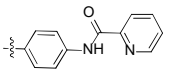
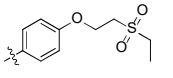
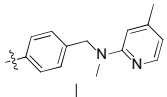
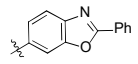
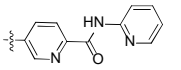
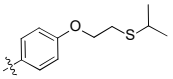
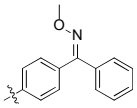
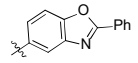
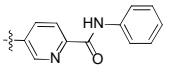
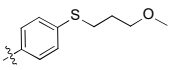
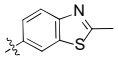
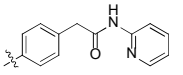
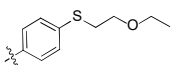
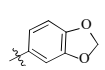
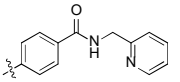
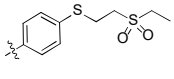
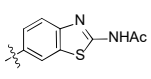
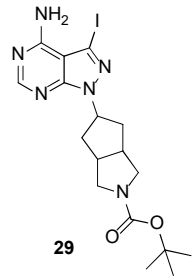
a		k		u		ae	
b		l		v		af	
c		m		w		ag	
d		n		x		ah	
e		o		y		ai	
f		p		z		aj	
g		q		aa		ak	
h		r		ab		al	
i		s		ac		am	
j		t		ad		an	

Step I: Tert-butyl 5-(4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta [c]pyrrole-2(1H)-carboxylate **(29)**.



29

3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine (2 g, 7.66 mmol) was dissolved in dry tetrahydrofuran (50 mL), then triphenylphosphine (4.02 g, 15.32 mmol) and tert-butyl 5-hydroxyhexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate (1.72 g 7.66 mmol) were added. The reaction mixture was cooled to 0 ºC after 15 minutes of stirring, then diisopropyl diazodicarboxylate (2.98 mL, 15.32 mmol) was added, and the reaction mixture was stirred for 18 hours at room temperature. After the completion of the reaction, the mixture was diluted with water (50 mL) and extracted with ethyl acetate (2 x 50 mL). The organic layer was then washed with water (25 mL) and brine (25 mL), dried over sodium sulphate, concentrated, and dried *in vacuo*. Through flash column chromatography, the crude product was purified to yield tert-butyl-5-(4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c] pyrrole-2(1H)-carboxylate as white solid (2.4 g, 5.10 mmol, 66% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 1.41 (s, 9H), 1.95-1.99 (m, 2H), 2.17-2.24 (m, 2H), 2.88-2.91 (m, 2H), 3.10-3.13 (m, 2H), 3.45-3.50 (m, 2H), 5.26-5.30 (m, 1H), 8.18 (s, 1H). **ESI-MS** (m/z): 470.85 $[M+H]^+$.

Step II: General procedure for the preparation of compounds **30a-an**.

Tert-butyl 5-(4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c] pyrrole-2(1H)-carboxylate (0.4 g, 0.85 mmol) was dissolved in dry N,N-dimethylformamide (8 mL), followed by the addition of

substituted boronic acid **28a-an** (0.90 mmol), bis(triphenylphosphine)palladium(II) chloride (0.058 g, 0.085), and an aqueous potassium bicarbonate solution (2.55 mL, 5.10 mmol). The reaction mixture was then nitrogen-purged for 10 minutes. The reaction mixture was heated for 3 hours at 90 °C in nitrogen-containing conditions. After cooling to ambient temperature, the mixture was filtered through Celite, diluted with cold water (50 mL), and extracted with ethyl acetate (3 x 25 mL). The mixed organic layer was washed with water (25 mL) and brine (25 mL), dried on sodium sulphate, then concentrated and dried *in vacuo*. The crude product was purified by flash column chromatography to yield the intended product **30a-an**.

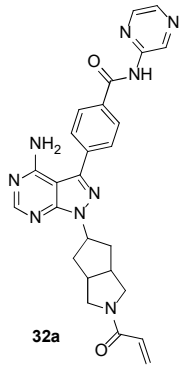
Step III: General procedure for the preparation of compounds **31a-an**.

Following the procedure described in step VI of Section 4.1.4, **31a-an** were synthesised from their respective starting materials**.**

Step III: General procedure for the preparation of compounds **32a-an**.

Following the procedure described in step VII of Section 4.1.4, **32a-an** were synthesised from their respective starting materials**.**

4.1.6.1. 4-(1-(2-acryloyloctahydrocyclopenta[c]pyrrol-5-yl)-4-amino-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-N-(pyrazin-2-yl)benzamide (32a).



32a

32a was isolated as a light-yellow solid with a 58% yield.

Purity by UPLC: 96.48%

Melting point: 258-260 ^{o}C

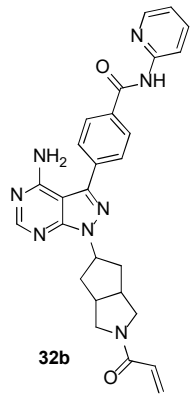
^{1}H NMR (400 MHz , DMSO-d_6) δ ppm 2.06-2.09 (m, 2H), 2.31-2.39 (m, 2H), 3.07-3.11 (m, 2H), 3.34-3.39 (m, 1H), 3.53-3.54 (m, 1H), 3.61-3.64 (m, 1H), 3.76-3.79 (m, 1H), 5.43-5.46 (m, 1H), 5.67 (dd, *J* =10, 2.4 Hz, 1H), 6.14 (dd, *J* =16.8, 2.8 Hz, 1H), 6.62 (dd, *J* =16.8, 10.4 Hz, 1H), 7.82 (d, *J* =8.4 Hz, 2H), 8.22 (d, *J* =8.4 Hz, 2H), 8.26 (s, 1H), 8.44 (d, *J* =2.4 Hz, 1H), 8.49-8.50 (m, 1H), 9.45 (d, *J* =1.6 Hz, 1H), 11.22 (s, 1H).

^{13}C NMR (100 MHz, DMSO-*d*6) δ ppm 37.83, 38.10, 42.71, 51.14, 52.06, 58.17, 97.63, 127.24, 128.63, 129.04, 129.35, 129.59, 130.05, 134.53, 135.52, 136.70, 137.46, 139.76, 145.39, 147.33, 149.85, 152.01, 154.09, 163.87, 166.17.

Elemental (CHNS) analysis: Calculated for $C_{26}H_{25}N_9O_2$: C, 63.02; H, 5.09; N, 25.44; found: C, 63.14; H, 5.08; N, 25.40;

ESI-MS (m/z): 496.25 $[M+H]^+$

4.1.6.2. 4-(1-(2-acryloyloctahydrocyclopenta[c]pyrrol-5-yl)-4-amino-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-N-(pyridin-2-yl)benzamide (32b).



32b

32b was isolated as a white solid with a 71% yield.

Purity by UPLC: 99.72%

Melting point: 255-257 °C

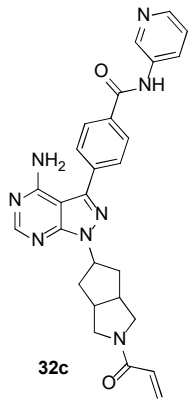
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.99-2.05 (m, 2H), 2.35-2.38 (m, 2H), 3.00-3.07 (m, 2H), 3.35-3.39 (m, 1H), 3.51-3.54 (m, 1H), 3.61-3.66 (m, 1H), 3.76-3.81 (m, 1H), 5.42-5.46 (m, 1H), 5.67 (dd, *J* =10.4, 2.4 Hz, 1H), 6.14 (dd, *J* =16.8, 2.4 Hz, 1H), 6.62 (dd, *J* =16.8, 10.4 Hz, 1H), 7.12-7.17 (m, 1H), 7.79 (d, *J* =8.4 Hz, 2H), 7.84-7.88 (m, 1H), 8.20 (d, *J* =8.4 Hz, 2H), 8.21-8.23 (m, 1H), 8.26 (s, 1H), 8.40-8.41 (m, 1H), 10.87 (s, 1H).

^{13}C NMR (100 MHz, DMSO-*d*6) δ ppm 37.95, 38.19, 42.53, 51.58, 52.08, 58.09, 97.58, 115.63, 120.60, 127.20, 128.83, 129.02, 129.48, 129.82, 130.05, 134.53, 135.46, 139.76, 145.39, 147.33, 149.85, 152.01, 152.34, 154.09, 163.87, 166.17.

Elemental (CHNS) analysis: Calculated for $C_{27}H_{26}N_8O_2$: C, 65.57; H, 5.30; N, 22.66; found: C, 65.64; H, 5.31; N, 22.69;

ESI-MS (m/z): 495.80 $[M+H]^+$

4.1.6.3. 4-(1-(2-acryloyloctahydrocyclopenta[c]pyrrol-5-yl)-4-amino-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-N-(pyridin-3-yl)benzamide (32c).



32c

32c was isolated as a white solid with a 70% yield.

Purity by UPLC: 95.24%

Melting point: 250-252 °C

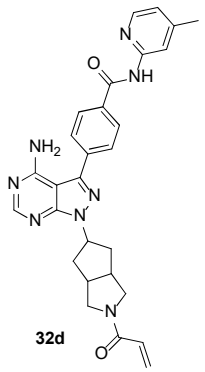
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.05-2.08 (m, 2H), 2.31-2.37 (m, 2H), 2.98-3.08 (m, 2H), 3.35-3.39 (m, 1H), 3.51-3.55 (m, 1H), 3.61-3.66 (m, 1H), 3.77-3.79 (m, 1H), 5.43-5.45 (m, 1H), 5.67 (dd, *J* =10.4, 2.4 Hz, 1H), 6.14 (dd, *J* = 16.8, 2.4 Hz, 1H), 6.62 (dd, *J* = 16.4, 10.0 Hz, 1H), 7.40-7.43 (m, 1H), 7.84 (d, *J* = 8.4 Hz, 2H), 8.16 (d, *J* = 8.4 Hz, 2H), 8.21-8.23 (m, 1H), 8.26 (s, 1H), 8.32-8.33 (m, 1H), 8.96 (d, *J* = 2.4 Hz, 1H), 10.54 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 37.99, 38.18, 42.48, 51.57, 52.01, 58.18, 97.61, 119.22, 122.01, 127.25, 128.94, 129.09, 129.40, 129.83, 130.01, 134.53, 134.61, 135.68, 139.49, 140.61, 145.34, 147.37, 152.33, 154.14, 163.91, 166.15.

Elemental (CHNS) analysis: Calculated for $C_{27}H_{26}N_8O_2$: C, 65.57; H, 5.30; N, 22.66; found: C, 65.62; H, 5.28; N, 22.71;

ESI-MS (m/z): 495.50 $[M+H]^+$

4.1.6.4. 4-(1-(2-acryloyloctahydrocyclopenta[c]pyrrol-5-yl)-4-amino-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-N-(4-methylpyridin-2-yl)benzamide (32d).



32d

32d was isolated as a white solid with a 62% yield.

Purity by UPLC: 96.67%

Melting point: 239-241 ºC

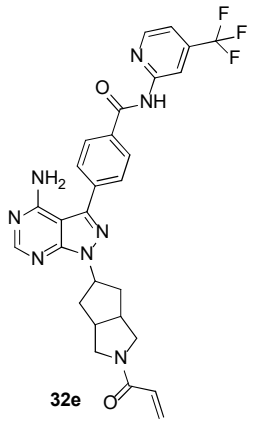
^{1}H NMR (400 MHz, $CDCL_3$) δ ppm 2.09-2.20 (m, 2H), 2.43 (s, 3H), 2.54-2.59 (m, 2H), 3.12-3.23 (m, 2H), 3.46-3.56 (m, 2H), 3.83-3.86 (m, 2H), 5.44 (bs, 2H), 5.56-5.60 (m, 1H), 5.69 (dd, *J* = 10.0, 2.4 Hz, 1H), 6.36-6.50 (m, 2H), 6.94 (d, *J* = 5.2 Hz, 1H), 7.86 (d, *J* = 8.0Hz, 2H), 8.10 (d, *J* = 8.4Hz, 2H), 8.19 (d, *J* = 5.2 Hz, 2H), 8.26 (s, 1H), 8.39 (s, 1H), 8.59 (bs, 1H).

^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 20.20, 37.95, 38.19, 42.53, 51.58, 52.02, 58.15, 97.50, 113.78, 120.73, 127.18, 128.78 128.94, 129.40, 129.76, 130.04, 134.61, 135.49, 140.18, 145.37, 147.29, 152.35, 154.11, 158.22, 158.76, 163.83, 166.15.

Elemental (CHNS) analysis: Calculated for $C_{28}H_{28}N_8O_2$: C, 66.13; H, 5.55; N, 22.03; found: C, 66.12; H, 5.48; N, 22.15;

ESI-MS (m/z): 509.15 $[M+H]^+$

4.1.6.5. 4-(1-(2-acryloyloctahydrocyclopenta[c]pyrrol-5-yl)-4-amino-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-N-(4-(trifluoromethyl)pyridin-2-yl)benzamide (32e).



32e

32e was isolated as a white solid with a 65% yield.

Purity by UPLC: 99.55%

Melting point: 236-238 ºC

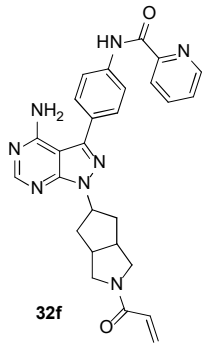
^{1}H NMR (400 MHz, $CDCL_3$) δ ppm 2.15-2.18 (m, 2H), 2.53-2.57 (m, 2H), 3.12-3.20 (m, 2H), 3.47-3.56 (m, 2H), 3.83-3.87 (m, 2H), 5.56-5.60 (m, 3H), 5.70 (dd, *J* = 9.6, 2.4Hz, 1H), 6.36-6.51 (m,2H), 7.34 (d, *J* =5.2 Hz, 1H), 7.88 (d, *J* = 8.4Hz, 2H), 8.14 (d, *J* = 8.4 Hz, 2H), 8.39 (s, 1H), 8.51 (d, *J* = 5.2 Hz, 1H), 8.73 (s, 1H), 9.02 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 37.90, 38.27, 42.55, 51.58, 52.12, 58.01, 97.60, 114.15, 119.41, 123.87, 127.22, 128.85, 129.12, 129.23, 129.84, 130.02, 134.59, 135.41, 145.22, 147.19, 147.37, 149.62, 152.37 154.12, 155.55 163.77, 166.19.

Elemental (CHNS) analysis: Calculated for $C_{28}H_{25}F_3N_8O_2$: C, 59.78; H, 4.48; F, 10.13; N, 19.92; found: C, 59.71; H, 4.52; N, 10.18;

ESI-MS (m/z): 563.25 $[M+H]^+$

4.1.6.6. N-(4-(1-(2-acryloyloctahydrocyclopenta[c]pyrrol-5-yl)-4-amino-1H-pyrazolo[3,4-d]pyrimidin-3-yl)phenyl)picolinamide (32f).



32f

32f was isolated as a white solid with a 65% yield.

Purity by UPLC: 98.33%

Melting point: 260-262 ºC

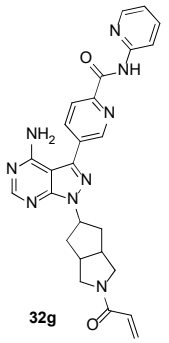
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.04-2.08 (m, 2H), 2.32-2.37 (m, 2H), 2.98-3.08 (m, 2H), 3.35-3.39 (m, 1H), 3.51-3.55 (m, 1H), 3.61-3.66 (m, 1H), 3.77-3.79 (m, 1H), 5.41-5.42 (m, 1H), 5.67 (dd, *J* = 10.4, 2.4 Hz, 1H), 6.14 (dd, *J* =16.4, 2.4 Hz, 1H), 6.62 (dd, *J* = 16.8, 10.0 Hz, 1H), 7.65-7.71 (m, 3H), 8.09-8.12 (m, 3H), 8.19 (d, *J* = 8.0Hz,1H), 8.23 (s, 1H), 8.77 (d, *J* = 4.4 Hz, 1H), 10.12 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 37.95, 38.17, 42.53, 51.56, 52.06, 58.14, 97.59, 119.28, 119.32, 120.37, 121.56, 127.17, 128.89, 128.95, 130.17, 134.44, 135.63, 139.21, 145.48, 147.35, 149.24, 151.80, 152.10, 154.05, 163.82, 166.14.

Elemental (CHNS) analysis: Calculated for $C_{27}H_{26}N_8O_2$: C, 65.57; H, 5.30; N, 22.66; found: C, 65.68; H, 5.33; N, 22.75;

ESI-MS (m/z): 495.15 $[M+H]^+$

4.1.6.7. 5-(1-(2-acryloyloctahydrocyclopenta[c]pyrrol-5-yl)-4-amino-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-N-(pyridin-2-yl)picolinamide (32g).



32g

32g was isolated as a white solid with a 52% yield.

Purity by UPLC: 99.00%

Melting point: 228-230 °C

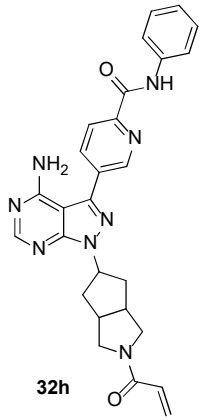
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.11-2.08 (m, 2H), 2.32-2.39 (m, 2H), 2.95-3.08 (m, 2H), 3.35-3.39 (m, 1H), 3.54-3.65 (m, 1H), 3.61-3.63 (m, 1H), 3.76-3.78 (m, 1H), 5.44-5.46 (m, 1H), 5.65-5.68 (m, 1H), 6.14 (dd, *J* =16.8, 2.0 Hz, 1H), 6.62 (dd, *J* = 16.8, 10.4 Hz, 1H), 7.21-7.24 (m, 3H), 7.92-7.94 (m, 1H), 8.28-8.33 (M, 4H), 8.41-8.42 (m, 1H), 8.99 (s, 1H), 10.47 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d_6) δ ppm (100 MHz, DMSO-*d*6) δ ppm 37.97, 38.16, 42.63, 51.57, 52.06, 58.11, 97.65, 115.42, 120.25, 124.65, 127.20, 128.83, 131.13, 134.47, 135.84, 138.71, 141.65, 147.74, 149.72, 152.17, 152.29, 153.95, 163.61, 165.98.

Elemental (CHNS) analysis: Calculated for $C_{26}H_{25}N_9O_2$: C, 63.02; H, 5.09; N, 25.44; found: C, 63.09; H, 5.11; N, 25.53;

ESI-MS (m/z): 496.15 $[M+H]^+$

4.1.6.8. 5-(1-(2-acryloyloctahydrocyclopenta[c]pyrrol-5-yl)-4-amino-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-N-phenylpicolinamide (32h).



32h

32h was isolated as a white solid with a 69% yield.

Purity by UPLC: 98.88%

Melting point: 245-247 ºC

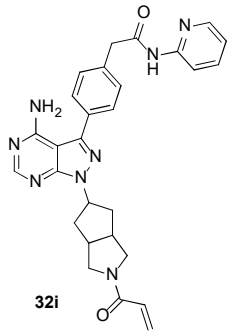
^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ ppm 2.06-2.11 (m, 2H), 2.33-2.41 (m, 2H), 2.99-3.11 (m, 2H), 3.35-3.39 (m, 1H), 3.51-3.55 (m, 1H), 3.61-3.66 (m, 1H), 3.76-3.81 (m, 1H), 5.44-5.47 (m, 1H), 5.67 (dd, *J* =10.4, 2.4 Hz, 1H), 6.14 (dd, *J* =16.8, 2.4 Hz, 1H), 6.63 (dd, *J* =16.4, 10.4 Hz, 1H), 7.07-7.17 (m, 3H), 7.35-7.39 (m, 2H), 7.95 (d, *J* =7.6 Hz, 2H), 8.29 (d, *J* =7.6 Hz, 3H), 8.96 (s, 1H), 10.75 (s, 1H).

^{13}C NMR (100 MHz, DMSO-*d*6) δ ppm 37.91, 38.17, 42.54, 51.56, 52.05, 58.08, 97.54, 119.85, 120.12, 124.74, 128.78, 128.96, 129.31, 129.88, 129.95, 134.35, 135.86, 137.76, 141.59, 149.75, 152.04, 152.38, 154.05, 163.92, 166.18.

