ELECTRONIC SUPPORTING INFORMATION

Ligand-Centred Phenotype-Driven Development of Potent Kinase Inhibitors against Oesophageal Cancer

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List of Contents

1. General Chemistry Methods

All the experiments of this work were performed in a Biotage® Initiator Third Generation microwave synthesiser or they were performed on a heating magnetic stirrer with temperature sensor, using commercially available anhydrous solvents. Chemicals that were commercially available were purchased from different suppliers, including Acros Organics, Alfa Aesar, Fisher Scientific, Fluorochem, Sigma Aldrich and VWR International. For the analysis of compounds during reactions, thin-layer chromatography (TLC) was used, using Merck TLC Silica gel 60 F254 plates, that were cut to be approximately 5 cm x 10 cm. Detection of the spots was done using a potassium permanganate dip and drying using a heat gun, or by visualization using UV light at 254 nm using a UV-plate reader. Compound purification was performed through one of three techniques. Flash column chromatography using 220-240 mesh silica gel, purchased from Sigma Aldrich, and commercially available solvents, was carried out in a glass column fitted with frit and PET tap. Preparative-TLC was also used to purify final compounds. Two different silica TLC plates were used: Analtech Uniplate 1000 µm Silica TLC plates on glass, for crude mixtures of 100-200 mg, or Merck Millipore TLC Silica gel 60 F254 plates, for up to 50 mg of crude product.

NMR spectra were obtained at ambient temperature on 500 MHz, 400 MHz or 600MHz Bruker spectrometers. Samples were dissolved in deuterated solvents commercially available from Sigma-Aldrich, VWR or Fluorochem and placed into glass Norrell s400 NMR tubes. 1H NMR spectra: chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane. The data is presented as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants (in Hertz, Hz) and interpretation. 13C NMR spectra were referenced to the solvent carbon peak. The data is presented as follows: chemical shift and assignment; and was analysed using MestReNova.

The compounds used in the biological experiments were determined to be >90% pure by analytical HPLC with evaporative light scattering detection (Agilent). Low resolution mass (LRMS) and purity values were obtained using an Agilent 1260 Infinity II LC-MS system equipped with a SQ-MS with an API Electrospray Source and an ELSD. Data from this device was analysed using Agilent data analysis software. High resolution mass spectra (HRMS) were obtained for the compounds that underwent further evaluation using a Bruker 12T SolariX FT-ICR-MS. Data for these compounds are reported, along with the calculated value for the relative ion and the molecular formula.

2. Synthesis and characterization of Compounds 2-12

Synthesis of 1*H*-Pyrazolo[3,4-*d*]pyrimidin-4-amine (2). 5-amino-1*H*-pyrazole-4-carbonitrile (**1,** commercially available from Fluorochem or Sigma Aldrich) (4.31 g, 39.96 mmol, 1 equiv) was mixed with formamide (15 mL, 443 mmol, 16 eq) in a microwave vial. The reaction was heated at 180 °C using microwave radiation for 75 min. Subsequently, a precipitate was formed and was collected by vacuum filtration and washed with water. This was then dried overnight at 40 °C in a vacuum oven to give a white solid (5.08 g, 37.60 mmol, 97 %).**^{1}H NMR** (500 MHz, DMSO-d_6) δ 13.31 (s, 1H), 8.13 (s, 1H), 8.07 (s, 1H), 7.57 (s, 2H). **^{13}C NMR** (126 MHz, DMSO) δ 157.9(C), 155.8 (CH), 154.7 (C), 132.6 (CH), 99.5 (C).

Synthesis of 3-Iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (3). 1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **(2)** (3g , 22.3 mmol) was suspended in 13 mL of DMF and *N*-iodosuccinimide (6 g, 26.2 mmol, 1.2 eq) was added. The reaction was heated at 180 °C using microwave radiation for 60 min. Upon removal from the microwave, ethanol (10 mL) was added dropwise to the mixture, and this was allowed to cool down for 1 hr. The precipitate was filtrated and washed with ethanol. The product was then dried overnight at 40 °C in a vacuum oven, to afford the desired product (2.60 g, 9.96 mmol, 45 %). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 13.79 (s, 1H), 8.17 (s, 1H), 7.80 – 6.39 (m, 2H). **^{13}C NMR** (151 MHz, DMSO) δ 157.5 (C), 156.0 (CH), 155.0 (C), 102.5 (C), 89.7 (C).

Synthesis of 1-(cyclopentylmethyl)-3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (4). 3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **(3)** (2.2 g, 0.269 mmol, 1 eq) was suspended in DMF (13 mL), and sodium hydride was added (506.6mg, 12.64 mmol, 1.5 eq, 60% dispersion in mineral oil), and the mixture stirred until gas evolution subsided. To this, iodomethyl cyclopentane (1.32 mL, 10.12 mmol, 1.2 eq) was added, and the mixture was heated at 150 °C using microwave irradiation for 85 min. Then, the mixture was partitioned between EtOAc and water. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified by silica gel flash chromatography using MeOH/DCM (0-3 %) to afford the desired product (3.25 mg, 9.47 mol, 56%). **^{1}H NMR** (500 MHz, DMSO-d_6) δ = 8.19 (s, 1H), 7.92 – 6.26 (m, 2H), 4.18 (d, *J*=7.5, 2H), 2.43 (dt, *J*=14.6, 7.3, 1H), 1.60 – 1.53 (m, 4H), 1.49 (dddd, *J*=9.2, 6.4, 4.6, 3.4, 2H), 1.28 – 1.22 (m, 2H).**^{13}C NMR** (126 MHz, DMSO) δ 157.7 (C), 156.0 (CH), 153.5 (C), 102.9 (C), 88.5 (C), 51.0 (CH_2),40.1 (CH) 29.5(2 x CH_2), 24.5 (2 x CH_2).

Synthesis of 6-chloro-3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidine (6). 6-chloro-1*H*-pyrazolo[3,4-*d*]pyrimidine **(5,** commercially available from Fluorochem**)** (1 g, 6.5 mmol) was suspended in DMF (14ml). To this, *N*-iodosuccinimide (1.8 g, 7.8 mmol, 1.2 equiv) was added. The reaction was heated at 120 °C using microwave radiation for 60 min. The reaction mixture was partitioned between water and EtOAc. The organic layers were combined and washed with water and brine, dried over anhydrous $MgSO_4$, and concentrated under vacuum. The crude product was purified by silica gel flash chromatography using MeOH/DCM (0-2%) to afford the desired product (853 mg, 0.003 mmol, 47%). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 14.64 (s, 1H), 9.02 (s, 1H). **^{13}C NMR** (126 MHz, DMSO) δ 157.8 (C), 155.8 (CH), 155.2 (C), 117.6 (C), 94.16. **LRMS** (*m*/*z*) $[M + H]^+$: 281.00.

Synthesis of 3-iodo-*N*-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (7). 6-chloro-3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidine **(6)** (500 mg, 1.78 mmol) was dissolved in THF (3 ml). Methylamine 2N in THF (12 ml) was added and then the mixture was heated at 150 °C using microwave radiation for 60 min. The precipitated was dried by vacuum filtration and washed with water, no further purification was performed (302.5 mg, 1.2 mmol, 62 %). **^{1}H NMR** (500 MHz, DMSO-d_6) δ = 13.28 (s, 1H), 8.45 (s, 1H), 7.53 (s, 1H), 2.82 (d, *J*=4.6, 3H). **^{13}C NMR** (126 MHz, DMSO) δ 162.7 (C), 157.4 (C), 156.8 (CH), 153.9 (C), 93.3 (C), 28.4 (CH_3).

Synthesis of 1-(cyclopentylmethyl)-3-iodo-*N*-methyl-1*H*pyrazolo[3,4-*d*] pyrimidin-6-amine (8). 3-iodo-*N*-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine **(7)** (303 mg, 1.10 mmol, 1 equiv), was suspended in DMF (4 ml), and sodium hydride