Elemental (CHNS) analysis: Calculated for $C_{27}H_{26}N_8O_2$: C, 65.57; H, 5.30; N, 22.66; found: C, 65.55; H, 5.27; N, 22.74;

ESI-MS (m/z): 495.15 $[M+H]^+$

4.1.6.9. 2-(4-(1-(2-acryloyloctahydrocyclopenta[c]pyrrol-5-yl)-4-amino-1H-pyrazolo[3,4-d]pyrimidin-3-yl)phenyl)-N-(pyridin-2-yl)acetamide (32i).



32i

32i was isolated as a white solid with a 65% yield.

Purity by UPLC: 99.22%

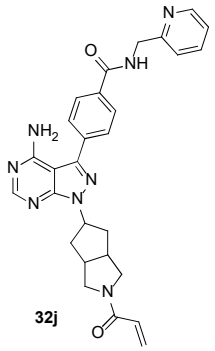
Melting point: 220-222 ºC

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.02-2.07 (m, 2H), 2.28-2.37 (m, 2H), 2.96-3.08 (m, 2H), 3.36-3.37 (m, 1H), 3.49-3.53 (m, 1H), 3.59-3.64 (m, 1H), 3.75-3.81 (m, 3H), 5.37-5.44 (m, 1H), 5.66 (dd, *J* =10.4, 2.4 Hz, 1H), 6.14 (dd, *J* =16.8, 2.4 Hz, 1H), 6.61 (dd, *J* =16.8, 10.4 Hz, 1H), 7.08-7.11 (m, 1H), 7.52 (d, *J* =8.0 Hz, 2H), 7.63 (d, *J* =8.0 Hz, 2H), 7.74-7.78 (m, 1H), 8.08 (d, *J* =8.0 Hz, 2H), 8.22 (s, 1H), 8.31-8.32 (m, 1H), 10.76 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{28}H_{28}N_8O_2$: C, 66.13; H, 5.55; N, 22.03; found: C, 66.28; H, 5.59; N, 22.16

ESI-MS (m/z): 509.55 $[M+H]^+$

4.1.6.10. 4-(1-(2-acryloyloctahydrocyclopenta[c]pyrrol-5-yl)-4-amino-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-N-(pyridin-2-ylmethyl)benzamide (32j).



32j

32j was isolated as a white solid with a 67% yield.

Purity by UPLC: 99.09%

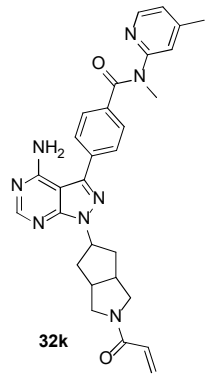
Melting point: 235-237 °C

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.04-2.08 (m, 2H), 2.32-2.38 (m, 2H), 2.98-3.09 (m, 2H), 3.34-3.38 (m, 1H), 3.50-3.54 (m, 1H), 3.61-3.66 (m, 1H), 3.76-3.81 (m, 1H), 4.60-4.61 (m, 2H), 5.41-5.45 (m, 1H), 5.67 (dd, *J* =10.4, 2.0 Hz, 1H), 6.14 (dd, *J* =16.8, 2.4 Hz, 1H), 6.62 (dd, *J* =16.8, 10.4 Hz, 1H), 7.25-7.28 (m, 1H), 7.34 (d, *J* =8.0 Hz, 1H), 7.74-7.78 (m, 3H), 8.08 (d, *J* =8.0 Hz, 2H), 8.25 (s, 1H), 8.51-8.52 (m, 1H), 9.22 (t, *J* =6.0 Hz, 1H).

Elemental (CHNS) analysis: Calculated for $C_{28}H_{28}N_8O_2$: C, 66.13; H, 5.55; N, 22.03; found: C, 66.15; H, 5.54; N, 22.14;

ESI-MS (m/z): 509.55 $[M+H]^+$

4.1.6.11. 4-(1-(2-acryloyloctahydrocyclopenta[c]pyrrol-5-yl)-4-amino-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-N-methyl-N-(4-methylpyridin-2-yl)benzamide (32k).



32k

32k was isolated as a white solid with a 59% yield.

Purity by UPLC: 98.29%

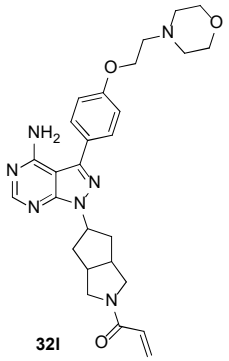
Melting point: 228-230 ºC

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.03-2.06 (m, 2H), 2.21 (s, 3H), 2.31-2.34 (m, 2H), 2.96-3.08 (m, 2H), 3.36-3.41 (m, 1H), 3.43 (s, 3H), 3.49-3.53 (m, 1H), 3.59-3.62 (m, 1H), 3.75-3.77 (m, 1H), 5.38-5.40 (m, 1H), 5.67 (dd, *J* =10.4, 2.4 Hz, 1H), 6.14 (dd, *J* =16.8, 2.4 Hz, 1H), 6.62 (dd, *J* =16.4, 10.0 Hz, 1H), 7.02-7.03 (m, 1H), 7.12 (s, 1H), 7.43 (d, *J* =8.4 Hz, 2H), 7.57 (d, *J* =8.4 Hz, 2H), 8.21 (d, *J* =4.8 Hz, 1H), 8.23 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{29}H_{30}N_8O_2$: C, 66.65; H, 5.79; N, 21.44; found: C, 66.68; H, 5.74; N, 21.38;

ESI-MS (m/z): 523.35 $[M+H]^+$

4.1.6.12. 1-(5-(4-amino-3-(4-(2-morpholinoethoxy)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32l).



32l

32l was isolated as a white solid with a 53% yield.

Purity by UPLC: 98.73%

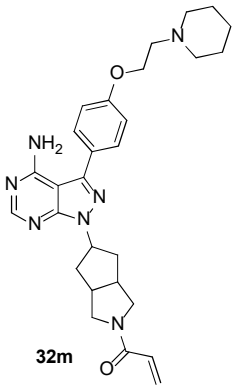
Melting point: 183-185 ^{o}C

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.01-2.07 (m, 2H), 2.28-2.37 (m, 2H), 2.48-2.50 (m, 4H), 2.70-2.73 (m, 2H), 2.95-3.09 (m, 2H), 3.35-3.41 (m, 1H), 3.48-3.53 (m, 1H), 3.57-3.65 (m, 5H), 3.75-3.80 (m, 1H), 4.14-4.16 (m, 2H), 5.37-5.41 (m, 1H), 5.67 (dd, J =10.0, 2.4 Hz, 1H), 6.14 (dd, J =16.8, 2.4 Hz, 1H), 6.62 (dd, J =16.4, 10.4 Hz, 1H), 7.10 (d, J =8.8 Hz, 2H), 7.57 (d, J =8.8 Hz, 2H), 8.21 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{27}H_{33}N_7O_3$: C, 64.39; H, 6.61; N, 19.47; found: C, 64.51; H, 6.65; N, 19.62;

ESI-MS (m/z): 504.55 $[M+H]^+$

4.1.6.13. 1-(5-(4-amino-3-(4-(2-(piperidin-1-yl)ethoxy)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32m).



32m

32m was isolated as an off white solid with a 47% yield.

Purity by UPLC: 97.53%

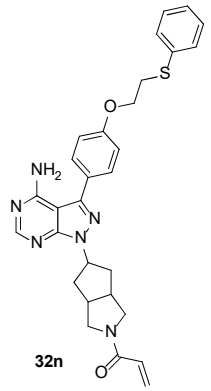
Melting point: 166-168 °C

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.26-1.34 (m, 2H), 1.43-1.51 (m, 4H), 2.07-2.33 (m, 2H), 2.41-2.46 (m, 4H), 2.69-2.73 (m, 2H), 2.96-3.08 (m, 2H), 3.35-3.42 (m, 1H), 3.48-3.53 (m, 1H), 3.57-3.61 (m, 1H), 3.75-3.79 (m, 1H), 4.04-4.16 (m, 2H), 5.37-5.40 (m, 1H), 5.67 (dd, J =10.4, 2.4 Hz, 1H), 6.14 (dd, J =16.8, 2.4 Hz, 1H), 6.62 (dd, J =16.4, 10.4 Hz, 1H), 7.10 (d, J =8.8 Hz, 2H), 7.57 (d, J =8.4 Hz, 2H), 8.21 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{28}H_{35}N_7O_2$: C, 67.04; H, 7.03; N, 19.55; found: C, 66.82; H, 7.05; N, 19.51;

ESI-MS (m/z): 502.25 $[M+H]^+$

4.1.6.14. 1-(5-(4-amino-3-(4-(2-(phenylthio)ethoxy)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32n).



32n

32n was isolated as a white solid with a 47% yield.

Purity by UPLC: 96.03%

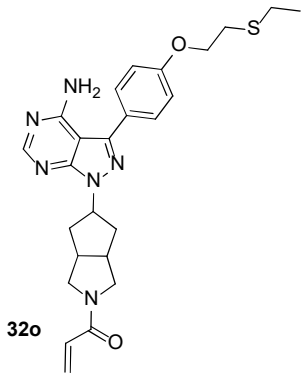
Melting point: 168-170 °C

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.05 – 2.03 (m, 2H), 2.36 – 2.28 (m, 2H), 2.97 – 2.95 (m, 1H), 3.07 – 3.06 (m, 1H), 3.42 – 3.62 (m, 3H), 3.52 – 3.49 (m, 1H), 3.65 – 3.59 (m, 1H), 3.80 – 3.75 (m, 1H), 4.24 (t, J = 6.0 Hz, 2H), 5.42 – 5.37 (m, 1H), 5.68 (dd, J = 2.4 Hz, 10.4 Hz, 1H), 6.16 (dd, J = 2.0 Hz, 16.8 Hz, 1H), 6.65 (dd, J = 10.4 Hz, 16.8 Hz, 1H), 7.07 (d, J = 8.8 Hz, 2H), 7.23 (t, J = 7.2 Hz, 1H), 7.36 (t, J = 7.6 Hz, 2H), 7.43 (d, J = 7.2 Hz, 2H), 7.57 (d, J = 8.4 Hz, 2H), 8.21 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{28}H_{35}N_7O_2$: C, 67.04; H, 7.03; N, 19.55; found: C, 66.82; H, 7.05; N, 19.51;

ESI-MS (m/z): 527.15 $[M+H]^+$, 549.15 $[M+Na]^+$

4.1.6.15. 1-(5-(4-amino-3-(4-(2-(ethylthio)ethoxy)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32o).



32o

32o was isolated as a yellow solid with a 55% yield.

Purity by UPLC: 95.11%

Melting point: 128-130 ºC

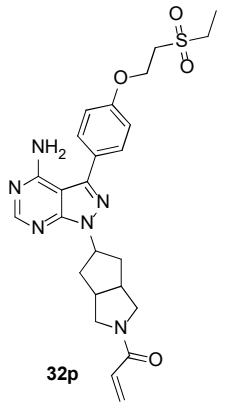
^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ ppm 1.23 (t, 3H, *J* = 7.6 Hz), 2.01-2.07 (m, 2H), 2.30-2.37 (m, 2H), 2.66 (q, 2H, *J* = 7.6 Hz), 2.93 (t, *J* = 6.4 Hz, 2H), 2.96-2.99 (m, 1H), 3.05-3.08 (m, 1H), 3.36-3..37 (m, 1H), 3.49-3.53 (m, 1H), 3.60-3.65 (m, 1H), 3.75-3.80 (m, 1H), 4.21 (t, 2H, *J* = 6.4 Hz), 5.38-5.41 (m, 1H), 5.68 (dd, *J* = 10.4, 2.4 Hz, 1H), 6.16 (dd, *J* = 16.8, 2.4 Hz, 1H), 6.65 (dd, *J* = 16.8, 10.4 Hz, 1H), 7.11 (d, *J* = 8.8 Hz, 2H), 7.59 (d, *J* = 8.8 Hz, 2H), 8.21 (s, 1H).

^{13}C NMR (100 MHz, DMSO-*d*6) δ ppm 14.55, 26.07, 33.44, 37.85, 38.07, 42.57, 51.59, 52.08, 58.37, 67.19, 97.91, 115.60, 126.07, 127.20, 128.91, 130.11, 143.75, 151.85, 154.14, 155.96, 158.62, 166.20.

Elemental (CHNS) analysis: Calculated for $C_{25}H_{30}N_6O_2S$: C, 62.74; H, 6.32; N, 17.56; S, 6.70; found: C, 62.89; H, 6.33; N, 17.63; S, 6.58;

ESI-MS (m/z): 479.65 $[M+H]^+$

4.1.6.16. 1-(5-(4-amino-3-(4-(2-(ethylsulfonyl)ethoxy)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32p).



32p

32p was isolated as a white solid with a 64% yield.

Purity by UPLC: 96.34%

Melting point: 132-134 °C

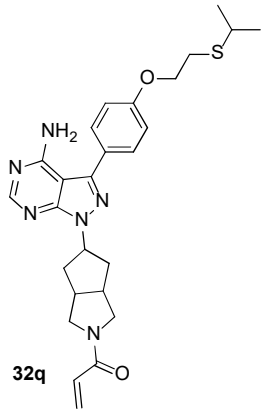
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.29 (t, J = 7.2 Hz, 3H), 1.89-2.08 (m, 2H), 2.28-2.37 (m, 2H), 2.96-2.99 (m, 1H), 3.06-3.08 (m, 1H), 3.22 (q, J = 7.2 Hz, 2H), 3.36-3.37 (m, 1H), 3.49-3.53 (m, 1H), 3.60-3.65 (m, 3H), 3.75-3.80 (m, 1H), 4.43 (t, J = 5.6 Hz, 2H), 5.40-5.41 (m, 1H), 5.68 (dd, J = 10.4, 2.4 Hz, 1H), 6.16 (dd, J = 16.8, 2.0 Hz, 1H), 6.65 (dd, J = 16.8, 10.4 Hz, 1H), 7.16 (d, J = 8.8 Hz, 2H), 7.61 (d, J = 8.4 Hz, 2H), 8.22 (s, 1H).

^{13}C NMR (100 MHz, DMSO-d6) δ ppm 9.36, 37.96, 38.20, 42.45, 51.5, 51.91, 53.78, 58.19, 62.18, 63.77, 67.58, 98.11, 115.42, 125.90, 127.19, 128.97, 130.07, 143.10, 152.51, 154.03, 155.96, 158.62, 166.14.

Elemental (CHNS) analysis: Calculated for $C_{25}H_{30}N_6O_4S$: C, 58.81; H, 5.92; N, 16.46; S, 6.28; found: C, 58.97; H, 5.88; N, 16.50; S, 6.32;

ESI-MS (m/z): 533.15 $[M+H]^+$

4.1.6.17. 1-(5-(4-amino-3-(4-(2-(isopropylthio)ethoxy)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32q).



32q

32q was isolated as a white solid with a 51% yield.

Purity by UPLC: 95.65%

Melting point: 115-117 ºC

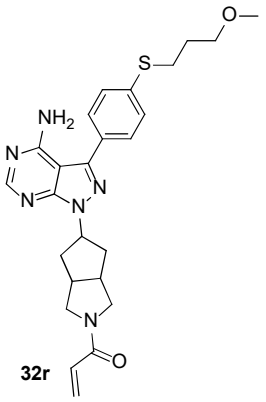
^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ ppm 1.24 (d, *J* = 6.4 Hz, 6H), 1.97-2.07 (m, 2H), 2.28-2.37 (m, 2H), 2.90-3.00 (m, 3H), 3.02-3.11 (m, 2H), 3.34-3.37 (m, 1H), 3.49-3.53 (m, 1H), 3.60-3.65 (m, 1H), 3.75-3.80 (m, 1H), 4.20 (t, *J* = 6.4 Hz, 2H), 5.37-5.41 (m, 1H), 5.68 (dd, *J* = 10.4, 2.8 Hz, 1H), 6.16 (dd, *J* = 16.8, 2.4 Hz, 1H), 6.65 (dd, *J* = 16.8, 10.4 Hz, 1H), 7.10 (d, *J* = 8.8 Hz, 2H), 7.59 (d, *J* = 8.8 Hz, 2H), 8.21 (s, 1H).

^{13}C NMR (100 MHz, DMSO-*d*6) δ ppm 24.34, 26.78, 37.78, 38.17, 42.62, 51.61, 51.99, 58.09, 66.43, 97.91, 115.55, 126.09, 127.19, 128.93, 130.08, 143.44, 152.12, 154.15, 155.96, 158.60, 166.05.

Elemental (CHNS) analysis: Calculated for $C_{26}H_{32}N_6O_2S$: C, 63.39; H, 6.55; N, 17.06; S, 6.51; found: C, 63.35; H, 6.60; N, 17.12; S, 6.41;

ESI-MS (m/z): 493.23 $[M+H]^+$

4.1.6.18. 1-(5-(4-amino-3-(4-((3-methoxypropyl)thio)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32r).



32r

32r was isolated as a light pink solid with a 58% yield.

Purity by UPLC: 95.59%

Melting point: 105-107 °C

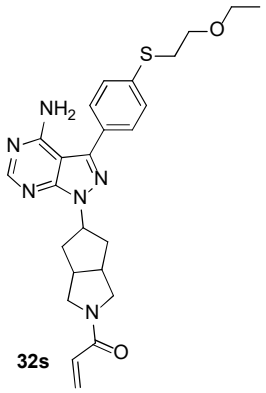
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.83-1.86 (m, 2H), 2.03-2.07 (m, 2H), 2.30-2.35 (m, 2H), 2.96-2.99 (m, 1H), 3.08 (t, J = 7.2 Hz, 3H), 3.23 (s, 3H), 3.36-3.37 (m, 1H), 3.45 (t, J = 6.0 Hz, 2H), 3.52-3.53 (m, 1H), 3.60-3.62 (m, 1H), 3.75-3.78 (m, 1H), 5.37-5.40 (m, 1H), 5.68 (dd, J = 10.4, 2.4 Hz, 1H), 6.16 (dd, J = 16.8, 2.4 Hz, 1H), 6.65 (dd, J = 16.8, 10.0 Hz, 1H), 7.46 (d, J = 8.4 Hz, 2H), 7.61 (d, J = 8.4 Hz, 2H), 8.22 (s, 1H).

^{13}C NMR (100 MHz, DMSO-*d*6) δ ppm 27.12, 34.53, 37.97, 38.10, 42.58, 51.59, 52.05, 58.10, 60.28, 74.92, 97.90, 127.07, 128.60, 129.26, 130.04, 131.07, 134.24, 143.12, 151.88, 154.42, 156.09, 166.04.

Elemental (CHNS) analysis: Calculated for $C_{25}H_{30}N_6O_2S$: C, 62.74; H, 6.32; N, 17.56; S, 6.70; found: C, 62.83; H, 6.39; N, 17.52; S, 6.78;

ESI-MS (m/z): 479.15 $[M+H]^+$

4.1.6.19. 1-(5-(4-amino-3-(4-((2-ethoxyethyl)thio)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32s).



32s

32s was isolated as a white solid with a 49% yield.

Purity by UPLC: 95.45%

Melting point: 111-113 ºC

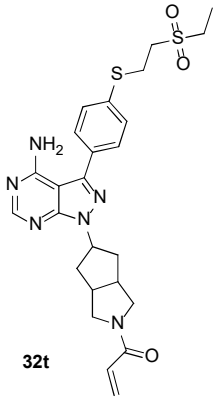
^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ ppm 1.10 (t, *J* = 6.8 Hz, 3H), 2.02-2.07 (m, 2H), 2.30-2.35 (m, 2H), 2.96-2.99 (m, 1H), 3.05-3.09 (m, 1H), 3.20 (t, *J* = 6.4 Hz, 2H), 3.53-3.43 (m, 4H), 3.65-3.58 (m, 3H), 3.80-3.75 (m, 1H), 5.43-5.38 (m, 1H), 5.66 (dd, *J* = 10.0, 2.4 Hz, 1H), 6.14 (dd, *J* = 16.4, 2.4 Hz, 1H), 6.62 (dd, *J* = 16.4, 10.0 Hz, 1H),7.48 (d, *J* = 8.4 Hz, 2H), 7.60 (d, *J* = 8.4 Hz, 2H), 8.22 (s, 1H).