was added (37 mg, 1.54 equivalents, 1.4 mmol 60% dispersion in mineral oil). The mixture was stirred until gas evolution subsided, and then (iodomethyl)cyclopentane (0.216 ml, 1.7 mmol 1.5 equiv) was added. The mixture was heated at 150 °C using microwave radiation for 85 min. Upon cooling, EtOAc and water were added to the mixture, and the organic layers separated. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The crude product was purified by silica gel flash chromatography using MeOH/DCM (0−5%) (327 mg, 0.92 mmol, 83.3 %). **^{1}H NMR** (500 MHz, DMSO-*d*$_6$) δ 8.43 (s, 1H), 7.60 (s, 1H), 4.13 – 4.07 (m, 2H), 2.85 (d, J = 4.6 Hz, 3H), 2.47 – 2.41 (m, 1H), 1.58 (d, *J* = 10.9 Hz, 4H), 1.48 (dq, *J* = 6.0, 1.9 Hz, 2H), 1.27 (dd, *J* = 12.8, 6.8 Hz, 2H). **^{13}C NMR** (126 MHz, DMSO) δ 162.5 (C), 155.8 (C), 154.2 (CH), 124.0 (C), 92.3 (C), 50.7 (CH2), 31.1 (CH), 30.0 (2 x CH_2), 28.4 (CH3), 24.9 (2 x CH_2). **LRMS** (*m*/*z*) [M + H]$^+$: 358.10.

Synthesis of methyl (2*E*)-3-[4-Amino-1-(cyclopentylmethyl)-1*H*-pyrazolo[3,4-*d*] pyrimidin-3-yl]prop-2-enoate (9). To a solution of 1-(cyclopentylmethyl)-3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **(4)** (1.7 g, 4.95 mmol, 1 equiv) in DMF/water/TEA (3.0 mL/2.86 mL/2.86 mL) was added TBAI (3.66 g, 9.9 mmol, 2 equiv), methyl acrylate (4.5 ml, 49.5 mmol, 10 equiv), and $Pd(PPh_3)_2Cl_2$ (700 mg, 0.996 mmol, 0.2 eq). The reaction vial was flushed with N_2 for 10 minutes and then heated at 80 °C for 24 hours. Upon cooling to r.t, the mixture was partitioned between water and DCM. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered through a plug of Celite ® and concentrated under vacuum. The crude product was purified by silica gel flash chromatography (EtOAc/Hexane 0-70 %) to afford the desired product (761.4 mg, 2.53 mmol, 51%). **^{1}H NMR** (601 MHz, Methanol-*d*$_4$) δ 8.21 (s, 1H), 8.00 (d, *J* = 15.7 Hz, 1H), 6.80 (d, *J* = 15.7 Hz, 1H), 4.31 (d, *J* = 7.6 Hz, 2H), 3.83 (s, 3H), 2.57 (p, *J* = 7.5 Hz, 1H), 1.71 – 1.65 (m, 4H), 1.60 – 1.55 (m, 2H), 1.39 – 1.32 (m, 2H).**^{13}C NMR** (151 MHz, MeOD) δ 168.8 (C), 160.0 (C), 156.7 (C), 155.6 (CH), 140.1 (C), 135.0 (CH), 122.0 (CH), 100.3 (C), 53.0 (CH_2), 52.4 (CH_3), 41.4 (CH), 31.0 (2 x CH_2), 26.0 (2 x CH_2). **LRMS** (*m*/*z*) [M + H]$^+$: 302.30.

Synthesis of (2*E*)-3-[4-Amino-1-(cyclopentylmethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoic acid (10). Methyl (2*E*)-3-[4-amino-1-(cyclopentylmethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoate **(9)** (450 mg, 1.52 mmol, 1 equiv) was dissolved in THF (18 mL) and 6M $NaOH_{aq}$ solution (1.26 ml, 7.6 mmol, 5 equiv) was added. The reaction was heated at 60°C for 24 hours. Then, the solvent was evaporated under reduced pressure, the residue was partitioned between water/EtOAc (1:1) and acidified (pH 4) with 1M HCl. The product was extracted with EtOAc, and the combined organic layers were concentrated under vacuum. The product was allowed to dry in an oven at 40 °C overnight, no further purification was performed (500 mg, 1.74 mmol, 100%).**^{1}H NMR** (601 MHz, DMSO-*d*$_6$) δ 12.47 (s,1H), 8.19 (s, 1H), 7.97 (d, *J* = 15.6 Hz, 1H), 7.53 (s, 2H), 6.64 (d, *J* = 15.6 Hz, 1H), 4.23 (d, *J* = 7.5 Hz, 2H), 2.49 – 2.43 (m, 1H), 1.62 – 1.53 (m, 4H), 1.52 – 1.44 (m, 2H), 1.34 – 1.24 (m, 2H). **^{13}C NMR** (151 MHz, DMSO) δ 167.3 (C), 158.1 (C), 155.7 (C), 154.5 (CH), 138.2 (C), 133.3 (CH), 122.2 (CH), 98.5 (C), 51.0 (CH_2), 40.1 (CH), 29.6 (2 x CH_2), 24.5 (2 x CH_2). **LRMS** (*m*/*z*) [M + H]$^+$: 288.30