^{13}C NMR (100 MHz, DMSO-*d*6) δ ppm 15.55, 32.18, 37.76, 38.18, 41.12, 41.59, 50.85, 53.77, 57.19, 65.90, 68.82, 74.92, 97.90, 127.10, 128.63, 129.28, 130.05, 131.09, 137.46, 143.44, 154.27, 156.00, 158.61, 166.04.

Elemental (CHNS) analysis: Calculated for $C_{25}H_{30}N_6O_2S$: C, 62.74; H, 6.32; N, 17.56; S, 6.70; found: C, 62.81; H, 6.38; N, 17.52; S, 6.73;

ESI-MS (m/z): 479.65 $[M+H]^+$

4.1.6.20. 1-(5-(4-amino-3-(4-((2-(ethylsulfonyl)ethyl)thio)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32t).



32t

32t was isolated as a white solid with a 47% yield.

Purity by UPLC: 95.26%

Melting point: 120-122 °C

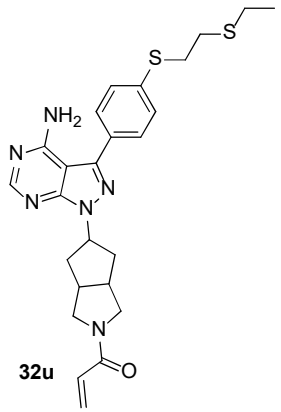
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.43 (t, J = 7.6 Hz, 3H), 2.19-2.13 (m, 2H), 2.58-2.53 (m, 2H), 3.06 (q, J = 7.6 Hz, 2H), 3.28-3.11 (m, 4H), 3.44-3.40 (m, 2H), 3.55-3.47 (m, 2H), 3.88-3.93 (m, 2H), 5.64-5.55 (m, 3H), 5.71 (dd, J = 10.0, 2.8 Hz, 1H), 6.38-6.51 (m, 2H), 7.54 (d, J = 8.0 Hz, 2H), 7.69 (d, J = 8.4 Hz, 2H), 8.41 (s, 1H).

^{13}C NMR (100 MHz, DMSO-*d*6) δ ppm 8.59, 26.37, 37.94, 38.17, 42.62, 50.84, 51.80, 52.22, 57.36, 60.28, 98.11, 127.11, 128.59, 129,26, 130.06, 131.20, 134.43, 143.75, 154.18, 155.87, 158.59, 166.04.

Elemental (CHNS) analysis: Calculated for $C_{25}H_{30}N_6O_3S_2$: C, 57.01; H, 5.74; N, 15.96; S, 12.17; found: C, 57.09; H, 5.73; N, 15.96; S, 12.22;

ESI-MS (m/z): 527.15 $[M+H]^+$

4.1.6.21. 1-(5-(4-amino-3-(4-((2-(ethylthio)ethyl)thio)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32u).



32u

32u was isolated as a white solid with a 55% yield.

Purity by UPLC: 99.52%

Melting point: 131-133 °C

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.17 (t, *J* = 7.4 Hz, 3H), 2.04-2.07 (m, 2H), 2.30-2.35 (m, 2H), 2.59 (q, *J* = 7.4 Hz, 2H), 2.74-2.78 (m, 2H), 2.96-3.01 (m, 1H), 3.04-3.08 (m, 1H), 3.22-3.26 (m, 2H), 3.34 – 3.38 (m, 1H), 3.51-3.53 (m, 1H), 3.59-3.63 (m, 1H), 3.75-3.79 (m, 1H), 5.38-5.43 (m, 1H), 5.66 (dd, *J* = 10.4, 2.4 Hz, 1H), 6.14 (dd, *J*

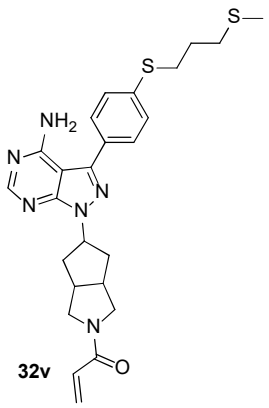
= 16.8, 2.4 Hz, 1H), 6.62 (dd, *J* = 16.8, 10.4 Hz, 1H), 7.49 (d, *J* = 8.0 Hz, 2H), 7.61 (d, *J* = 8.0 Hz, 2H), 8.22 (s, 1H).

^{13}C NMR (100 MHz, DMSO-*d*6) δ ppm 15.23, 25.41, 30.10, 34.59, 37.95, 38.19, 42.53, 51.58, 52.08, 58.09, 98.07, 127.10, 128.64, 129.24, 130.05, 131.22, 134.70, 143.44, 154.13, 155.90, 158.63, 166.05.

Elemental (CHNS) analysis: Calculated for $C_{25}H_{30}N_6OS_2$: C, 60.70; H, 6.11; N, 16.99; S, 12.96; found: C, 60.84; H, 6.02; N, 17.05; S, 12.93;

ESI-MS (m/z): 495.50 $[M+H]^+$

4.1.6.22. 1-(5-(4-amino-3-(4-((3-(methylthio)propyl)thio)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32v).



32v

32v was isolated as a white solid with a 69% yield.

Purity by UPLC: 95.45%

Melting point: 122-124 °C

^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ ppm 1.91-1.93 (m, 2H), 2.05-2.07 (m, 5H), 2.30-2.35 (m, 2H), 2.62 (t, *J* = 7.2 Hz, 2H), 2.96-2.98 (m, 1H), 3.06-3.08 (m, 1H), 3.13 (t, *J*

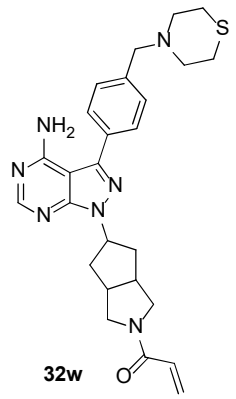
= 7.2 Hz, 2H), 3.33-3.37 (m, 1H), 3.49-3.53 (m, 1H), 3.60-3.65 (m, 1H), 3.75-3.80 (m, 1H), 5.39-5.42 (m, 1H), 5.68 (dd, *J* = 10.0, 2.4 Hz, 1H), 6.16 (dd, *J* = 16.8, 2.4 Hz, 1H), 6.65 (dd, *J* = 16.8, 10.0 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 2H), 7.62 (d, *J* = 8.4 Hz, 2H), 8.22 (s, 1H).

^{13}C NMR (100 MHz, DMSO-*d*6) δ ppm 15.08, 26.94, 32.84, 33.26, 37.93, 38.11, 42.32, 58.19, 67.23, 97.87, 127.20, 128.73, 128.92, 129.38, 129.72, 130.09, 130.27, 135.58, 143.31, 154.24, 154.96, 158.47, 166.11.

Elemental (CHNS) analysis: Calculated for $C_{25}H_{30}N_6OS_2$: C, 60.70; H, 6.11; N, 16.99; S, 12.96; found: C, 60.78; H, 6.08; N, 17.11; S, 12.94;

ESI-MS (m/z): 495.15 $[M+H]^+$

4.1.6.23. 1-(5-(4-amino-3-(4-(thiomorpholinomethyl)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32w).



32w

32w was isolated as a white solid with a 54% yield.

Purity by UPLC: 96.94%

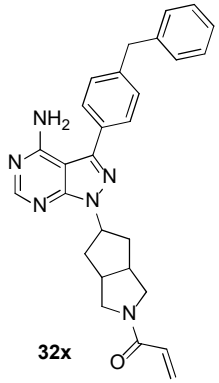
Melting point: 144-146 ºC

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.01-2.08 (m, 2H), 2.28-2.33 (m, 2H), 2.35-2.49 (m, 4H), 2.95-3.08 (m, 2H), 3.34-3.37 (m, 1H), 3.49-3.65 (m, 8H), 3.75-3.80 (m, 1H), 5.39-5.42 (m, 1H), 5.67 (dd, *J* = 10.4, 2.4 Hz, 1H), 6.14 (dd, *J* = 16.8, 2.4 Hz, 1H), 6.60 (dd, *J* = 16.8, 10.4 Hz, 1H), 7.46 (d, *J* = 8.4 Hz, 2H), 7.62 (d, *J* = 8.0 Hz, 2H), 8.23 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{26}H_{31}N_7OS$: C, 63.78; H, 6.38; N, 20.02; S, 6.55; found: C, 63.81; H, 6.39; N, 20.22; S, 6.59;

ESI-MS (m/z): 490.20 $[M+H]^+$

4.1.6.24. 1-(5-(4-amino-3-(4-benzylphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32x).



32x

32x was isolated as a white solid with a 68% yield.

Purity by UPLC: 99.21%

Melting point: 158-160 °C

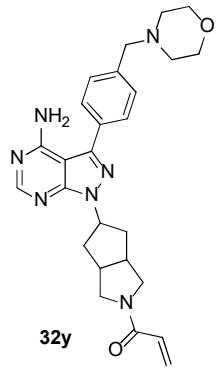
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.12-2.18 (m, 2H), 2.52-2.59 (m, 2H), 3.10-3.22 (m, 2H), 3.42-3.55 (m, 2H), 3.82-3.88 (m, 2H), 4.07 (s, 2H), 5.40-5.41 (m, 2H), 5.54-

5.58 (m, 1H), 5.70 (dd, *J* = 9.6, 2.4 Hz, 1H), 6.37-6.51 (m, 2H), 7.23-7.38 (m, 9H), 7.62 (d, *J* = 8.0 Hz, 2H), 8.37 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{28}H_{28}N_6O$: C, 72.39; H, 6.08; N, 18.09; found: C, 72.35; H, 6.15; N, 18.14;

ESI-MS (m/z): 465.15 $[M+H]^+$

4.1.6.25. 1-(5-(4-amino-3-(4-(morpholinomethyl)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32y).



32y

32y was isolated as a white solid with a 65% yield.

Purity by UPLC: 96.60%

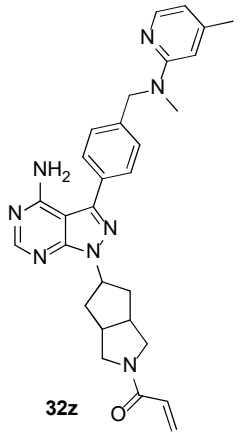
Melting point: 133-135 °C

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.11-2.19 (m, 2H), 2.50-2.60 (m, 6H), 3.10-3.22 (m, 2H), 3.42-3.55 (m, 2H), 3.59 (s, 2H), 3.73-3.76 (m, 4H), 3.83-3.89 (m, 2H), 5.44 (s, 2H), 5.40-5.41 (m, 1H), 5.53-5.59 (m, 1H), 5.71 (dd, *J* = 10.0, 2.4 Hz, 1H), 6.37-6.51 (m, 2H), 7.52 (d, *J* = 8.0 Hz, 2H), 7.66 (d, *J* = 8.0 Hz, 2H), 8.38 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{26}H_{31}N_7O_2$: C, 65.94; H, 6.60; N, 20.70; found: C, 65.85; H, 6.64; N, 20.73;

ESI-MS (m/z): 474.25 $[M+H]^+$

4.1.6.26. 1-(5-(4-amino-3-(4-((methyl(4-methylpyridin-2-yl)amino)methyl)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32z).



32z

32z was isolated as a white solid with a 55% yield.

Purity by UPLC: 98.42%

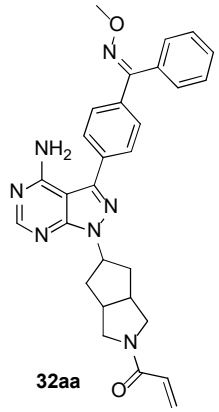
Melting point: 148-150 °C

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.03-2.05 (m, 2H), 2.21 (s, 3H), 2.28-2.34 (m, 3H), 2.96-3.08 (m, 5H), 3.35-3.37 (m, 1H), 3.49-3.53 (m, 1H), 3.60-3.65 (m, 1H), 3.75-3.80 (m, 1H), 4.86 (s, 2H), 5.39-5.42 (m, 1H), 5.66 (dd, J = 10.0, 2.4 Hz, 1H), 6.13 (dd, J = 16.8, 2.4 Hz, 1H), 6.44 (d, J = 5.2 Hz, 1H), 6.50 (s, 1H), 6.62 (dd, J = 16.8, 10.4 Hz, 1H), 7.34 (d, J = 8.0 Hz, 2H), 7.60 (d, J = 8.0 Hz, 2H), 7.95 (d, J = 5.2 Hz, 2H), 8.22 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{29}H_{32}N_8O$: C, 68.48; H, 6.34; N, 22.03; found: C, 68.60; H, 6.32; N, 22.06;

ESI-MS (m/z): 509.40 $[M+H]^+$

4.1.6.27. (E)-1-(5-(4-amino-3-(4-((methoxyimino)(phenyl)methyl)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32aa).



32aa

32aa was isolated as a white solid with a 60% yield.

Purity by UPLC: 96.82%

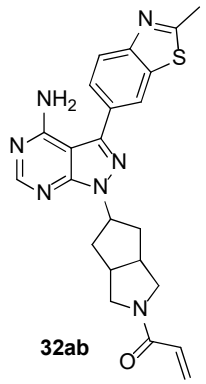
Melting point: 180-182 °C

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.13-2.22 (m, 2H), 2.53-2.61 (m, 2H), 3.12-3.26 (m, 2H), 3.48-3.57 (m, 2H), 3.84-3.90 (m, 2H), 3.97 (s, 3H), 5.58 (s, 2H), 5.60-5.64(m, 1H), 5.72 (dd, *J* = 9.6 , 2.4 Hz, 1H), 6.38-6.52 (m, 2H), 7.52-7.55 (m, 2H), 7.63-7.67 (m, 1H), 7.84-7.87 (m, 4H), 8.00 (d, *J* = 8.4 Hz, 2H), 8.42 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{29}H_{29}N_7O_2$: C, 68.62; H, 5.76; N, 19.32; found: C, 68.70; H, 5.72; N, 19.38;

ESI-MS (m/z): 508.25 $[M+H]^+$

4.1.6.28. 1-(5-(4-amino-3-(2-methylbenzo[d]thiazol-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32ab).



32ab

32ab was isolated as a white solid with a 51% yield.

Purity by UPLC: 95.09%

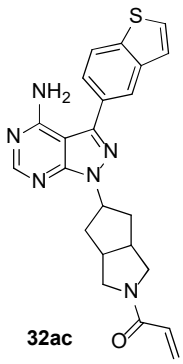
Melting point: 194-196 ^{o}C

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.14-2.20 (m, 2H), 2.55-2.59 (m, 2H), 2.91 (m, 3H), 3.12-3.24 (m, 2H), 3.47-3.56 (m, 2H), 3.83-3.89 (m, 2H), 5.53 (s, 2H), 5.57-5.61 (m, 1H), 5.71 (dd, *J* = 9.6, 2.4 Hz, 1H), 6.37-6.51 (m, 2H), 7.77 (dd, *J* = 8.4, 2.0 Hz, 1H), 8.11 (d, *J* = 8.4 Hz, 1H), 8.19 (d, *J* = 2.0 Hz, 1H), 8.40 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{23}H_{23}N_7OS$: C, 62.00; H, 5.20; N, 22.01; S, 7.20; found: C, 62.15; H, 5.21; N, 19.15; S, 7.25;

ESI-MS (m/z): 446.00 $[M+H]^+$

4.1.6.29. 1-(5-(4-amino-3-(benzo[b]thiophen-5-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32ac).



32ac

32ac was isolated as a light-yellow solid with a 57% yield.

Purity by UPLC: 96.53%

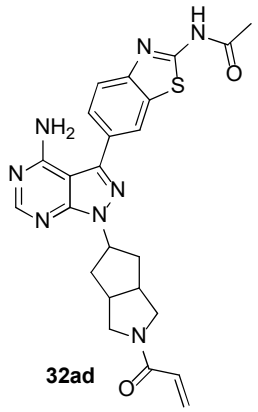
Melting point: 194-196 °C

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.04-2.09 (m, 2H), 2.31-2.38 (m, 2H), 2.98-3.10 (m, 2H), 3.35-3.39 (m, 1H), 3.42-3.54 (m, 1H), 3.60-3.66 (m, 1H), 3.76-3.81 (m, 1H), 5.41-5.45 (m, 1H), 5.67 (dd, J = 10.0, 2.4 Hz, 1H), 6.14 (dd, J = 16.8, 2.4 Hz, 1H), 6.62 (dd, J = 16.8, 10.4 Hz, 1H), 7.59 (d, J = 5.2 Hz, 1H), 7.65 (dd, J = 8.4, 1.6 Hz, 1H), 7.85 (d, J = 5.6 Hz, 1H), 8.15-8.17 (m, 2H), 8.24 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{23}H_{22}N_6OS$: C, 64.17; H, 5.15; N, 19.52; S, 7.45; found: C, 64.11; H, 5.18; N, 19.49; S, 7.44;

ESI-MS (m/z): 431.00 $[M+H]^+$

4.1.6.30. N-(6-(1-(2-acryloyloctahydrocyclopenta[c]pyrrol-5-yl)-4-amino-1H-pyrazolo[3,4-d]pyrimidin-3-yl)benzo[d]thiazol-2-yl)acetamide (32ad).



32ad

32ad was isolated as a white solid with a 49% yield.

Purity by UPLC: 96.29%

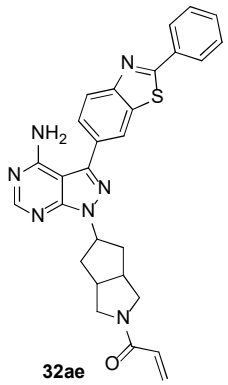
Melting point: 266-268 °C

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.03-2.09 (m, 2H), 2.23 (m, 3H), 2.37-2.49 (m, 2H), 2.99-3.11 (m, 2H), 3.34-3.39 (m, 1H), 3.50-3.54 (m, 1H), 3.61-3.69 (m, 1H), 3.73-3.81 (m, 1H), 5.41-5.44 (m, 1H), 5.66 (dd, *J* = 10.4, 2.4 Hz, 1H), 6.14 (dd, *J* = 16.8, 2.4 Hz, 1H), 6.62 (dd, *J* = 16.8, 10.4 Hz, 1H), 7.69 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.86 (d, *J* = 8.4 Hz, 1H), 8.24 (s, 2H), 12.41 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{24}H_{24}N_8O_2S$: C, 59.00; H, 4.95; N, 22.94; S, 6.56; found: C, 59.14; H, 4.93; N, 22.89; S, 6.58;

ESI-MS (m/z): 489.35 $[M+H]^+$

4.1.6.31. 1-(5-(4-amino-3-(2-phenylbenzo[d]thiazol-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32ae).



32ae

32ae was isolated as a white solid with a 47% yield.

Purity by UPLC: 97.51%

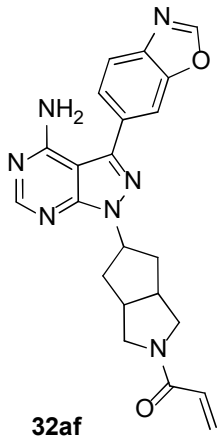
Melting point: 272-274 °C

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.13-2.22 (m, 2H), 2.55-2.63 (m, 2H), 3.14-3.25 (m, 2H), 3.48-3.56 (m, 2H), 3.84-3.90 (m, 2H), 5.49 (s, 2H), 5.59-5.69 (m, 1H), 5.71 (dd, *J* = 10.0, 2.4 Hz, 1H), 6.38-6.52 (m, 2H), 7.56-7.53 (m, 3H), 7.82 (dd, *J* = 8.4 , 2.0 Hz, 1H), 8.13-8.15 (m, 2H), 8.23 (d, *J* = 8.0 Hz, 1H), 8.27 (s, 1H), 8.41 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{28}H_{25}N_7OS$: C, 66.25; H, 4.96; N, 19.32; S, 6.32; found: C, 66.18; H, 4.95; N, 19.30; S, 6.39;

ESI-MS (m/z): 508.25 $[M+H]^+$

4.1.6.32. 1-(5-(4-amino-3-(benzo[d]oxazol-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32af).



32af

32af was isolated as a white solid with a 60% yield.