Synthesis of methyl (2*E*)-3-[1-(cyclopentylmethyl)-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoate (11). To a solution of 1-(cyclopentylmethyl)-3-iodo-*N*-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine **(8)** (315.1 mg, 0.992 mmol, 1 equiv) in DMF/water/TEA (14.07 mL/1.48 mL/1.48mL) at r.t were added TBAI (651.57 mg, 1.76 mmol, 2 equiv), methyl acrylate (0.794 ml, 8.82 mmol, 10 equiv) and $Pd(PPh_3)_2Cl_2$ (123.82mg, 0.176 mmol, 0.2 eq). The reaction vial was flushed with N_2 for 10 minutes and then heated at 80 °C for 24 hours. After cooling to r.t, the mixture was extracted with DCM. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered through a plug of Celite ® and concentrated under vacuum. The crude product was purified by silica gel flash chromatography using EtOAc/Hexane (0-50 %) to afford the desired product (203 mg, 0.644 mmol, 73 %). **^{1}H NMR** (500 MHz, Methanol-*d*$_4$) δ 8.97 (s, 1H), 7.72 (d, *J* = 16.4 Hz, 1H), 6.73 (d, *J* = 16.3 Hz, 1H), 4.22 (d, *J* = 7.6 Hz, 2H), 3.82 (s, 3H), 2.99 (s, 3H), 2.57 (p, *J* = 7.4 Hz, 1H), 1.72 – 1.64 (m, 4H), 1.59 (dddd, *J* = 7.7, 5.7, 3.9, 2.5 Hz, 2H), 1.37 (ttd, *J* = 13.7, 6.5, 3.0 Hz, 2H). **^{13}C NMR** (126 MHz, MeOD) δ 168.6 (C), 163.1 (C), 157.7 (CH), 154.5 (C), 141.7 (C), 136.5 (CH), 121.9 (CH), 106.9 (C), 52.3 (CH_3), 52.0 (CH_2), 41.3 (CH), 31.1 (2 x CH_2), 28.5 (CH_3), 25.9 (2 x CH_2).

Synthesis of (2*E*)-3-[1-(cyclopentylmethyl)-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoic acid (12). To a solution of methyl (2*E*)-3-[1-(cyclopentylmethyl)-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoate **(11)** (203 mg, 0.644 mmol, 1 equiv) in THF (10 mL) 6M $NaOH_{aq}$ solution (0.540 mL, 3.22 mmol, 5 equiv) was added. The reaction was heated at 60°C for 24 hours. Then, the solvent was evaporated under reduced pressure; the residue obtained was dissolved in water/EtOAc (1:1) and acidified (pH 4) with 1M HCl. The product was extracted with

EtOAc, the combined organic layers were dried under vacuum. The product was allowed to dry in an oven at 40 °C overnight and no further purification was performed (82.1 mg, 0.272mmol, 42 %). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 8.97 (s, 1H), 7.68 (d, *J* = 16.3 Hz, 1H), 6.71 (d, *J* = 16.2 Hz, 1H), 4.22 (d, *J* = 7.5 Hz, 2H), 2.99 (s, 3H), 2.58 (p, *J* = 7.4 Hz, 1H), 1.73 – 1.65 (m, 4H), 1.59 (td, *J* = 8.6, 8.2, 3.2 Hz, 2H), 1.38 (tt, *J* = 12.7, 7.0 Hz, 2H). **^{13}C NMR** (126 MHz, MeOD) δ 170.0 (C),163.1 (C), 157.7 (CH), 154.4 (C), 141.9 (C), 136.3 (CH), 123.2 (CH), 106.7 (C), 51.9 (CH_2), 41.3 (CH), 31.1 (2 x CH_2), 28.5 (CH_3), 25.9 (2 x CH_2) . **LRMS** (*m*/*z*) $[M + H]^+$: 302.20

3. Synthesis and characterization of Compounds 1A 1-10

General Synthesis of Library 1A Compounds 1A 1-10

(2*E*)-3-[4-amino-1-(cyclopentylmethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoic acid **(10)** (0.015-0.038 mmol) was dissolved in anhydrous DMF (0.5 mL). To this, the appropriate secondary amine (4 eq.) and DIPEA (3 eq.) were then added. The resulting mixture was stirred for 5 min before adding HATU (1.2 eq.). The reaction vial was flushed with N_2, and the reaction stirred at r.t for 24 hours. The solvent was removed under vacuum and the residue partitioned between water and DCM. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The crude product was purified by silica gel TLC plate to afford the desired product.

Synthesis of (2*E*)-3-[4-Amino-1-(cyclopentylmethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]-1-(piperidin-1-yl)prop-2-en-1-one (1A1). The crude product was purified by silica gel TLC plate using MeOH/DCM (5%) to afford the desired product (5 mg, 0.0141 mmol, 94%). **1H NMR** (500 MHz, Methanol-d_4) δ 8.20 (s, 1H), 7.80 (d, *J* = 15.2 Hz, 1H), 7.39 (d, *J* = 15.2 Hz, 1H), 4.31 (d, *J* = 7.6 Hz, 2H), 3.70 (q, *J* = 6.4 Hz, 4H), 2.59 (p, *J* = 7.5 Hz, 1H), 1.75 – 1.72 (m, 2H), 1.70 – 1.61 (m, 8H), 1.60 – 1.55 (m, 2H), 1.38 – 1.32 (m, 2H).**13C NMR** (126 MHz, DMSO) δ 163.9 (C), 158.2 (C), 155.7 (C), 154.4 (CH), 139.1 (C), 130.7 (CH), 121.5 (CH), 98.3 (C), 51.0 (CH_2), 46.4 (2 x CH_2), 42.4 (CH), 29.6 (2 x CH_2), 25.4 (2 x CH_2), 24.6 (2 x CH_2), 24.1 (CH_2). **HRMS** (ESI, *m*/*z*) calcd for $C_{19}H_{27}N_6O_1$ $[M + H]^+$: 355.2241; found, 355.2254.

Synthesis (2*E*)-3-[4-Amino-1-(cyclopentylmethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]-1-(morpholin-4-yl)prop-2-en-1-one (1A2). The crude product was purified by silica gel TLC plate using MeOH/DCM (4%) to afford the desired product (6.2 mg, 0.0174 mmol, 46%). **1H NMR** (500 MHz, Methanol-d_4) δ 8.21 (s, 1H), 7.86 (d, *J* = 15.2 Hz, 1H), 7.38 (d, *J* = 15.2 Hz, 1H), 4.30 (d, *J* = 7.6 Hz, 2H), 3.75 (d, *J* = 13.2 Hz, 8H), 2.58 (p, *J* = 7.4 Hz, 1H), 1.71 – 1.64 (m, 4H), 1.60 – 1.56 (m, 2H), 1.35 (q, *J* = 6.3, 5.7 Hz, 2H). **13C NMR** (126 MHz, MeOD) δ 167.3 (C), 160.0 (C), 156.8 (C), 155.5 (CH), 140.8 (C), 132.9 (CH), 121.5 (CH), 100.4 (C), 68.0 (2 x CH_2), 53 (CH_2), 44.0 (2 x CH_2), 41.5 (CH), 30.7 (2 x CH_2), 26.0 (2 x CH_2). **HRMS** (ESI, *m*/*z*) calcd for $C_{18}H_{25}N_6O_2$ $[M + H]^+$: 357.2034; found, 357.2058.