Purity by UPLC: 95.38%

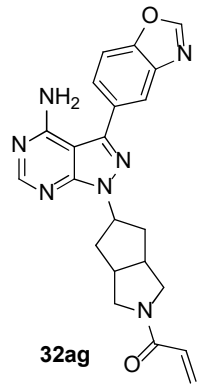
Melting point: 167-169 °C

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.14-2.23 (m, 2H), 2.55-2.62 (m, 2H), 3.12-3.23 (m, 2H), 3.48-3.56 (m, 2H), 3.84-3.90 (m, 2H), 5.59-5.65 (m, 1H), 5.71 (dd, *J* = 10.0, 2.4 Hz, 1H), 6.42-6.47 (m, 2H), 7.72-7.74 (m, 1H), 7.97-7.94 (m, 2H), 8.22 (s, 1H), 8.40 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{22}H_{21}N_7O_2$: C, 63.60; H, 5.10; N, 23.60; found: C, 63.74; H, 5.09; N, 23.56;

ESI-MS (m/z): 416.10 $[M+H]^+$

4.1.6.33. 1-(5-(4-amino-3-(benzo[d]oxazol-5-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32ag).



32ag

32ag was isolated as a white solid with a 49% yield.

Purity by UPLC: 96.64%

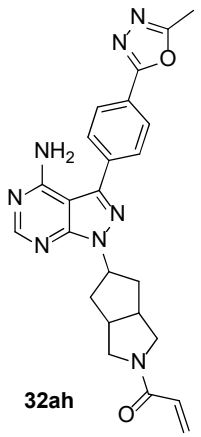
Melting point: 174-176 ºC

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.05-2.07 (m, 2H), 2.32-2.39 (m, 2H), 2.98-3.15 (m, 2H), 3.35-3.40 (m, 1H), 3.50-3.53 (m, 1H), 3.60-3.66 (m, 1H), 3.76-3.78 (m, 1H), 5.41-5.44 (m, 1H), 5.66 (dd, *J* = 10.4, 2.4 Hz, 1H), 6.14 (dd, *J* = 16.8, 2.4 Hz, 1H), 6.62 (dd, *J* = 16.8, 10.4 Hz, 1H), 7.72 (d, *J* = 8.4, 1H), 7.92 (d, *J* = 8.4 Hz, 1H), 8.01 (s, 1H), 8.24 (s, 1H), 8.84 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{22}H_{21}N_7O_2$: C, 63.60; H, 5.10; N, 23.60; found: C, 63.80; H, 5.04; N, 23.62;

ESI-MS (m/z): 416.10 $[M+H]^+$

4.1.6.34. 1-(5-(4-amino-3-(4-(5-methyl-1,3,4-oxadiazol-2-yl)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32ah).



32ah

32ah was isolated as a white solid with a 53% yield.

Purity by UPLC: 95.87%

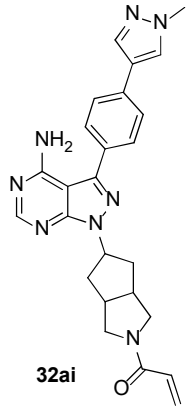
Melting point: 151-153 °C

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.05-2.07 (m, 2H), 2.32-2.38 (m, 2H), 2.61 (s, 3H), 2.98-3.15 (m, 2H), 3.34-3.39 (m, 1H), 3.50-3.54 (m, 1H), 3.61-3.64 (m, 1H), 3.76-3.78 (m, 1H), 5.44-5.46 (m, 1H), 5.66 (dd, *J* = 10.0, 2.4 Hz, 1H), 6.14 (dd, *J* = 16.4, 2.4 Hz, 1H), 6.62 (dd, *J* = 16.8, 10.4 Hz, 1H), 7.88 (d, *J* = 8.0, 1H), 8.12 (d, *J* = 8.0 Hz, 1H), 8.25 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{24}H_{24}N_8O_2$: C, 63.15; H, 5.30; N, 24.55; found: C, 63.08; H, 5.34; N, 24.53;

ESI-MS (m/z): 457.10 $[M+H]^+$

4.1.6.35. 1-(5-(4-amino-3-(4-(1-methyl-1H-pyrazol-4-yl)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32ai).



32ai

32ai was isolated as a white solid with a 68% yield.

Purity by UPLC: 96.98%

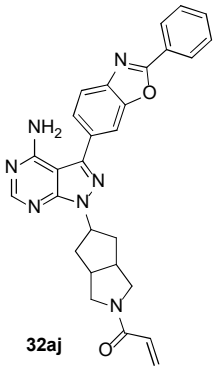
Melting point: 173-175 ^{o}C

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.14-2.23 (m, 2H), 2.55-2.62 (m, 2H), 3.12-3.23 (m, 2H), 3.46-3.56 (m, 2H), 3.83-3.89 (m, 2H), 5.54-5.59 (m, 3H), 5.71 (dd, *J* = 9.6, 2.4 Hz, 1H), 6.37-6.51 (m, 2H), 7.63-7.71 (m, 5H), 7.83 (s, 1H), 8.38 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{25}H_{26}N_8O$: C, 66.06; H, 5.77; N, 24.65; found: C, 66.20; H, 5.83; N, 24.65;

ESI-MS (m/z): 455.10 $[M+H]^+$

4.1.6.36. 1-(5-(4-amino-3-(2-phenylbenzo[d]oxazol-6-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32aj).



32aj

32aj was isolated as a white solid with a 61% yield.

Purity by UPLC: 95.63%

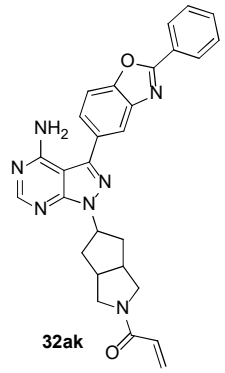
Melting point: 203-205 ºC

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.17-2.20 (m, 2H), 2.55-2.62 (m, 2H), 3.12-3.23 (m, 2H), 3.48-3.58 (m, 2H), 3.84-3.90 (m, 2H), 5.50 (s, 2H), 5.59-5.62 (m, 1H), 5.71 (dd, *J* = 9.6, 2.4 Hz, 1H), 6.38-6.52 (m, 2H), 7.57-7.59 (m, 3H), 7.72 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.93-7.96 (m, 2H), 7.72 (dd, *J* = 8.0, 2.4 Hz, 1H), 8.41 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{28}H_{25}N_7O_2$: C, 68.42; H, 5.13; N, 19.95; found: C, 68.30; H, 5.16; N, 19.91;

ESI-MS (m/z): 492.35 $[M+H]^+$

4.1.6.37. 1-(5-(4-amino-3-(2-phenylbenzo[d]oxazol-5-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32ak).



32ak

32ak was isolated as a white solid with a 62% yield.

Purity by UPLC: 97.40%

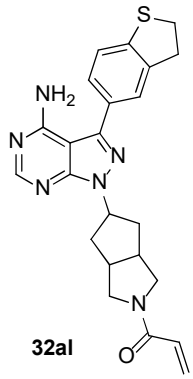
Melting point: 186-188 ºC

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.04-2.09 (m, 2H), 2.31-2.38 (m, 2H), 3.00-3.10 (m, 2H), 3.35-3.39 (m, 1H), 3.50-3.55 (m, 1H), 3.61-3.66 (m, 1H), 3.76-3.81 (m, 1H), 5.42-5.45 (m, 1H), 5.67 (dd, *J* = 10.4, 2.4 Hz, 1H), 6.14 (dd, *J* = 16.8, 2.4 Hz, 1H), 6.62 (dd, *J* = 16.4, 10.0 Hz, 1H), 7.63-7.73 (m, 4H), 7.94 (d, *J* = 8.8 Hz, 1H), 8.01 (m, 1H), 8.24-8.27 (m, 1H).

Elemental (CHNS) analysis: Calculated for $C_{28}H_{25}N_7O_2$: C, 68.42; H, 5.13; N, 19.95; found: C, 68.49; H, 5.18; N, 20.02;

ESI-MS (m/z): 492.05 $[M+H]^+$

4.1.6.38. 1-(5-(4-amino-3-(2,3-dihydrobenzo[b]thiophen-5-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32al).



32al

32al was isolated as a light-yellow solid with a 72% yield.

Purity by UPLC: 95.80%

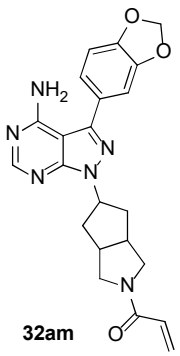
Melting point: 146-148 °C

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.04-2.06 (m, 2H), 2.29-2.33 (m, 2H), 2.96-3.06 (m, 2H), 3.35-3.37 (m, 1H), 3.50-3.55 (m, 1H), 3.61-3.66 (m, 1H), 3.76-3.81 (m, 1H), 5.38-5.40 (m, 1H), 5.66 (dd, J = 10.0, 2.4 Hz, 1H), 6.14 (dd, J = 16.4, 2.4 Hz, 1H), 6.62 (dd, J = 16.4, 10.4 Hz, 1H), 7.38-7.39 (m, 2H), 7.51 (s, 1H), 8.21 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{23}H_{24}N_6OS$: C, 63.87; H, 5.59; N, 19.43; S, 7.41; found: C, 64.01; H, 5.55; N, 19.47; S, 7.34;

ESI-MS (m/z): 433.05 $[M+H]^+$

4.1.6.39. 1-(5-(4-amino-3-(benzo[d][1,3]dioxol-5-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32am).



32am

32am was isolated as a white solid with a 53% yield.

Purity by UPLC: 96.58%

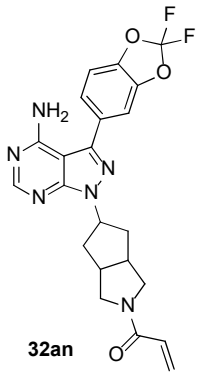
Melting point: 135-137 ºC

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.01-2.05 (m, 2H), 2.30-2.35 (m, 2H), 2.96-3.08 (m, 2H), 3.34-3.37 (m, 1H), 3.49-3.52 (m, 1H), 3.60-3.65 (m, 1H), 3.75-3.80 (m, 1H), 5.37-5.40 (m, 1H), 5.66 (dd, J = 10.0, 2.4 Hz, 1H), 6.09 (s, 2H), 6.14 (dd, J = 16.8, 2.4 Hz, 1H), 6.61 (dd, J = 16.8, 10.0 Hz, 1H), 7.05-7.15 (m, 3H), 8.21 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{22}H_{22}N_6O_3$: C, 63.15; H, 5.30; N, 20.08; found: C, 63.17; H, 5.28; N, 20.14;

ESI-MS (m/z): 419.10 $[M+H]^+$

4.1.6.40. 1-(5-(4-amino-3-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)prop-2-en-1-one (32an).



32an

32an was isolated as a white solid with a 46% yield.

Purity by UPLC: 96.58%

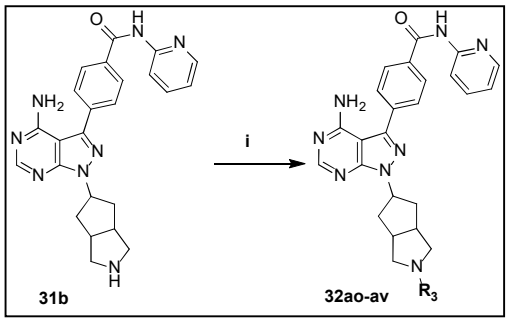
Melting point: 128-130 ºC

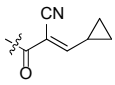
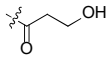
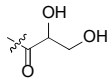
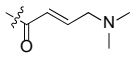
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.04-2.07 (m, 2H), 2.28-2.36 (m, 2H), 2.96-3.08 (m, 2H), 3.34-3.37 (m, 1H), 3.49-3.54 (m, 1H), 3.60-3.65 (m, 1H), 3.75-3.80 (m, 1H), 5.35-5.42 (m, 1H), 5.67 (dd, *J* = 10.4, 2.4 Hz, 1H), 6.09-6.16 (m, 3H), 6.61 (dd, *J* = 16.4, 10.0 Hz, 1H), 7.05-7.15 (m, 3H), 8.21 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{22}H_{20}F_2N_6O_3$: C, 58.15; H, 4.44; N, 18.49; found: C, 58.20; H, 4.43; N, 18.56;

ESI-MS (m/z): 455.10 $[M+H]^+$

4.1.7. Preparation of compound 32ao-av Series 3

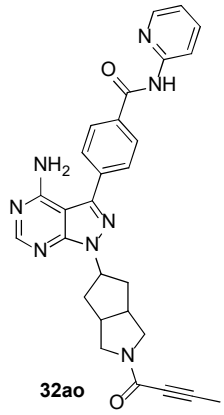


No.	R_3	No.	R_3	No.	R_3
ao		ar		au	
ap		as		av	
aq		at			

General procedure for the synthesis of compounds **32ao** and **32aq-av.**

To the solution of **31b** (0.485 mmol), in N,N-dimethylformamide (10 mL), were added the corresponding acids (0.533 mmol) and N,N-diisopropylethylamine (0.203 mL, 1.45 mmol) and cooled at 0 ºC, followed by the addition of O-(1H-Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (0.533 mmol). The reaction mixture was stirred for 18 hours. The mixture was diluted with water (10 mL) and extracted with ethyl acetate (3 x 10 mL). The combined organic layer was washed with water (10 mL) and brine (10 mL), dried over sodium sulphate, and concentrated *in vacuo*. The obtained crude product was purified by preparative HPLC to get the desired products **31ao** and **31aq-av**.

4.1.7.1. 4-(4-amino-1-(2-(but-2-ynoyl)octahydrocyclopenta[c]pyrrol-5-yl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-N-(pyridin-2-yl)benzamide (32ao).



32ao

32ao was isolated as a white solid with a 70% yield.

Purity by UPLC: 99.60%

Melting point: 246-248 ^{o}C

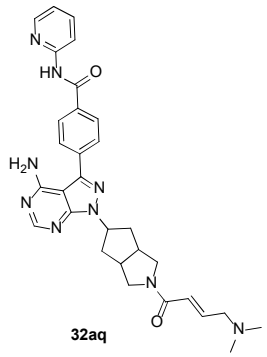
^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ ppm 2.01 (s, 3H), 2.04-2.08 (m, 2H), 2.32-2.36 (m, 2H), 3.04-3.05 (m, 2H), 3.27- 3.28 (m, 1H), 3.54-3.55 (m, 2H), 3.81-3.82 (m, 1H),5.42-5.44 (m, 1H), 7.17-7.20 (m, 1H), 7.79 (d, *J* =8.4 Hz, 2H), 7.86-7.87 (m, 1H), 8.18-8.23 (m,3H), 8.26 (s, 1H), 8.40-8.41 (m, 1H), 10.87 (s, 1H).

^{13}C NMR (100 MHz, DMSO-*d*6) δ ppm 3.72, 37.78, 38.17, 41.14, 41.61, 50.85, 53.78, 57.36, 74.92, 88.03, 98.11, 115.24, 120.36, 128.60, 129.26, 134.24, 136.71, 138.61, 143.12, 148.43, 151.88, 152.62, 154.42, 156.09, 158.61, 166.04.

Elemental (CHNS) analysis: Calculated for $C_{28}H_{26}N_8O_2$: C, 66.39; H, 5.17; N, 22.12; found: C, 66.54; H, 5.21; N, 22.08;

ESI-MS (m/z): 507.95 $[M+H]^+$

4.1.7.2. (E)-4-(4-amino-1-(2-(4-(dimethylamino)but-2-enoyl)octahydrocyclopenta[c] pyrrol-5-yl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-N-(pyridin-2-yl)benzamide (32aq).



32aq was isolated as a light-yellow solid with a 41% yield.

Purity by UPLC: 96.14%

Melting point: 195-197 ºC

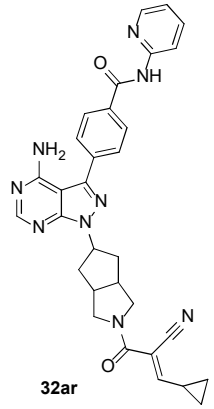
^{1}H NMR (400 MHz, MEOD) δ ppm 2.14-2.19 (m, 2H), 2.30 (s, 6H), 2.49-2.53(m, 2H), 3.14-3.28 (m, 4H), 3.53-3.54 (m, 1H), 3.62-3.62 (m, 1H), 3.76-3.80 (m, 1H), 3.89-3.92 (m, 1H), 5.54-5.55 (m, 1H), 6.52 (dd, *J* = 15.2, 1.2 Hz, 1H), 6.83-6.86 (m, 1H), 7.18-7.21 (m, 1H), 7.84-7.90 (m, 3H), 8.15-8.17 (m, 2H) 8.25-8.27 (m, 2H), 8.38-8.40 (m, 1H).

^{13}C NMR (100 MHz, DMSO-*d*6) δ ppm 34.59, 37.87, 38.06, 42.44, 45.46, 49.02, 51.69, 52.70, 57.21, 60.28, 98.07, 115.26, 116.24, 120.37, 128.59, 129.28, 134.24, 136.70, 138.63, 143.17, 148.46, 152.60, 154.40, 156.10, 158.59, 158.90, 166.05.

Elemental (CHNS) analysis: Calculated for $C_{30}H_{33}N_9O_2$: C, 65.32; H, 6.03; N, 22.85; found: C, 65.36; H, 6.00; N, 22.88;

ESI-MS (m/z): 552.35 $[M+H]^+$

4.1.7.3. (E)-4-(4-amino-1-(2-(2-cyano-3-cyclopropylacryloyl)octahydrocyclopenta[c] pyrrol-5-yl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-N-(pyridin-2-yl)benzamide (32ar).



32ar

32ar was isolated as a white solid with a 56% yield.

Purity by UPLC: 95.50%

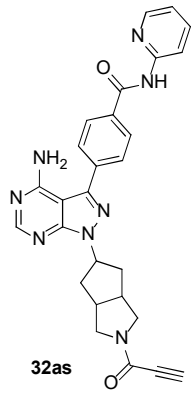
Melting point: 229-231 ºC

^{1}H NMR (400 MHz, $CDCL_3$) δ ppm 0.95-0.98 (m, 2H), 1.26-1.30 (m, 2H), 1.48-1.53 (m, 1H), 2.11-2.19 (m, 2H), 2.52-2.59 (m, 2H), 3.12-3.18 (m, 2H), 3.54- 3.68 (m, 2H), 3.88-4.01 (m, 2H), 5.41 (bs, 2H), 5.57-5.59 (m, 1H), 6.86 (d, *J* = 11.6 Hz, 1H), 7.17-7.20 (m, 1H), 7.78 (d, *J* = 8.4 Hz, 2H), 7.85-7.87 (m, 1H), 8.18-8.22 (m, 3H), 8.26 (s, 1H), 8.40-8.41 (m, 1H), 10.61 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{31}H_{29}N_9O_2$: C, 66.53; H, 5.22; N, 22.53; found: C, 66.68; H, 5.25; N, 22.59;

ESI-MS (m/z): 560.25 $[M+H]^+$

4.1.7.4. 4-(4-amino-1-(2-propioloyloctahydrocyclopenta[c]pyrrol-5-yl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-N-(pyridin-2-yl)benzamide (32as).



32as

32as was isolated as a white solid with a 45% yield.

Purity by UPLC: 95.78%

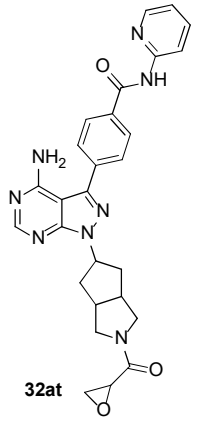
Melting point: 228-230 ºC

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.01-2.05 (m, 2H), 2.31-2.36 (m, 2H), 2.96-3.05 (m, 3H), 3.27- 3.28 (m, 1H), 3.52-3.55 (m, 2H), 3.75-3.79 (m, 1H), 5.42-5.44 (m, 1H), 7.17-7.20 (m, 1H), 7.79 (d, J = 8.4 Hz, 2H), 7.86-7.87 (m, 1H), 8.18-8.23 (m,3H), 8.26 (s, 1H), 8.40-8.41 (m, 1H), 10.87 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{27}H_{24}N_8O_2$: C, 65.84; H, 4.91; N, 22.75; found: C, 65.79; H, 4.93; N, 22.78;

ESI-MS (m/z): 493.10 $[M+H]^+$

4.1.7.5. 4-(4-amino-1-(2-(oxirane-2-carbonyl)octahydrocyclopenta[c]pyrrol-5-yl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-N-(pyridin-2-yl)benzamide (32at).