Synthesis of (2*E*)-3-[4-Amino-1-(cyclopentylmethyl)-1*H*-pyrazolo[3,4-*d*] pyrimidin-3-yl]-1-(4-methylpiperidin-1-yl)prop-2-en-1-one (1A3). The crude product was purified by silica gel TLC plate using MeOH/DCM (10%) to afford the desired product (9 mg, 0.024 mmol, 70%). **1H NMR** (601 MHz, Methanol-d_4) δ 8.20 (s, 1H), 7.80 (d, *J* = 15.2 Hz, 1H), 7.39 (d, *J* = 15.2 Hz, 1H), 4.61 (ddt, *J* = 13.1, 4.7, 2.4 Hz, 1H), 4.30 (d, *J* = 7.6 Hz, 2H), 4.21 (ddd, *J* = 13.6, 4.3, 2.2 Hz, 1H), 3.24 – 3.18 (m, 1H), 2.79 (td, *J* = 12.8, 2.9 Hz, 1H), 2.58 (hept, *J* = 7.6 Hz, 1H), 1.79 (ddt, *J* = 27.5, 13.0, 2.6 Hz, 2H), 1.74 – 1.64 (m, 5H), 1.58 (dtd, *J* = 9.2, 7.6, 7.0, 4.9 Hz, 2H), 1.39 – 1.32 (m, 2H), 1.23 – 1.11 (m, 2H), 0.99 (d, *J* = 6.5 Hz, 3H). **13C NMR** (151 MHz, MeOD) δ 167.0 (C), 160.0 (C), 156.8 (C), 155.4 (CH), 141.0 (C), 132.3 (CH), 122.3 (CH), 100.3 (C), 53.0 (CH_2), 47.6 (2 x CH_2), 44.1 (CH), 36.0 (CH_2), 35.1 (CH_2), 32.2 (2 x CH_2), 31.0 (CH), 26.0 (2 x CH_2), 22.0 (CH_3). **HRMS** (ESI, m/z) calcd for C20H29N6O1 [M + H]+: 369.2397; found, 369.2403.

Synthesis of (2*E*)-3-[4-Amino-1-(cyclopentylmethyl)-1*H*-pyrazolo[3,4-*d*] pyrimidin-3-yl]-1-[3-(dimethylamino)piperidin-1-yl]prop-2-en-1-one (1A4). The crude product was purified by silica gel TLC plate using MeOH/DCM (10%) to afford the desired product (9.1 mg, 0.023 mmol, 66%). **1H NMR** (500 MHz, Methanol-d_4) δ 8.20 (s, 1H), 7.82 (d, *J* = 15.2 Hz, 1H), 7.39 (dd, *J* = 15.2, 8.3 Hz, 1H), 4.66 – 4.60 (m, 1H), 4.30 (d, *J* = 7.6 Hz, 2H), 4.11 – 4.05 (m, 1H), 3.28 – 3.14 (m, 1H), 2.96 (dd, *J* = 10.0, 6.4 Hz, 1H), 2.57 (dq, *J* = 13.8, 7.2 Hz, 1H), 2.45 (s, 3H), 2.40 (s, 2H), 2.36 (d, *J* = 15.4 Hz, 1H), 2.09 (dd, *J* = 11.6, 4.9 Hz, 1H), 1.95 – 1.85 (m, 1H), 1.71 – 1.64 (m, 5H), 1.60 – 1.55 (m, 3H), 1.38 – 1.32 (m, 2H).**13C NMR** (126 MHz, DMSO) δ 164.2 (C), 158.2 (C), 155.7 (C), 154.4 (CH), 139.1 (C), 131 (CH), 121.4 (CH), 98.4 (C), 60.6 (CH), 50.8 (CH_2), 48.3 (CH_2), 45.9 (CH_2), 44.0 (2 x CH_3), 41.6 (CH), 29.6 (2 x CH_2), 26.7 (CH_2), 24.6 (2 x CH_2), 23.7 (CH_2). **HRMS** (ESI, *m*/*z*) calcd for $C_{21}H_{32}N_7O_1$ $[M + H]^+$: 398.2663; found, 398.2671.

Synthesis of (2*E*)-3-[4-Amino-1-(cyclopentylmethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]-1-(4-hydroxypiperidin-1-yl)prop-2-en-1-one (1A5). The crude product was purified by silica gel TLC plate using MeOH/DCM (10%) to afford the

desired product (9.1 mg, 0.023 mmol, 70%).**1H NMR** (500 MHz, Methanol-d_4) δ 8.20 (s, 1H), 7.82 (d, *J* = 15.2 Hz, 1H), 7.41 (d, *J* = 15.2 Hz, 1H), 4.31 (d, *J* = 7.6 Hz, 2H), 4.21 – 4.15 (m, 1H), 4.04 (d, *J* = 13.4 Hz, 1H), 3.92 (tt, *J* = 8.1, 3.8 Hz, 1H), 3.51 – 3.45 (m, 1H), 2.59 (p, *J* = 7.5 Hz, 1H), 1.93 (d, *J* = 21.0 Hz, 2H), 1.68 (qd, *J* = 10.9, 10.0, 5.1 Hz, 4H), 1.60 – 1.56 (m, 2H), 1.53 (td, *J* = 9.1, 4.3 Hz, 2H), 1.36 (dd, *J* = 12.7, 6.2 Hz, 2H). **13C NMR** (126 MHz, DMSO) δ 164.0 (C), 158.2 (C), 155.6 (C), 154.4 (CH), 139.1 (C), 130.8 (CH), 121.4 (CH), 98.3 (C), 65.5 (CH), 51.0 (CH_2), 43.01 (2 x CH_2), 34.9 (CH), 33.9 (2 x CH_2), 29.6 (2 x CH_2), 24.6 (2 x CH_2). **HRMS** (ESI, *m*/*z*) calcd for $C_{19}H_{27}N_6O_2$ $[M + H]^+$: 371.2190; found, 371.2204.

Synthesis of (2*E*)-3-[4-Amino-1-(cyclopentylmethyl)-1*H*-pyrazolo[3,4-*d*] pyrimidin-3-yl]-1-(4-phenylpiperidin-1-yl)prop-2-en-1-one (1A6). The crude product was purified by silica gel TLC plate using MeOH/DCM (10%) to afford the desired product (8.3 mg, 0.019 mmol, 55%). **1H NMR** (601 MHz, DMSO-d_6) δ 8.19 (s, 1H), 7.84 (d, *J* = 15.1 Hz, 1H), 7.46 (s, 2H), 7.32 (d, *J* = 3.4 Hz, 1H), 7.31 – 7.29 (m, 2H), 7.27 – 7.24 (m, 2H), 7.21 – 7.18 (m, 1H), 4.67 (d, *J* = 13.0 Hz, 1H), 4.23 (d, *J* = 7.5 Hz, 2H), 4.19 (d, *J* = 14.1 Hz, 1H), 3.29 (s, 1H), 3.24 (t, *J* = 12.8 Hz, 1H), 2.85 – 2.79 (m, 1H), 2.79 – 2.72 (m, 1H), 1.86 (s, 2H), 1.67 – 1.55 (m, 6H), 1.53 – 1.45 (m, 2H), 1.35 – 1.25 (m, 2H).**13C NMR** (151 MHz, DMSO) δ 164.1 (C), 158.2 (C), 155.7 (C), 154.4 (CH), 145.6 (C), 139.1 (C), 130.8 (2 x CH), 128.4 (2 x CH), 126.7 (CH), 126.2 (CH), 121.6 (CH), 98.3 (C), 51.0 (CH_2), 46.0 (2 x CH_2), 42.1 (CH), 41.8 (CH), 33.6 (CH), 32.8 (CH), 29.6 (2 x CH_2), 24.6 (2 x CH_2). **HRMS** (ESI, *m*/*z*) calcd for $C_{25}H_{31}N_6O_1$ $[M + H]^+$: 431.2554; found, 431.2560.