32at

32at was isolated as a white solid with a 46% yield.

Purity by UPLC: 95.30%

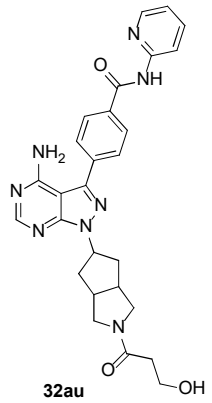
Melting point: 247-249 °C

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.05-2.10 (m, 2H), 2.29-2.41 (m, 3H), 2.75-2.82 (m, 2H), 2.97-3.12 (m, 2H), 3.32-3.38 (m, 1H), 3.53-3.57 (m, 1H), 3.60-3.63 (m, 1H), 3.69-3.74 (m, 1H), 5.44-5.47 (m, 1H), 7.17-7.20 (m, 1H), 7.80 (d, J =8.0 Hz, 2H), 7.84-7.88 (m, 1H), 8.19-8.23 (m, 3H), 8.26 (s, 1H), 8.40-8.41 (m, 1H), 10.87 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{27}H_{26}N_8O_3$: C, 63.52; H, 5.13; N, 21.95; found: C, 63.57; H, 5.16; N, 21.92;

ESI-MS (m/z): 493.25 $[M+H]^+$

4.1.7.6. 4-(4-amino-1-(2-(3-hydroxypropanoyl)octahydrocyclopenta[c]pyrrol-5-yl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-N-(pyridin-2-yl)benzamide (32au).



32au

32au was isolated as a white solid with a 71% yield.

Purity by UPLC: 97.70%

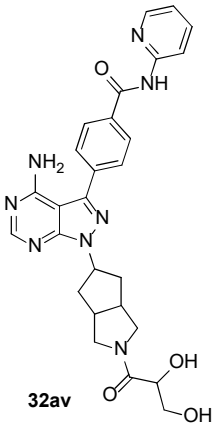
Melting point: 225-227 ºC

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.05-2.08 (m, 2H), 2.32-2.49 (m, 4H), 2.97-3.05 (m, 2H), 3.25-3.29 (m, 2H), 3.37-3.42 (m, 1H), 3.53-3.58 (m, 1H), 3.62-3.65 (m, 1H), 3.67-3.73 (m, 3H), 4.52 (t. J = 5.2 Hz, 1H), 5.42-5.46 (m, 1H), 7.17-7.20 (m, 1H), 7.79 (d, J =8.4 Hz, 2H), 7.84-7.88 (m, 1H), 8.18-8.23 (m, 3H), 8.26 (s, 1H), 8.40-8.41 (m, 1H), 10.87 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{27}H_{28}N_8O_3$: C, 63.27; H, 5.51; N, 21.86; found: C, 63.38; H, 5.47; N, 21.85;

ESI-MS (m/z): 513.90 $[M+H]^+$

4.1.7.7. 4-(4-amino-1-(2-(2,3-dihydroxypropanoyl)octahydrocyclopenta[c]pyrrol-5-yl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-N-(pyridin-2-yl)benzamide (32av).



32av

32av was isolated as a white solid with a 43% yield.

Purity by UPLC: 98.63%

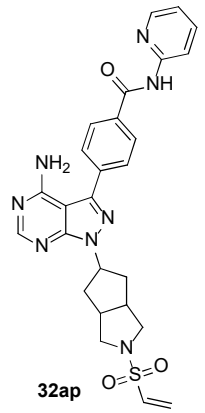
Melting point: 239-241 ºC

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.01-2.09 (m, 2H), 2.32-2.37 (m, 2H), 2.95-3.05 (m, 2H), 3.32-3.64 (m, 4H), 4.19-4.22 (m, 1H), 4.68-4.734 (m, 1H), 4.84-4.90 (m, 1H), 5.42-5.44 (m, 1H), 7.17-7.20 (m, 1H), 7.79 (d, J =8.4 Hz, 2H), 7.84-7.88 (m, 1H), 8.18-8.23 (m, 3H), 8.26 (s, 1H), 8.40-8.41 (m, 1H), 10.87 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{27}H_{28}N_8O_4$: C, 61.35; H, 5.34; N, 21.20; found: C, 61.36; H, 5.32; N, 21.29;

ESI-MS (m/z): 529.40 $[M+H]^+$

4.1.7.8. 4-(4-amino-1-(2-(vinylsulfonyl)octahydrocyclopenta[c]pyrrol-5-yl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-N-(pyridin-2-yl)benzamide (32ap).



32ap

To the solution of **31b** (150 mg, 0.341 mmol) in dichloromethane (5 mL), was added triethyl amine (0.142 mL, 1.022 mmol). The reaction was cooled to -50 ºC, followed by the dropwise addition of 2-chloroethanesulfonyl chloride (83 mg, 0.511 mmol). The reaction mixture was ambientally taken at room temperature and stirred for 3 hours. The mixture was diluted with water (5 mL), stirred for 5 minutes, the organic layer was separated, and the aqueous layer was extracted with dichloromethane (2 x 5 mL). The combined organic layer was washed with brine (5 mL), dried over sodium sulphate, and concentrated *in vacuo*. The obtained crude product was purified by preparative HPLC. **32ap** was isolated as a white solid with a 53% yield.

Purity by UPLC: 98.22%

Melting point: 276-278 ºC

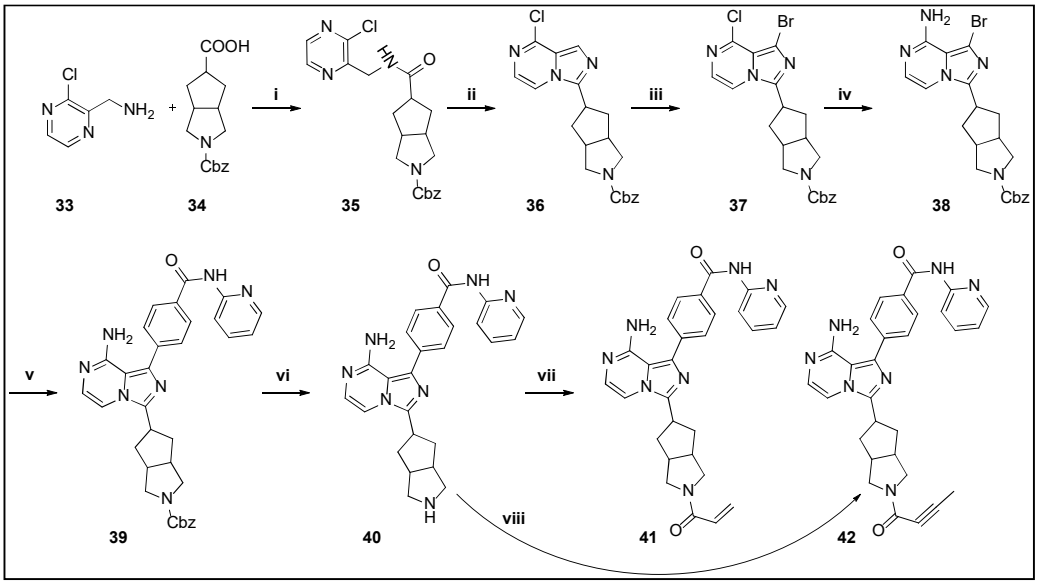
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.95-2.02 (m, 2H), 2.32-2.34 (m, 2H), 3.00-3.04 (m, 4H), 3.24-3.29 (m, 2H), 5.42-5.43 (m, 1H), 6.15 (d, *J* =16.8 Hz, 1H), 6.22 (d, *J* =10 *Hz*, 1H), 6.90 (dd, *J* = 16.4, 10.0 Hz, 1H), 7.16-7.11 (m, 1H), 7.77 (d, *J* = 8.4 Hz,

2H), 7.84-7.88 (m, 1H), 8.21 (d, *J* = 8.4 Hz, 2H), 8.22-8.24 (m, 1H) 8.26 (s, 1H), 8.40-8.41 (m, 1H), 10.84 (s, 1H).

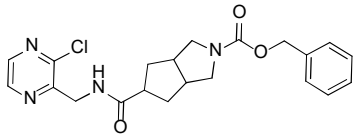
Elemental (CHNS) analysis: Calculated for $C_{26}H_{26}N_8O_3S$: C, 58.85; H, 4.94; N, 21.12; S, 6.04; found: C, 58.99; H, 4.90; N, 21.20; S, 6.06;

ESI-MS (m/z): 531.00 $[M+H]^+$

4.1.8. Preparation of compound 41 and 42 of Series 4



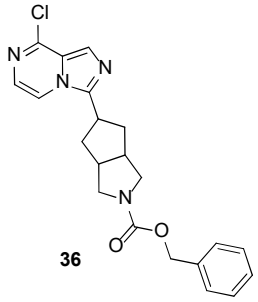
Step I: Benzyl 5-(((3-chloropyrazin-2-yl)methyl)carbamoyl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate **(35)**.



To the solution of (3-chloropyrazin-2-yl)methanamine (5 g, 34.8 mmol), in N,N-dimethylformamide (100 mL), were added 2-((benzyloxy)carbonyl)octahydrocyclopenta[c] pyrrole-5-carboxylic acid (10.08 g, 34.8 mmol) and N,N-diisopropylethylamine (18.25 mL, 104 mmol) and cooled at 0 °C,

followed by the addition of O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (15.85 g, 41.8 mmol). The reaction mixture was ambientally taken at room temperature and stirred for 18 hours. The mixture was diluted with water (500 mL) and extracted with ethyl acetate (3 x 150 mL). The combined organic layer was washed with water (150 mL) and brine (100 mL), dried over sodium sulphate, and concentrated *in vacuo*. To obtain benzyl 5-(((3-chloropyrazin-2-yl)methyl)carbamoyl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate as a thick oil (11.2 g, 27.0 mmol, 78% yield), which was used directly in the next step. **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 1.63-1.68 (m, 2H), 1.86-1.91 (m, 2H), 2.68-2.73 (m, 2H), 2.89-2.93 (m, 1H), 3.08-3.11 (m, 2H), 3.51-3.53 (m, 2H), 4.49 (d, J = 5.2 Hz, 2H), 5.04 (s, 2H), 7.28-7.38 (m, 5H), 8.30-8.35 (m, 1H), 8.42 (d, J = 2.4 Hz, 1H), 8.61 (d, J = 2.8 Hz, 1H). **ESI-MS** (m/z): 415.10 $[M+H]^+$.

Step II: Benzyl 5-(8-chloroimidazo[1,5-a]pyrazin-3-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate **(36)**.

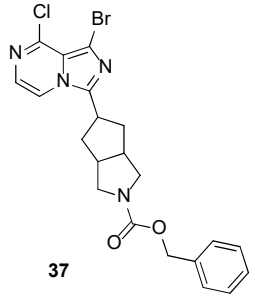


36

Benzyl 5-(((3-chloropyrazin-2-yl)methyl)carbamoyl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate (10.5 g, 25.3 mmol) was dissolved in acetonitrile (105 mL), phosphorus oxychloride (11.79 mL, 127 mmol) was added, and the mixture was stirred for 5 h at 80 °C. The reaction mixture was cooled to room temperature and concentrated *in vacuo* to remove the solvent. The obtained slurry was carefully poured into 150 mL of ice water. The aqueous phase was basified up to a pH of 8 using 25% aqueous ammonia. The resulting suspension was stirred for another 15 minutes, after which it

was extracted with ethyl acetate (2 x 100 mL). The combined organic layers were washed with water (100 mL) and brine (50 mL), dried over sodium sulphate, and concentrated *in vacuo*. The crude product was purified using flash chromatography to give benzyl 5-(8-chloroimidazo[1,5-a]pyrazin-3-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate as light yellow solid (6.2 g, 15.62 mmol, 61.7% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 1.96-2.01 (m, 2H), 2.07-2.15 (m, 2H), 2.68-2.73 (m, 2H), 2.89-2.93 (m, 2H), 3.24-3.26 (m, 2H), 3.82-3.89 (m, 1H), 5.07 (s, 2H), 7.28-7.39 (m, 6H), 7.78 (d, J = 1.6 Hz, 1H), 8.61 (dd, J = 0.8, 5.2 Hz, 1H). **ESI-MS** (m/z): 397.15 $[M+H]^+$.

Step III: Benzyl 5-(1-bromo-8-chloroimidazo[1,5-a]pyrazin-3-yl)hexahydrocyclopenta[c] pyrrole-2(1H)-carboxylate **(37)**.

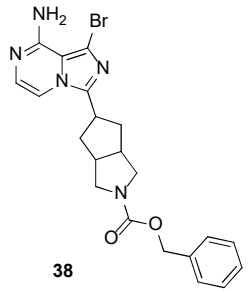


37

N-bromosuccinimide (2.56 g, 14.36 mmol) was added to a stirred solution of benzyl 5-(8-chloroimidazo[1,5-a]pyrazin-3-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate (5.7 g, 14.36 mmol) in N,N-dimethylformamide (85.5 mL). The reaction was stirred for 2 hours at room temperature. The reaction mixture was diluted with cold water (57 mL). After 10 minutes of stirring, an aqueous sodium thiosulfate solution (20% w/v, 57 mL) was added. After 30 minutes of stirring, the mixture was filtered and washed with water (2 x 30 mL). The resultant solid product dried *in vacuo* to afford benzyl 5-(1-bromo-8-chloroimidazo[1,5-a]pyrazin-3-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate as a light yellow solid (6.5 g, 13.66 mmol, 95% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 1.96-2.15 (m, 4H),

2.86-2.88 (m, 2H), 3.16-3.21 (m, 2H), 3.57-3.73 (m, 3H), 5.06 (s, 2H), 7.29-7.39 (m, 6H), 8.40 (d, *J* = 5.2 Hz, 1H). **ESI-MS** (m/z): 475.0 $[M]^+$, 477 $[M+2]^+$.

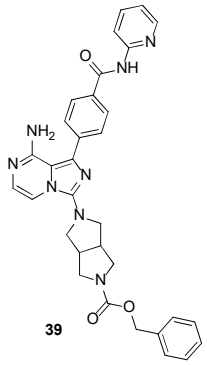
Step IV: Benzyl 5-(8-amino-1-bromoimidazo[1,5-a]pyrazin-3-yl)hexahydrocyclopenta[c] pyrrole-2(1H)-carboxylate **(38)**.



38

Benzyl 5-(1-bromo-8-chloroimidazo[1,5-a]pyrazin-3-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate (3 g, 6.31 mmol) and ethanolic ammonia (45 mL) were heated for 18 hours at 85 °C in a seal tube. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. The obtained slurry was dissolved in ethyl acetate (100 mL) and washed with water (50 mL) and brine (50 mL). The organic layer was dried over sodium sulphate and concentrated to give benzyl 5-(8-amino-1-bromoimidazo[1,5-a]pyrazin-3-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate as a white solid (2.7 g, 5.92 mmol, 94% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 1.91-1.95 (m, 2H), 2.00-2.07 (m, 2H), 2.86-2.88 (m, 2H), 3.22-3.24 (m, 2H), 3.61-3.74 (m, 3H), 5.06 (s, 2H), 6.70 (s, 2H), 6.96 (d, *J* = 5.2 Hz, 1H), 7.29-7.37 (m, 5H), 7.64 (d, *J* = 5.2 Hz, 1H). **ESI-MS** (m/z): 475.0 $[M]^+$, 477 $[M+2]^+$.

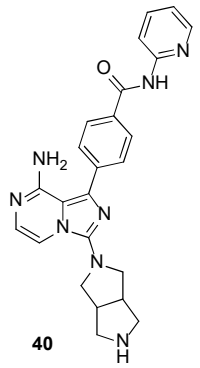
Step V: Benzyl 5-(8-amino-1-(4-(pyridin-2-ylcarbamoyl)phenyl)imidazo[1,5-a]pyrazin-3-yl)hexahydropyrrolo[3,4-c]pyrrole-2(1H)-carboxylate **(39)**.



39

Benzyl 5-(8-amino-1-bromoimidazo[1,5-a]pyrazin-3-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate (1.2 g, 2.63 mmol) was dissolved in dry N,N-dimethylformamide (30 mL), followed by the addition of (4-(pyridin-2-ylcarbamoyl)phenyl)boronic acid (0.700 g, 2.89 mmol), bis(triphenylphosphine)palladium(II) chloride (0.185 g, 0.263 mmol) and an aqueous potassium bicarbonate solution (7.89 mL, 15.78 mmol). The reaction mixture was then nitrogen-purged for 10 minutes. The reaction mixture was heated for 6 hours at 90 °C in nitrogen-containing conditions. After cooling to ambient temperature, the mixture was filtered through Celite, diluted with cold water (150 mL), and extracted with ethyl acetate (3 x 50 mL). The mixed organic layer was washed with water (50 mL) and brine (50 mL), dried on sodium sulphate, then concentrated and dried *in vacuo*. The crude product was purified by flash column chromatography to yield benzyl 5-(8-amino-1-(4-(pyridin-2-ylcarbamoyl)phenyl)imidazo[1,5-a]pyrazin-3-yl)hexahydropyrrolo[3,4-c]pyrrole-2(1H)-carboxylate as a white solid (1.2 g, 2.088 mmol, 79% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 1.98-2.03 (m, 2H), 2.14-2.21 (m, 2H), 2.89-2.91 (m, 2H), 3.27-3.29 (m, 2H), 3.64-3.66 (m, 2H), 3.81-3.85 (m, 1H), 5.08 (s, 2H), 6.82 (s, 2H), 7.09 (d, J = 5.2 Hz, 1H), 7.16-7.18 (m, 1H), 7.18-7.19(m, 1H), 7.30-7.38 (m, 5H), 7.74-7.88 (m, 4H), 8.15-8.21 (m, 3H), 8.41 (dd, J = 0.8, 5.0 Hz, 1H), 10.84 (s, 1H). **ESI-MS** (m/z): 574.20 $[M+H]^+$.

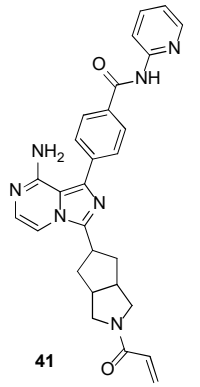
Step VI: 4-(8-amino-3-(hexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl)imidazo[1,5-a]pyrazin-1-yl)-N-(pyridin-2-yl)benzamide **(40)**.



40

Benzyl 5-(8-amino-1-(4-(pyridin-2-ylcarbamoyl)phenyl)imidazo[1,5-a]pyrazin-3-yl)hexahydropyrrolo[3,4-c]pyrrole-2(1H)-carboxylate (1.1 g, 1.914 mmol) was dissolved in methanol (16.5 mL), followed by the addition of 10% palladium on carbon (10.19 mg, 0.096 mmol). The reaction mixture was hydrogenated using a hydrogen gas balloon for 2 hours. After completion of the reaction, the reaction mixture was filtered through a Hyflow bed and washed with methanol (2 x 10 mL). The combined filtrate was concentrated and dried *in vacuo* to provide 4-(8-amino-3-(hexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl)imidazo[1,5-a]pyrazin-1-yl)-N-(pyridin-2-yl)benzamide (0.830 g, 1.884 mmol, 98% yield). The product was used for the next reaction without further purification.

4.1.8.1. 4-(3-(2-acryloyloctahydrocyclopenta[c]pyrrol-5-yl)-8-aminoimidazo[1,5-a]pyrazin-1-yl)-N-(pyridin-2-yl)benzamide (41).



41

Following the procedure described in step VII of Section 4.1.4, **41** was synthesised from 4-(8-amino-3-(hexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl)imidazo[1,5-a]pyrazin-1-yl)-N-(pyridin-2-yl)benzamide. **41** was isolated as a white solid with a 65% yield.

Purity by UPLC: 98.01%

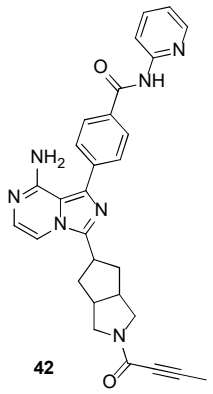
Melting point: 251-253 ^{o}C

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.00 – 1.99 (m, 2H), 2.23 – 2.22 (m, 2H), 2.89 – 2.88 (m, 1H), 2.99 – 2.98 (m, 1H), 3.36 – 3.31 (m, 1H), 3.51 – 3.47 (m, 1H), 3.74 – 3.69 (m, 1H), 3.88 – 3.81 (m, 2H), 5.68 (dd, *J* = 2.4 Hz, 10.0 Hz, 1H), 6.10 (s, 2H), 6.16 (dd, *J* = 2.4 Hz, 16.8 Hz, 1H), 6.66 (dd, *J* = 10.0 Hz, 16.8 Hz, 1H), 7.08 (d, *J* = 4.8 Hz, 1H), 7.19 – 7.16 (m, 1H), 7.70 (d, *J* = 4.8 Hz, 1H), 7.75 (d, *J* = 8.4 Hz, 2H), 7.87 – 7.83 (m, 1H), 8.15 (d, *J* = 8.4 Hz, 2H), 8.22 (d, *J* = 8.4 Hz, 1H), 8.41 – 8.39 (m, 1H), 10.81 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{28}H_{27}N_7O_2$: C, 68.14; H, 5.51; N, 19.87; found: C, 68.01; H, 5.54; N, 19.83;

ESI-MS (m/z): 494.20 $[M+H]^+$

4.1.8.2. 4-(8-amino-3-(2-(but-2-ynoyl)octahydrocyclopenta[c]pyrrol-5-yl)imidazo[1,5-a]pyrazin-1-yl)-N-(pyridin-2-yl)benzamide (42).



42

Following the procedure described in Section 4.1.7, **42** was synthesised from 4-(8-amino-3-(hexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl)imidazo[1,5-a]pyrazin-1-yl)-N-(pyridin-2-yl)benzamide and but-2-ynoic acid. **42** was isolated as a light yellow solid with a 59% yield.

Purity by UPLC: 98.90%

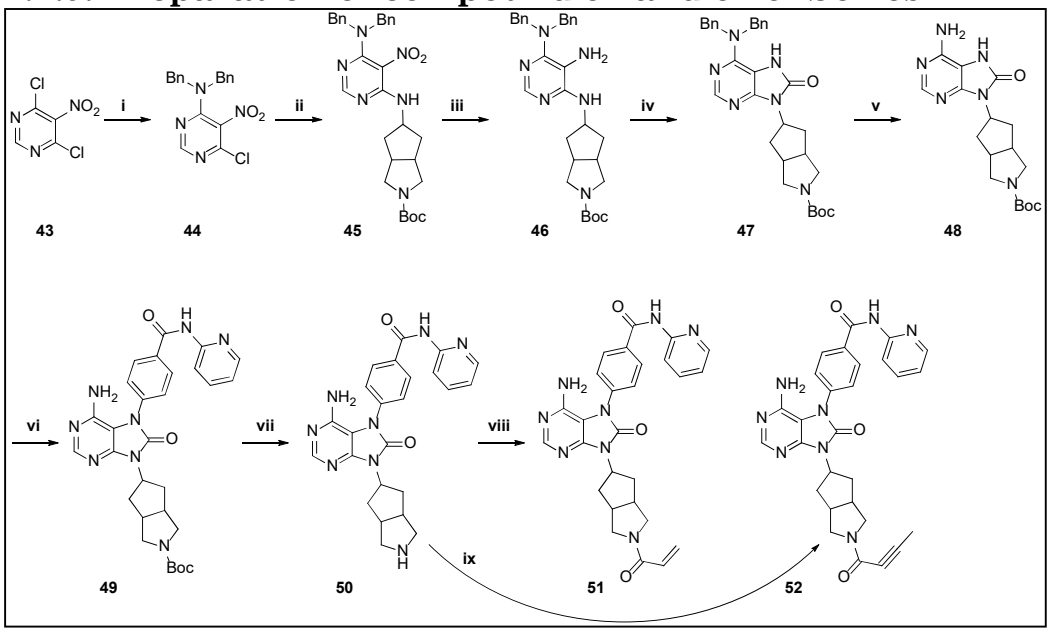
Melting point: 248-250 ^{o}C

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.01 – 1.99 (m, 5H), 2.18 – 2.15 (m, 2H), 2.94 – 2.92 (m, 2H), 3.27 – 3.25 (m, 1H), 3.51 – 3.48 (m, 1H), 3.69 – 3.63 (m, 1H), 3.84 – 3.82 (m, 1H), 3.89 – 3.87 (m, 1H), 6.10 (s, 2H), 7.09 (d, J = 5.2 Hz, 1H), 7.19 – 7.15 (m, 1H), 7.75 – 7.70 (m, 3H), 7.86 – 7.85 (m, 1H), 8.15 (d, J = 8.4 Hz, 2H), 8.22 (d, J = 8.4 Hz, 1H), 8.41 – 8.39 (m, 1H), 10.81 (s, 1H).

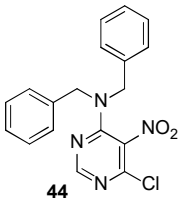
Elemental (CHNS) analysis: Calculated for $C_{29}H_{27}N_7O_2$: C, 68.89; H, 5.38; N, 19.39; found: C, 68.96; H, 5.41; N, 19.43;

ESI-MS (m/z): 506.20 $[M+H]^+$.

4.1.9. Preparation of compound 51 and 52 of Series 4

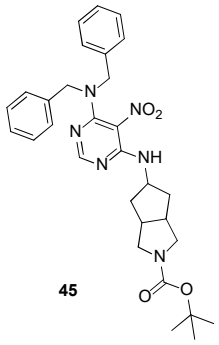


Step I: N,N-Dibenzyl-6-chloro-5-nitropyrimidin-4-amine **(44)**.



To a dichloromethane (50 mL) solution of 4,6-dichloro-5-nitropyrimidine (5 g, 25.8 mmol) at 0 ºC, dibenzylamine (5.09 g, 25.8 mmol) was added dropwise. Triethylamine (7.19 mL, 51.6 mmol) was then added and stirred for an additional hour at 0 ºC. The reaction mixture was quenched with water (50 mL) and agitated for 5 minutes. The organic layer was separated, rinsed with brine (25 mL), and dried over anhydrous sodium sulphate. Concentrated and dried *in vacuo* to obtain N,N-dibenzyl-6-chloro-5-nitropyrimidin-4-amine (9 g, 25.4 mmol, 98% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm 4.66 (s, 4H), 7.13-7.17 (m, 4H), 7.29-7.36 (m, 6H), 8.47 (s, 1H).

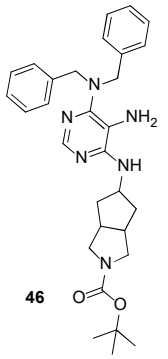
Step II: Tert-butyl 5-((6-(dibenzylamino)-5-nitropyrimidin-4-yl)amino)hexahydrocyclopenta [c]pyrrole-2(1H)-carboxylate **(45)**.



45

N,N-dibenzyl-6-chloro-5-nitropyrimidin-4-amine (6.5 g, 18.32 mmol) and tert-butyl 5-aminohexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate (4.15 g, 18.32 mmol) were dissolved in 1,4-dioxane (65 mL), and triethylamine was added to the reaction mixture and heated at 50 ºC for 6h. The reaction mixture was concentrated, and the resulting slurry was diluted with ethyl acetate. The organic phase was washed with water and brine and then dried over anhydrous sodium sulphate, concentrated and dried *in vacuo*. The crude product obtained was purified using flash column chromatography to yield tert-butyl 5-((6-(dibenzylamino)-5-nitropyrimidin-4-yl)amino)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate (6.9 g, 12.67 mmol, 69.1% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 1.39 (s, 9H), 1.81-1.84 (m, 4H), 2.73-2.75 (m, 2H), 3.01-3.05 (m, 2H), 3.42-3.46 (m, 2H), 4.60 (s, 4H), 4.63-4.65 (m, 1H), 7.14-7.15 (m, 4H), 7.24-7.32 (m, 6H), 8.13 (s, 1H), 8.16-8.18 (m, 1H). **ESI-MS (m/z)**: 545.45 $[M+H]^+$.

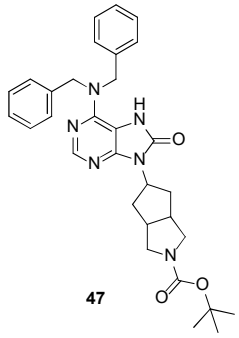
Step III: Tert-butyl 5-((5-amino-6-(dibenzylamino)pyrimidin-4-yl)amino)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate **(46)**.



46

A ethyl acetate (130 mL) solution of tert-butyl 5-((6-(dibenzylamino)-5-nitropyrimidin-4-yl)amino)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate (6.5 g, 11.93 mmol) was added dropwise to the mixture of zinc (7.80 g, 119 mmol) and 3.0 M aqueous ammonium chloride solution (23.87 mL, 71.6 mmol) at 0 ºC. After completion of the addition, the reaction mixture was warmed to room temperature and stirred for 2 hours. The reaction mixture was filtered through a Hyflow bed, and the residue was washed with ethyl acetate (2 x 25 mL). The obtained filtrate was concentrated and dried *in vacuo*. The crude product obtained was purified using flash column chromatography to yield tert-butyl 5-((5-amino-6-(dibenzylamino)pyrimidin-4-yl)amino)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate (5.1 g, 9.91 mmol, 83% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ ppm 1.47 (s, 9H), 1.74-1.79 (m, 2H), 2.01-2.05 (s, 2H), 2.80-2.81 (m, 2H), 3.02-3.05 (m, 2H), 3.18-3.21 (m, 2H), 3.59-3.61 (m, 2H), 4.33 (s, 4H), 4.44-4.46 (m, 1H), 4.56-4.61 (m, 1H), 7.22-7.32 (m, 10H), 8.15 (s, 1H). **ESI-MS (m/z)**: 541.50 $[M+H]^+$.

Step IV: Tert-butyl 5-(6-(dibenzylamino)-8-oxo-7,8-dihydro-9H-purin-9-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate **(47)**.

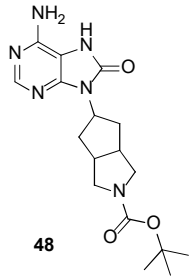


47

Tert-butyl 5-((5-amino-6-(dibenzylamino)pyrimidin-4-yl)amino)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate (4.5 g, 8.74 mmol) was dissolved in tetrahydrofuran (67.5 mL), followed by the addition of 1,1'-

carbonyldiimidazole (2.84 g, 17.49 mmol). The reaction mixture was heated at 60 ºC for 15 hours. The reaction mixture was concentrated, diluted with water, and extracted with ethyl acetate (2 x 50 mL). The combined organic phase was washed with brine (30 mL) and dried over anhydrous sodium sulphate, then concentrated and dried *in vacuo*. The crude product obtained was purified using flash column chromatography to yield tert-butyl 5-(6-(dibenzylamino)-8-oxo-7,8-dihydro-9H-purin-9-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate (4.1 g, 7.58 mmol, 87% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 1.40 (s, 9H), 1.76-1.81 (m, 2H), 2.44-2.48 (m, 2H), 2.92-2.94 (m, 2H), 3.04-3.08 (m, 2H), 4.78 (s, 4H), 4.86-4.95 (m, 1H), 7.19-7.33 (m, 10H), 8.15 (s, 1H). **ESI-MS (m/z)**: 541.50 $[M+H]^+$.

Step V: Tert-butyl 5-(6-amino-8-oxo-7,8-dihydro-9H-purin-9-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate **(48)**.

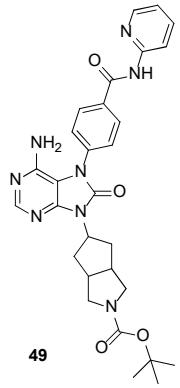


48

Tert-butyl 5-(6-(dibenzylamino)-8-oxo-7,8-dihydro-9H-purin-9-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate (3.5 g, 6.47 mmol) was dissolved in methanol (70 mL), followed by the addition of 20% palladium hydroxide on carbon (1.591 g, 2.266 mmol). The reaction mixture was hydrogenated at 50 psi for 24 hours using the Parr hydrogenation apparatus. After completion of the reaction, the reaction mixture was filtered through a Hyflow bed and washed with methanol (2 x 20 mL). The combined filtrate was concentrated and dried *in vacuo* to provide tert-butyl 5-(6-amino-8-oxo-7,8-dihydro-9H-purin-9-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate (2.25 g, 6.24 mmol, 96% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 1.40 (s, 9H),

1.75-1.80 (m, 2H), 2.41-2.48 (m, 2H), 2.92-2.94 (m, 2H), 3.04-3.08 (m, 2H), 3.45-3.49 (m, 2H), 4.81-4.85 (m, 1H), 6.48 (s, 2H), 7.99 (s, 1H). **ESI-MS (m/z)**: 382.95 $[M+Na]^+$.

Step VI: Tert-butyl 5-(6-amino-8-oxo-7-(4-(pyridin-2-ylcarbamoyl)phenyl)-7,8-dihydro-9H-purin-9-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate **(49)**.

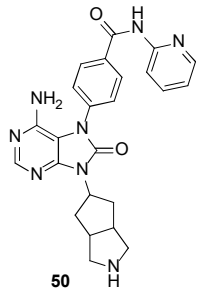


49

Tert-butyl 5-(6-amino-8-oxo-7,8-dihydro-9H-purin-9-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate (1 g, 2.77 mmol) was dissolved in dichloromethane (50 mL), followed by the addition of (4-(pyridin-2-ylcarbamoyl)phenyl)boronic acid (2.035 g, 8.41 mmol), copper(II) acetate (0.504 g, 2.77 mmol), molecular sieve 4A^o (1 g), and pyridine (1.571 mL, 19.42 mmol). The reaction mixture was stirred for 24 hours in the presence of air. The reaction mixture was filtered through a Hyflow bed and washed with dichloromethane (2 x 10 mL). The obtained filtrate was concentrated and dried *in vacuo*. The crude product obtained was purified using flash column chromatography to yield tert-butyl 5-(6-amino-8-oxo-7-(4-(pyridin-2-ylcarbamoyl)phenyl)-7,8-dihydro-9H-purin-9-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate (0.8 g, 1.437 mmol, 51.8% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 1.40 (s, 9H), 1.75-1.80 (m, 2H), 2.41-2.48 (m, 2H), 2.92-2.94 (m, 2H), 3.04-3.08 (m, 2H), 3.45-3.49 (m, 2H), 4.81-4.85 (m, 1H), 6.48 (s, 2H), 7.17-7.20 (m, 1H), 7.79 (d, J =8.4 Hz, 2H), 7.84-7.88 (m, 1H), 7.99

(s, 1H), 8.18-8.23 (m, 3H), 8.40-8.41 (m, 1H), 10.87 (s, 1H). **ESI-MS (m/z)**: 557.20 $[M+H]^+$.

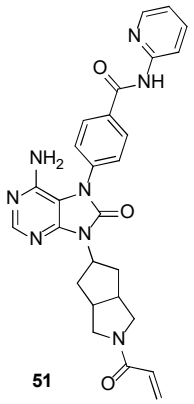
Step VII: 4-(6-amino-9-(octahydrocyclopenta[c]pyrrol-5-yl)-8-oxo-8,9-dihydro-7H-purin-7-yl)-N-(pyridin-2-yl)benzamide **(50)**.



50

Following the procedure described in step VI of Section 4.1.4., 4-(6-amino-9-(octahydrocyclopenta[c]pyrrol-5-yl)-8-oxo-8,9-dihydro-7H-purin-7-yl)-N-(pyridin-2-yl)benzamide was synthesised from tert-butyl 5-(6-amino-8-oxo-7-(4-(pyridin-2-ylcarbamoyl)phenyl)-7,8-dihydro-9H-purin-9-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate.

4.1.9.1. 4-(9-(2-acryloyloctahydrocyclopenta[c]pyrrol-5-yl)-6-amino-8-oxo-8,9-dihydro-7H-purin-7-yl)-N-(pyridin-2-yl)benzamide (51).



51

Following the procedure described in step VII of Section 4.1.4., **51** was synthesised from 4-(6-amino-9-(octahydrocyclopenta[c]pyrrol-5-yl)-8-oxo-8,9-dihydro-7H-purin-7-yl)-N-(pyridin-2-yl)benzamide. **51** was isolated as a white solid with a 53% yield.

Purity by UPLC: 96.56%

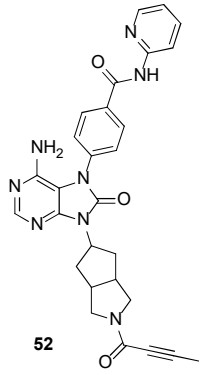
Melting point: 269-271 °C

^{1}H NMR (400 MHz, MeOD) δ ppm 2.02-2.07 (m, 3H), 2.66-2.74 (m, 3H), 3.41-3.49 (m, 1H), 3.54-3.57 (m, 1H), 3.77-3.91 (m, 2H), 5.19-5.23 (m, 1H), 5.76 (dd, *J* =10.4, 2.0 Hz, 1H), 6.29 (dd, *J* =16.4, 2.0 Hz, 1H), 6.65 (dd, *J* = 16.4, 10.4 Hz, 1H), 7.17-7.20 (m, 1H), 7.63 (d, *J* =8.4 Hz, 2H), 7.84-7.88 (m, 1H), 8.16-8.27 (m, 4H), 8.40-8.41 (m, 1H).

Elemental (CHNS) analysis: Calculated for $C_{27}H_{26}N_8O_3$: C, 63.52; H, 5.13; N, 21.95; found: C, 63.62; H, 5.16; N, 21.92;

ESI-MS (m/z): 511.35 $[M+H]^+$

4.1.9.2. 4-(6-amino-9-(2-(but-2-ynoyl)octahydrocyclopenta[c]pyrrol-5-yl)-8-oxo-8,9-dihydro-7H-purin-7-yl)-N-(pyridin-2-yl)benzamide (52).



52

Following the procedure described in Section 4.1.7, **52** was synthesised from 4-(6-amino-9-(octahydrocyclopenta[c]pyrrol-5-yl)-8-oxo-8,9-dihydro-7H-purin-7-yl)-N-(pyridin-2-yl)benzamide and but-2-ynoic acid. **52** was isolated as a white solid with a 62% yield.

Purity by UPLC: 96.90%

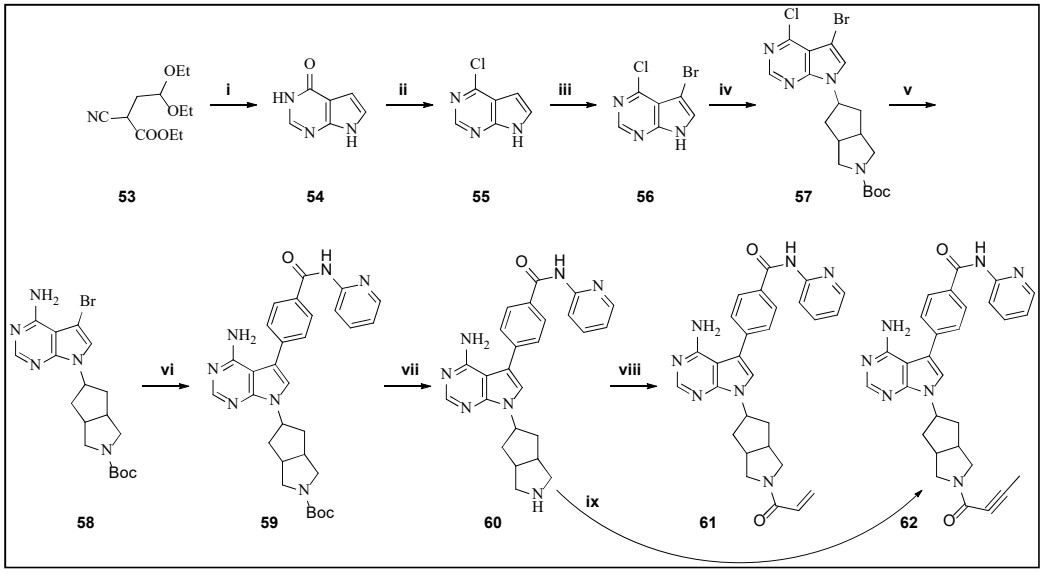
Melting point: 254-256 ^{o}C

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.01 (s, 3H), 2.04-2.07 (m, 2H), 2.32-2.36 (m, 2H), 3.04-3.05 (m, 2H), 3.24-3.27 (m, 1H), 3.54-3.55 (m, 2H), 3.80-3.82 (m, 1H), 5.41-5.43 (m, 1H), 7.19-7.20 (m, 1H), 7.52 (dd, J =6.8, 2.0 Hz, 2H), 7.86-7.88 (m, 1H), 8.18-8.21 (m, 4H), 8.39-8.41 (m, 1H), 10.88 (s, 1H).

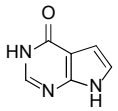
Elemental (CHNS) analysis: Calculated for $C_{28}H_{26}N_8O_3$: C, 64.36; H, 5.02; N, 21.44; found: C, 64.45; H, 5.08; N, 21.39;

ESI-MS (m/z): 523.25 $[M+H]^+$

4.1.10. Preparation of compound 61 and 62 of Series 4



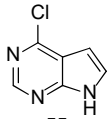
Step I: 3,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one **(54)**.



54

Formamidine acetate (22.70 g, 218 mmol) was added to the solution of sodium ethoxide in ethanol (200 mL, 436 mmol), and the resulting solution was stirred for 60 minutes. Ethyl 2-cyano-4,4-diethoxybutanoate (40 g, 174 mmol) was then added, and the resulting reaction mixture was refluxed for seven hours. The stirring was turned off after the solution was cooled and the solids were allowed to settle. The supernatant ethanol solution was removed, leaving the solids at the bottom of the reaction flask. The ethanol was evaporated, and the residue was added back to the solids remaining in the reaction flask with ice water (110 mL). A solution of 6 N aqueous hydrochloric acid (90 mL) was added to the resulting solution at 15 °C. The resulting solution was then heated at 45 °C for 1 hour. The solution was again cooled to 15 °C, and the pH was adjusted to 8.0 with the addition of aqueous ammonium hydroxide. The precipitated solids were collected by filtration, washed with water (3 x 25 mL), and suction dried. The solids were further washed with 1:1 ethyl acetate/hexane (25 mL), then hexane (2 x 25 mL), and dried in vacuum to afford 3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one as a yellow solid (15 g, 111 mmol, 63.6% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 6.42 (dd, J = 2.0, 3.2 Hz, 1H), 7.02 (dd, J = 2.4, 3.2 Hz, 1H), 7.81 (s, 1H), 11.75 (bs, 1H), 11.85 (bs, 1H). **ESI-MS (m/z)**: 135.60 $[M+H]^+$.

Step II: 4-chloro-7H-pyrrolo[2,3-d]pyrimidine **(55)**.



55

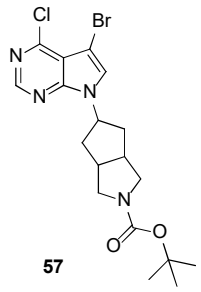
Phosphorous oxychloride (29.3 mL, 315 mmol) was added to 3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (8.5 g, 62.9 mmol), and the reaction was heated under nitrogen atmosphere to 100 °C for 1.5 hours. The reaction was allowed to cool to room temperature. Excess phosphorous oxychloride was removed *in vacuo*, the residue was cooled in an ice bath, and crushed ice was added with stirring. The mixture was diluted with water (100 mL) and extracted with ethyl acetate (2 x 100 mL). The combined organic extracts were washed with brine, then dried over anhydrous sodium sulphate, concentrated, and dried *in vacuo* to afford 4-chloro-7H-pyrrolo[2,3-d]pyrimidine as a white solid (6.27 g, 40.6 mmol, 64.5% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 6.60 (d, J = 3.6 Hz, 1H), 7.69 (d, J = 3.2 Hz, 1H), 7.58 (s, 1H), 12.57 (bs, 1H). **ESI-MS (m/z)**: 151.80 [M-H].

Step III: 5-bromo-4-chloro-7H-pyrrolo[2,3-d]pyrimidine **(56)**.

56

To a solution of 4-chloro-7H-pyrrolo[2,3-d]pyrimidine (6.2 g, 40.4 mmol) in N,N-dimethylformamide (62 mL), N-bromosuccinimide (7.90 g, 44.4 mmol) was added, and the mixture was stirred at room temperature for 3 hours. The mixture was poured into ice water (350 mL), and the precipitate was filtered, washed with water (3 x 50 mL), and dried in vacuum to afford 5-bromo-4-chloro-7H-pyrrolo[2,3-d]pyrimidine as an off white solid (8.68 g, 37.0 mmol, 92% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 7.94 (s, 1H), 8.61 (s, 1H), 12.96 (bs, 1H). **ESI-MS (m/z)**: 231.85 [M-H].

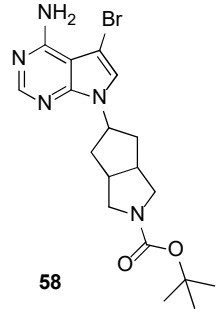
Step IV: Tert-butyl 5-(5-bromo-4-chloro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate (57).



57

5-bromo-4-chloro-7H-pyrrolo[2,3-d]pyrimidine (1 g, 4.30 mmol) was dissolved in N,N-dimethylformamide (15 mL), and cesium carbonate (3.50 g, 10.75 mmol) was added in one lot under nitrogen atmosphere. After stirring the reaction mixture for 30 minutes, tert-butyl 5-((methylsulfonyl)oxy)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate (1.445 g, 4.73 mmol) was added to it at room temperature. The reaction mixture was heated at 70 ºC for 18 hours. The mixture was poured into ice water (75 mL), and the precipitate was filtered, washed with water (3 x 25 mL), and dried in vacuum to afford tert-butyl 5-(5-bromo-4-chloro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate as a light yellow solid (1.5 g, 2.69 mmol, 62.6% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 1.41 (s, 1H), 2.01-2.06 (m, 2H), 2.27-2.29 (m, 2H), 2.92-2.94 (m, 2H), 3.10-3.14 (m, 2H), 3.48-3.53 (m, 2H), 5.28-5.32 (m, 1H), 8.14 (s, 1H), 8.66 (s, 1H). **ESI-MS (m/z)**: 441.15 $[M]^+$, 443.15 $[M+2]^+$.

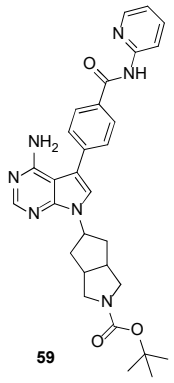
Step V: Tert-butyl 5-(4-amino-5-bromo-7H-pyrrolo[2,3-d]pyrimidin-7-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate **(58)**.



58

25% ammonium hydroxide (20 mL, 128 mmol) was added to the solution of tert-butyl 5-(5-bromo-4-chloro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate (1.1 g, 2.490 mmol) in 1,4-dioxane (30 mL), and the resulting solution was heated for 18 hours at 120 °C in a seal tube. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. The obtained slurry was dissolved in ethyl acetate (100 mL) and washed with water (50 mL) and brine (50 mL). The organic layer was dried over sodium sulphate and concentrated to give tert-butyl 5-(4-amino-5-bromo-7H-pyrrolo[2,3-d]pyrimidin-7-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate as a white solid (950 mg, 2.159 mmol, 87% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 1.40 (s, 1H), 1.93-1.98 (m, 2H), 2.11-2.19 (m, 2H), 2.89-2.91 (m, 2H), 3.07-3.11 (m, 2H), 3.48-3.53 (m, 2H), 5.13-5.17 (m, 1H), 6.70 (bs, 2H), 7.53 (s, 1H), 8.30 (s, 1H). **ESI-MS (m/z)**: 422.35 $[M]^+$, 424.35 $[M+2]^+$.

Step VI: Tert-butyl 5-(4-amino-5-(4-(pyridin-2-ylcarbamoyl)phenyl)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate **(59)**.

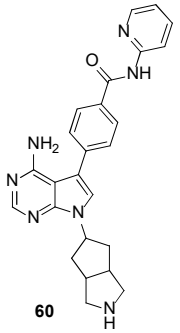


59

Following the procedure described in step V of Section 4.1.8., tert-butyl 5-(4-amino-5-(4-(pyridin-2-ylcarbamoyl)phenyl)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)hexahydrocyclopenta[c] pyrrole-2(1H)-carboxylate was synthesised from tert-butyl

5-(4-amino-5-bromo-7H-pyrrolo[2,3-d]pyrimidin-7-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate.

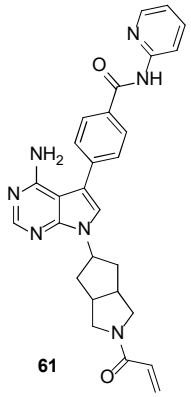
Step VII: 4-(4-amino-7-(octahydrocyclopenta[c]pyrrol-5-yl)-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-N-(pyridin-2-yl)benzamide **(60)**.



60

Following the procedure described in step VI of Section 4.1.4., 4-(4-amino-7-(octahydrocyclopenta[c]pyrrol-5-yl)-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-N-(pyridin-2-yl)benzamide was synthesised from tert-butyl 5-(4-amino-5-(4-(pyridin-2-ylcarbamoyl)phenyl)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)hexahydrocyclopenta[c] pyrrole-2(1H)-carboxylate.

4.1.10.1. 4-(7-(2-acryloyloctahydrocyclopenta[c]pyrrol-5-yl)-4-amino-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-N-(pyridin-2-yl)benzamide (61).



61

Following the procedure described in step VII of Section 4.1.4., **61** was synthesised from 4-(4-amino-7-(octahydrocyclopenta[c]pyrrol-5-yl)-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-N-(pyridin-2-yl)benzamide. **61** was isolated as a white solid with a 61% yield.

Purity by UPLC: 97.00%

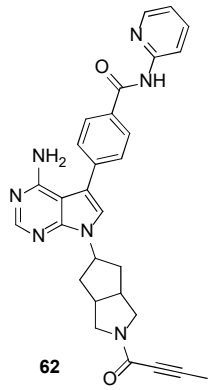
Melting point: 222-224 °C

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.06-2.07 (m, 2H), 2.27-2.32 (m, 2H), 2.96-3.06 (m, 2H), 3.27-3.28 (m, 1H), 3.49-3.53 (m, 1H), 3.65-3.70 (m, 1H), 3.79-3.84 (m, 1H), 5.26-5.30 (m, 1H), 5.67 (dd, *J* =10.0, 2.4 Hz, 1H), 6.13-6.17 (m, 3H), 6.63 (dd, *J* = 16.4, 10.0 Hz, 1H), 7.15-7.18 (m, 1H), 7.60-7.63 (m, 3H), 7.83-7.87 (m, 1H), 8.13-8.16 (m, 3H), 8.21 (d, *J* = 8.4 Hz, 1H), 8.39-8.40 (m, 1H), 10.76 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{28}H_{27}N_7O_2$: C, 68.14; H, 5.51; N, 19.87; found: C, 68.28; H, 5.55; N, 19.86;

ESI-MS (m/z): 494.15 $[M+H]^+$

4.1.10.2. 4-(4-amino-7-(2-(but-2-ynoyl)octahydrocyclopenta[c]pyrrol-5-yl)-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-N-(pyridin-2-yl)benzamide (62).



62

Following the procedure described in Section 4.1.7, **62b** was synthesised from 4-(4-amino-7-(octahydrocyclopenta[c]pyrrol-5-yl)-7H-pyrrolo[2,3-d]pyrimidin-5-yl)-N-(pyridin-2-yl)benzamide and but-2-ynoic acid. **62b** was isolated as a white solid with a 49% yield.

Purity by UPLC: 96.62%

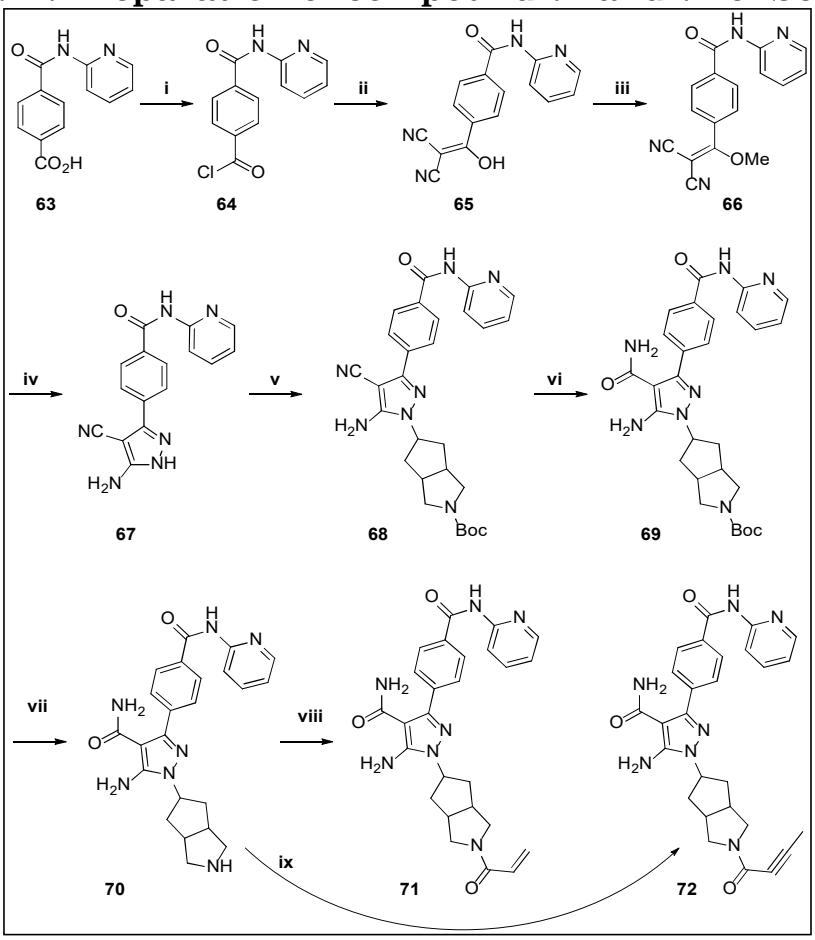
Melting point: 233-235 ^{o}C

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.03 (s, 3H), 2.25-2.27 (m, 2H), 2.35-2.41 (m, 2H), 3.02-3.07 (m, 2H), 3.50-3.55 (m, 2H), 3.87-3.93 (m, 2H), 5.26-5.29 (m, 1H), 6.32 (bs, 2H), 7.17-7.20 (m, 1H), 7.79 (d, *J* =8.4 Hz, 2H), 7.86-7.87 (m, 1H), 8.18-8.23 (m, 3H), 8.26 (s, 1H), 8.40-8.41 (m, 1H), 10.27 (s, 1H).

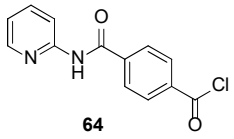
Elemental (CHNS) analysis: Calculated for $C_{29}H_{27}N_7O_2$: C, 68.89; H, 5.38; N, 19.39; found: C, 69.03; H, 5.26; N, 19.41;

ESI-MS (m/z): 506.15 $[M+H]^+$

4.1.11. Preparation of compound 71 and 72 of Series 4

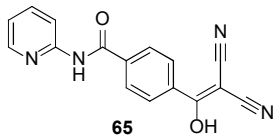


Step I: 4-(pyridin-2-ylcarbamoyl)benzoyl chloride **(64)**.



Thionyl chloride (9.94 mL, 136 mmol) was added to 4-(pyridin-2-ylcarbamoyl)benzoic acid (3 g, 12.38 mmol), and the resulting slurry was heated under reflux for 4 hours. The resulting solution was cooled to room temperature, and thionyl chloride was removed under reduced pressure to give 4-(pyridin-2-ylcarbamoyl)benzoyl chloride (3.2 g, 12.28 mmol, 99% yield) as a brown oil that was used immediately in the next step without purification.

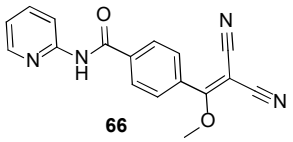
Step II: 4-(2,2-dicyano-1-hydroxyvinyl)-N-(pyridin-2-yl)benzamide **(65)**.



65

4-(pyridin-2-ylcarbamoyl)benzoyl chloride (3.2 g, 12.28 mmol) was dissolved in toluene (32 mL) and tetrahydrofuran (6.4 mL). Malononitrile (0.750 mL, 13.50 mmol) was added, and the solution was stirred at -10 °C. Diisopropylethylamine (3.22 mL, 18.41 mmol) was added to the reaction mixture while maintaining the temperature below 0 °C. After 1 hour at 0 °C, the mixture is stirred at room temperature for 18 hours. Amine hydrochloride is removed by filtration, and the filtrate evaporates *in vacuo*. The residue was taken up in ethyl acetate (100 mL) and washed with 1 M aqueous hydrochloric acid (50 mL), then with brine (50 mL), and dried over sodium sulphate. Evaporation of the solvents gives 4-(2,2-dicyano-1-hydroxyvinyl)-N-(pyridin-2-yl)benzamide (3.3 g, 11.37 mmol, 93% yield).

Step III: 4-(2,2-dicyano-1-methoxyvinyl)-N-(pyridin-2-yl)benzamide **(66)**.

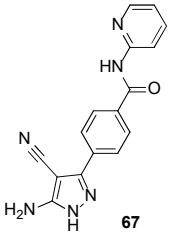


66

4-(2,2-dicyano-1-hydroxyvinyl)-N-(pyridin-2-yl)benzamide (3 g, 10.33 mmol) in acetonitrile (42 mL) and methanol (4.50 mL) is stirred under nitrogen at 0 °C while adding diisopropylethylamine (2.53 mL, 14.47 mmol) followed by a 2 M (Trimethylsilyl)diazomethane solution (7.23 mL, 14.47 mmol) in diethyl ether. The reaction is stirred for 24 hours at room temperature, and then 2 g of silica is added. The brown-red solution is evaporated *in vacuo*, and the residue is dissolved in ethyl acetate (100 mL) and washed well with water (50 mL), then brine (50 mL), dried over sodium sulphate, and concentrated. The residue was purified by flash chromatography to give

4-(2,2-dicyano-1-methoxyvinyl)-N-(pyridin-2-yl)benzamide (2.4 g, 7.89 mmol, 76% yield).

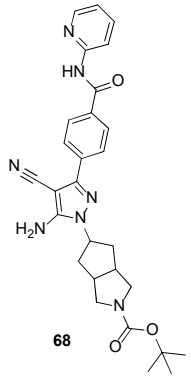
Step IV: 4-(5-amino-4-cyano-1H-pyrazol-3-yl)-N-(pyridin-2-yl)benzamide **(67)**.



67

4-(2,2-dicyano-1-methoxyvinyl)-N-(pyridin-2-yl)benzamide (2.3 g, 7.56 mmol) was treated with a solution of hydrazine hydrate (0.995 mL, 20.41 mmol) in ethanol (2.5 mL) and heated for 2 hours at 100 ºC. The mixture was poured into ice water (50 mL), and the precipitate was filtered, washed with water (2 x 25 mL), and dried in a vacuum to afford 4-(5-amino-4-cyano-1H-pyrazol-3-yl)-N-(pyridin-2-yl)benzamide as an orange solid (1.8 g, 5.91 mmol, 78% yield).

Step V: Tert-butyl 5-(5-amino-4-cyano-3-(4-(pyridin-2-ylcarbamoyl)phenyl)-1H-pyrazol-1-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate **(68)**.

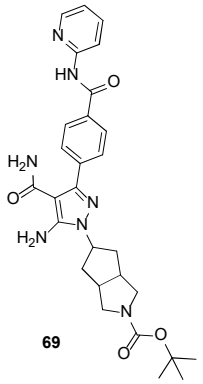


68

4-(5-amino-4-cyano-1H-pyrazol-3-yl)-N-(pyridin-2-yl)benzamide (1.5 g, 4.93 mmol) was dissolved in N,N-dimethylformamide (22.5 mL), and cesium carbonate (4.02 g, 12.32 mmol) was added in one lot under nitrogen atmosphere. After stirring the reaction mixture for 30 minutes, tert-butyl 5-

((methylsulfonyl)oxy)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate (1.445 g, 4.73 mmol) was added to it at room temperature. The reaction mixture was heated at 90 ºC for 18 hours. The mixture was poured into ice water (125 mL), and the precipitate was filtered, washed with water (3 x 25 mL), and dried in vacuum to afford tert-butyl 5-(5-amino-4-cyano-3-(4-(pyridin-2-ylcarbamoyl)phenyl)-1H-pyrazol-1-yl)hexahydrocyclopenta[c] pyrrole-2(1H)-carboxylate as a white solid (1.6 g, 3.12 mmol, 63% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ ppm 1.40 (s, 1H), 1.88-1.90 (m, 2H), 2.15-2.19 (m, 2H), 2.88-2.90 (m, 2H), 3.05-3.09 (m, 2H), 3.49-3.54 (m, 2H), 4.87-4.90 (m, 1H), 6.82 (bs, 2H), 7.17-7.20 (m, 1H), 7.62 (d, *J* = 8.4 Hz, 2H), 7.84-7.88 (m, 1H), 8.07 (d, *J* = 8.4 Hz, 2H), 8.19 (d, *J* = 8.4 Hz, 2H), 8.40-8.41 (m, 1H), 10.95 (s, 1H). **ESI-MS (m/z)**: 514.60 $[M+H]^+$.

Step VI: Tert-butyl 5-(5-amino-4-carbamoyl-3-(4-(pyridin-2-ylcarbamoyl)phenyl)-1H-pyrazol-1-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate **(69)**.

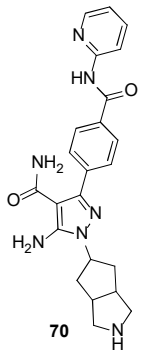


69

To a solution of tert-butyl 5-(5-amino-4-cyano-3-(4-(pyridin-2-ylcarbamoyl)phenyl)-1H-pyrazol-1-yl)hexahydrocyclopenta[c]pyrrole-2(1H)-carboxylate (1.1 g, 2.142 mmol) in dimethyl sulfoxide (27.5 mL) and potassium carbonate (0.888 g, 6.43 mmol). 48% aqueous hydrogen peroxide (2.461 mL, 38.6 mmol) was dropwise added while maintaining the reaction temperature between 20 and 25 ºC. The cooling was removed, and the reaction mixture was stirred at ambient temperature for 18 hours. The mixture

was partitioned between water (125 mL) and ethyl acetate (125 mL), the organic phase was separated, dried with sodium sulphate, filtered, and concentrated *in vacuo*. The crude material was purified by flash chromatography to afford tert-butyl 5-(5-amino-4-carbamoyl-3-(4-(pyridin-2-ylcarbamoyl)phenyl)-1H-pyrazol-1-yl)hexahydrocyclopenta[c] pyrrole-2(1H)-carboxylate as a white solid (0.750 g, 1.411 mmol, 65.9% yield). 1.40 (s, 1H), 1.87-1.91 (m, 2H), 2.20-2.26 (m, 2H), 2.88-2.90 (m, 2H), 3.05-3.09 (m, 2H), 3.48-3.54 (m, 2H), 4.87-4.90 (m, 1H), 6.33 (bs, 2H), 7.17-7.20 (m, 1H), 7.62 (d, J = 8.4 Hz, 2H), 7.84-7.88 (m, 1H), 8.07 (d, J = 8.4 Hz, 2H), 8.19 (d, J = 8.4 Hz, 2H), 8.41-8.42 (m, 1H), 10.94 (s, 1H). **ESI-MS (m/z)**: 532.90 $[M+H]^+$.

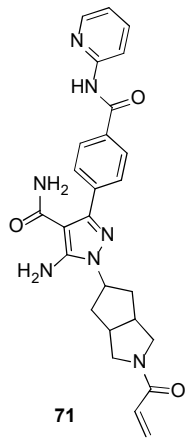
Step VII: 5-amino-1-(octahydrocyclopenta[c]pyrrol-5-yl)-3-(4-(pyridin-2-ylcarbamoyl)phenyl)-1H-pyrazole-4-carboxamide **(70)**.



70

Following the procedure described in step VI of Section 4.1.4., 5-amino-1-(octahydrocyclopenta[c]pyrrol-5-yl)-3-(4-(pyridin-2-ylcarbamoyl)phenyl)-1H-pyrazole-4-carboxamide was synthesised from tert-butyl 5-(5-amino-4-carbamoyl-3-(4-(pyridin-2-ylcarbamoyl)phenyl)-1H-pyrazol-1-yl)hexahydrocyclopenta[c] pyrrole-2(1H)-carboxylate.

4.1.11.1. 1-(2-acryloyloctahydrocyclopenta[c]pyrrol-5-yl)-5-amino-3-(4-(pyridin-2-ylcarbamoyl)phenyl)-1H-pyrazole-4-carboxamide (71).



71

Following the procedure described in step VII of Section 4.1.4., **71** was synthesised from 5-amino-1-(octahydrocyclopenta[c]pyrrol-5-yl)-3-(4-(pyridin-2-ylcarbamoyl)phenyl)-1H-pyrazole-4-carboxamide. **71** was isolated as a white solid with a 42% yield.

Purity by UPLC: 98.38%

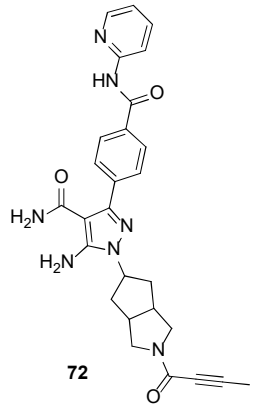
Melting point: 236-238 ºC

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.90-1.94 (m, 2H), 2.20-2.25 (m, 2H), 2.96-3.06 (m, 2H), 3.27-3.45 (m, 2H), 3.64-3.69 (m, 1H), 3.79-3.82 (m, 1H), 4.86-4.88 (m, 1H), 5.67 (dd, *J* =10.4, 2.4 Hz, 1H), 6.13 (dd, *J* = 16.8, 2.4 Hz, 1H), 6.32 (bs, 2H), 6.61 (dd, *J* = 16.4, 10.4 Hz, 1H), 7.15-7.18 (m, 1H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.83-7.87 (m, 1H), 8.06 (d, *J* = 8.4 Hz, 2H), 8.20 (d, *J* = 8.4 Hz, 2H), 8.38-8.40 (m, 1H), 10.81 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{26}H_{27}N_7O_3$: C, 64.32; H, 5.61; N, 20.19; found: C, 64.44; H, 5.63; N, 20.25;

ESI-MS (m/z): 486.90 $[M+H]^+$

4.1.11.2. 5-amino-1-(2-(but-2-ynoyl)octahydrocyclopenta[c]pyrrol-5-yl)-3-(4-(pyridin-2-ylcarbamoyl)phenyl)-1H-pyrazole-4-carboxamide (72).



72

Following the procedure described in Section 4.1.7., **72** was synthesised from 5-amino-1-(octahydrocyclopenta[c]pyrrol-5-yl)-3-(4-(pyridin-2-ylcarbamoyl)phenyl)-1H-pyrazole-4-carboxamid and but-2-ynoic acid. **72** was isolated as a white solid with a 50% yield.

Purity by UPLC: 98.35%

Melting point: 245-247 ºC

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.86-1.90 (m, 2H), 2.00 (s, 3H), 2.16-2.21 (m, 2H), 2.35-2.41 (m, 2H), 2.90-2.92 (m, 2H), 3.19-3.21 (m, 1H), 3.32-3.45 (m, 1H), 3.59-3.62 (m, 1H), 3.80-3.82 (m, 1H), 4.83-4.85 (m, 1H), 6.32 (bs, 2H), 7.17-7.19 (m, 1H), 7.62 (d, J = 8.4 Hz, 2H), 7.85-7.88 (m, 1H), 8.06 (d, J = 8.4 Hz, 2H), 8.20 (d, J = 8.4 Hz, 2H), 8.38-8.40 (m, 1H), 10.81 (s, 1H).

Elemental (CHNS) analysis: Calculated for $C_{27}H_{27}N_7O_3$: C, 65.18; H, 5.47; N, 19.71; found: C, 65.24; H, 5.46; N, 19.80;

ESI-MS (m/z): 498.35 $[M+H]^+$

4.2. Biology

4.2.1. BTK enzyme inhibition assay and kinase selectivity assay

The enzymatic activities of the tested compounds were assessed in a cell-free enzyme assay. Briefly, a fixed amount of recombinant purified human BTK (3 ng/reaction) was incubated with increasing concentrations of test compounds (0.01 nmol/L to10 µmol/L) in 1X kinase reaction buffer (40 mmol/L Tris-Cl, pH 7.5, 20 mmol/L $MgCl_2$, 2 mmol/L $MnCl_2$, 0.1 mg/mL BSA, and 50 µmol/L DTT). The enzymatic reaction was initiated by adding a substrate cocktail containing 50 µmol/L of ATP (final concentration) and 5 µg of polyGln4Tyr1 in 96-well plates. The reaction was incubated at room temperature for 2 hours, followed by quantification of the left-over ATP according to the manufacturer's protocol using the ADP-Glo reagent. Data were plotted using 'enzyme with no inhibitor' as 100% kinase activity. All the experiments were performed in duplicate. The IC_{50} values were calculated using linear regression analysis **[127]**.

The kinase selectivity of **32b** was conducted at Proquin ASE (GmbH). A radiometric protein kinase assay was used in 96-well Flash Plates ^{TM} from Perkin Elmer.

4.2.2. TMD8 cell anti proliferation assay

TMD8 cells were routinely grown in RPMI-1640 with 10% FBS and supplemented with 55 µmol/L β-mercaptoethanol (β-ME). For the cytotoxicity assay, defined numbers of cells were incubated in 96-well plates with increasing concentrations of test compounds (0.01 nmol/L to10 µmol/L) formulated in 100% DMSO (the final concentration of DMSO in the well is 0.2%) for 96 hours. Cell growth was measured using the MTT assay, and IC_{50} values were determined by nonlinear regression using the Graph Pad Prism 6 software **[128]**.

4.2.3. CYP inhibition assay

For CYP1A2, CYP2C8, CYP2C9, CYP2D6, CYP2C19, and CYP3A4 inhibition studies, human liver microsomes (0.2 mg/mL), Testosterone (50 μM) / Dextromethorphan (5 μM) respectively, were used as probe substrates, and 0.1 molar potassium phosphate buffer of pH 7.4 and 1 molar NADPH were incubated with different concentrations of test compounds (10 μM concentration) at 37ºC for 10 minutes. % Enzyme inhibition was determined with respect to the positive control **[122]**.

4.2.4. hERG inhibition assay

The Kv11.1 channel, a potassium ion channel encoded by the human ether-à-go-go-related (hERG) gene (thus also referred to as hERG channel), plays a very critical role in the cardiac action potential repolarization. Inhibition of hERG function can cause cardiotoxicities.

In this assay, CHO-hERG cells are used to specifically assess the effect of test compounds on the hERG channel. Pre-compound current and post-compound current are measured by patch clamp, and applied to the calculation of hERG inhibition as reported by Timm Danker **[123]**.

4.2.5. Pharmacokinetic studies

In vivo PK studies of target compounds and IBR were performed in male BALB/c mice and male Wister rats using a parallel study design (n = 3 per group). The oral dose was administered via gavage under an overnight fasted condition, and the intravenous dose was administered as a bolus via tail vein injection under a non-fasted condition. The oral dosing in either mice or rats was performed by a homogenous suspension formulation prepared with 1% Tween-80 and 0.5% methylcellulose in purified water.

The intravenous solution was prepared with 10% NMP, 5% ethanol, and 85% citric acid in purified water. Blood samples were collected serially from each animal at 0 h (pre-dose), 0.25, 0.5, 1, 2, 4, 6, 8, 24, 48, and 72 h post-dose. The blood samples were centrifuged to obtain plasma samples, which were stored below −70 °C. The concentrations of compounds in plasma were determined by LC-MS/MS (Shimadzu LC10AD, USA), using an YMC hydrosphere C18 (2.0 × 50 mm, 3 μM) column (YMC Inc., USA). PK parameters were derived using the non-compartmental analysis (NCA) module of Win Non Lin® software **[129]**.

4.2.6. *In vivo* efficacy studies

The *in vivo* efficacy studies were carried out in rats and mice. All animals were quarantined in the animal house of Zydus Research Centre for a seven-day period with a 12-hour dark/light cycle. During this period, the animals had free access to standard pellet feed and water ad libitum. The experiment protocols were approved by the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA), Government of India, and the Institutional Animal Ethics Committee (IAEC), Zydus Research Centre.

4.2.6.1. Protocol for TMD8 xenograft model studies

The xenograft studies were conducted in 6 to 8-week-old SCID mice. A total of 10×10^6 TMD-8 cells were suspended in 200 μL of phosphate buffer saline and subcutaneously injected into the flank. When tumours were palpable, animals were grouped so that the average tumour volume was around 100 mm^3. They were assigned to four groups: vehicle and three groups for 32b treatment (1.5, 3, and 15 mg/kg, BID), and treatment was continued for 20 days. The length and width of the tumour were

measured using a digital calliper, and the volume of the tumour was calculated using the formula: length × (width) ^{2}/2 **[130]**.

4.2.6.2. Protocol for CIA model studies

BA1/J male mice, 8–10 weeks old, were immunised on days 0 and 21 for induction of arthritis with bovine type II collagen via intradermal injection at the base of the tail. Injection volumes were 0.1 mL aliquots, consisting of a 1:1 (v/v) emulsion of Mycobacterium tuberculosis (2 mg/mL in mineral oil) and bovine type II collagen (2 mg/mL in 10 mmol/L acetic acid). **32b** (0.5 mg/kg) and IBR (0.6 mg/kg) were administered orally once a day for 4 weeks. Clinical scores, an index of arthritis, were assessed according to Scales HE et al. **[126]**, using the following criteria: 0, normal with no swelling or redness; 1, swelling and/or redness of the paw or one joint; 2, swelling in two or more joints; 3, gross swelling of the paw with more than two joints involved; and 4, severe arthritis of the entire paw and joints **[131]**.

4.2.7. Caco-2 permeability assay

The Caco-2 cell line, which was developed from a human colon cancer cell line, is frequently employed as an *in vitro* model for the assessment of intestinal drug permeability and absorption **[124]**. After 21 days of incubation, the cells were implanted in Trans wells (Millipore, 0.4 μM pore size) and produced a confluent monolayer. The test compound **32b** (50 μM) was introduced to the apical side of the membrane on day 21, and the concentration of the compound throughout the Caco-2 cell monolayer was assessed by UPLC after 1 hour of incubation at 37 °C. Transepithelial electrical resistance (TEER) should be measured before and after transport studies, and it should be greater than 500Ω × cm^{2}. The apparent permeability coefficient (Papp) of the compound was estimated using the following equation: (Papp

= (dQ/dt)/ (C_0A)). Where dQ/dt represents the rate of drug permeability across the cells, C_0 represents the initial concentration, and A represents the area of the cell monolayer.

4.2.8. Liver microsome stability assay

Mouse, rat, dog, and human liver microsome stability assay was carried out as specified **[125]**. In a nutshell, the mouse liver microsomal incubation contained microsomal protein (0.5 mg/mL), the lead compound **32b** (10 μmol/L), Tris-HCl buffer, and a NADPH-generating apparatus. At 37 °C for 30 minutes, duplicate incubations were conducted. The addition of the NADPH-generating apparatus triggered the reactions, which were subsequently terminated by adding a proportionate quantity of ice-cold acetonitrile bearing an internal standard. Centrifugation of the mixtures was followed by LC-MS/MS analysis of the supernatants. As a negative control, inactive liver microsomes were incubated.

4.2.9. Plasma protein binding

Equilibrium dialysis was utilised for quantifying the magnitude of plasma protein binding for the tested substance. Human plasma that had been warmed to 37 °C and combined with test compound (2.5 μM). 200 μL of the tested compound plasma solution (2.5 μM) was placed in the red compartment and 350 μL of phosphate buffer in the buffer compartment to generate a dialysis plate. Samples were taken from each compartment for LC-MS/MS analysis following a five-hour incubation period at 37 °C and 100 rpm on an orbital shaker **[132]**.

4.2.10. Acute toxicity studies

To assess the safety profile of **32b**, repeat-dose acute toxicity studies (14 days) were carried out in male Wistar rats **[133]**. Oral doses were administered to groups of five male and female rats. Once day for 14 days, at doses of 50, 100, and 300 mg/kg of

compound **32b**. Considering that the ED_{50} dose was 3 mg/kg as determined by the CIA model studies, these doses are 16x, 33x, and 100x of the ED_{50}, respectively. In order to determine whether there were any toxic effects that were delayed, persistent, or recovered, two groups—the high-dose group and the vehicle control group—were held for a 2-week recovery period.

The following parameters and observations were undertaken during the period of study:

Mortality and Clinical Signs: Twice daily, a mortality check was performed. On a daily basis, cage-side observation was carried out.

Ophthalmic Examination: During the last week of treatment and the last week of recovery, an ophthalmological examination was conducted with an ophthalmoscope.

Body Weight: For the purpose of weight stratification, body weights were taken at both the time of reception and before the animals were randomly assigned to dose groups. On day 1 (prior to dosing), weights were taken, and then every week until the termination of the treatment and the recovery period. Organ-body weight ratios were calculated using fasted body weights on the day of necropsy.

Feed Consumption: From the start of the treatment phase to the end of the recovery period, the amount of feed consumed was monitored every week.

Neurobehavioral Observations: Animals from the control and high dose groups underwent neurobehavioral observations after the modified Irwin test towards the completion of the treatment and the recovery period.

Clinical Pathology: At the point of completion of the treatment and recovery phases, clinical pathology examinations were done on all of the rats that had survived.

Haematology: The following haematological parameters were determined using an automated haematology analyzer (Cell-Dyn 3700).

Total Leukocyte Count (WBC), Erythrocyte Count (RBC), Platelet Count (PLT), Hematocrit (HCT), Haemoglobin Concentration (HGB), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Reticulocyte Count (RETIC), Absolute Differential Leukocyte Count, Monocyte, Eosinophil, Basophil, Neutrophil, Lymphocyte.

Clinical Chemistry: The following clinical chemistry parameters were determined using automated biochemical analyzer (Daytona).

Glucose (GLU), Urea, Triglycerides (TG), Creatinine (CREA), Total Cholesterol (TCHO), Total Bilirubin (TBIL), Aspartate aminotransferase (AST), Calcium (Ca), Alanine aminotransferase (ALT), Inorganic Phosphorus (Phos), Alkaline Phosphatase (ALP), Total Protein (TP), Albumin (ALB), Sodium (Na^+), Potassium (K^+), Chloride (Cl^-).

Terminal Procedures: Prior to euthanasia, all of the animals that survived fasted for the whole night. At the conclusion of the treatment and recovery periods, necropsies were done on all surviving animals. The animals were asphyxiated with carbon dioxide (CO_2) and then exsanguinated to end their lives.

Necropsy Procedures: During necropsy, the animals were visually inspected for any external abnormalities. The organs were pulled out, examined, and weighed, while the abdominal, thoracic, and cerebral chambers were examined for discrepancies. Organ and body weight ratios were calculated by weighing various organs, including the

adrenals, brain, heart, lung, liver, testicles, epididymides, spleen, thymus, ovary, uterus, and kidneys.

Statistical Analysis: The Graph Pad Prism programme was used to conduct statistical analysis. By using the appropriate statistical test, normality within groups and homogeneity of variances between groups were investigated, respectively. One-way ANOVA was employed for more than two groups to examine the equality of group means. For the pairwise comparison of groups, the appropriate post-hoc test was conducted. The Student's t-test was used for two groups to compare the equality of group means. The 5% and 1% levels of significance were adopted to assess all analyses and comparisons.

4.2.11. Molecular docking protocol

To explain the potent and selective BTK enzyme inhibitory activity of designed molecules, docking studies were carried out using CovDock **[120]**, a covalent docking program developed by Schrodinger. This mimics the multi-step binding process of covalent modifiers by simulating both pre- and post-reactive states. The geometry of compounds to be docked was subsequently optimized using the Ligprep **[121]**. The scoring function, binding mode and H-bonds were used to assess the binding affinity of the compounds. The BTK enzyme crystal structure was retrieved from the RCSB Protein Data Bank (PDB ID: 5P9M). The active site was defined to include residues within 5 A^{o} to any of the IBR atoms. All these molecules (IBR, **24e**, **32b** and **32ao**) use an electrophilic warhead to covalently react with Cys481.