Synthesis of (2*E*)-3-[4-Amino-1-(cyclopentylmethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]-1-(4-methoxypiperidin-1-yl)prop-2-en-1-one (1A7). The crude product was purified by silica gel TLC plate using MeOH/DCM (5%) to afford the desired product (4.8 mg, 0.012 mmol, 36%). **1H NMR** (500 MHz, Methanol-d_4) δ 8.20 (s, 1H), 7.81 (d, *J* = 15.2 Hz, 1H), 7.40 (d, *J* = 15.2 Hz, 1H), 4.30 (d, *J* = 7.6 Hz, 2H), 3.97 (dddd, *J* = 24.3, 10.9, 7.1, 3.9 Hz, 2H), 3.55 (ddt, *J* = 9.8, 6.1, 2.9 Hz, 2H), 3.49 (ddd, *J* = 12.7, 8.4, 3.6 Hz, 1H), 3.39 (s, 3H), 2.58 (p, *J* = 7.5 Hz, 1H), 2.00 – 1.89 (m, 2H), 1.71 – 1.55 (m, 8H), 1.38 – 1.33 (m, 2H). **13C NMR** (126 MHz, MeOD) δ 167.1 (C), 160 (C), 156.8 (C), 155.4 (CH), 141.0 (C), 132.5 (CH), 122.1 (CH), 100.3 (C), 76.6 (CH) , 56.1 (CH_3), 53.0 (CH_2), 41.5 (CH), 40.8 (2 x CH_2), 32.4 (2 x CH_2), 31.0 (2 x CH_2), 26.0 (2 x CH_2). **HRMS** (ESI, *m*/*z*) calcd for $C_{20}H_{29}N_6O_2$ $[M + H]^+$: 385.2347; found, 385.2367.

Synthesis of (2*E*)-3-[4-Amino-1-(cyclopentylmethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]-1-[4-(2-hydroxyethyl)piperidin-1-yl]prop-2-en-1-one (1A8). The crude product was purified by silica gel TLC plate using MeOH/DCM (10% +1% TEA) to afford the desired product (13.8 mg, 0.0346 mmol, 99%). **1H NMR** (601 MHz, DMSO-d_6) δ = 8.18 (s, 1H), 7.80 (d, *J*=15.1, 1H), 7.44 (s, 2H), 7.25 (d, *J*=15.1, 1H), 4.47 (d, *J*=13.0, 1H), 4.36 (t, *J*=5.1, 1H), 4.23 (d, *J*=7.6, 2H), 4.03 (d, *J*=13.4, 2H), 3.45 (tt, *J*=7.3, 3.6, 2H), 3.09 (t, *J*=12.9, 1H), 2.69 – 2.60 (m, 1H), 1.73 (t, *J*=13.0, 3H), 1.66 (dp, *J*=11.0, 3.3, 1H), 1.59 (dddd, *J*=17.0, 9.7, 7.2, 4.5, 4H), 1.52 – 1.46 (m, 3H), 1.39 – 1.35 (m, 2H), 1.32 – 1.25 (m, 2H). **13C NMR** (151 MHz, DMSO) δ 163.9 (C), 158.2 (C), 155.6 (C), 154.4 (CH), 139.1 (C), 130.7 (CH), 121.5 (CH), 98.3 (C), 58.1(CH_2), 51.0 (CH_2), 46.7 (2 x CH_2), 45.8 (CH_2), 41.8 (CH), 32.72 (CH), 32.2 (2 x CH_2), 29.6 (2 x CH_2), 24.6 (2 x CH_2). **HRMS** (ESI, *m*/*z*) calcd for $C_{21}H_{31}N_6O_2$ $[M + H]^+$: 399.2503; found, 399.2492.

Synthesis of (2*E*)-3-[4-Amino-1-(cyclopentylmethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]-1-[4-(dimethylamino)piperidin-1-yl]prop-2-en-1-one (1A9). The crude product was purified by silica gel TLC plate using MeOH/DCM (10% +1% TEA) to afford the desired product (5.7 mg, 0.0143 mmol, 41%). **1H NMR** (500 MHz, Methanol-d_4) δ 8.21 (s, 1H), 7.85 (d, *J* = 15.2 Hz, 1H), 7.42 (d, *J* = 15.2 Hz, 1H), 4.46 – 4.40 (m, 1H), 4.31 (d, *J* = 7.6 Hz, 2H), 3.38 – 3.34 (m, 1H), 3.27 (d, *J* = 12.2 Hz, 1H), 3.21 (q, *J* = 7.3 Hz, 1H), 2.86 – 2.80 (m, 1H), 2.79 (s, 6H), 2.58 (p, *J* = 7.5 Hz, 1H), 2.23 – 2.14 (m, 2H), 1.72 – 1.62 (m, 6H), 1.60 – 1.55 (m, 2H), 1.36 (ddd, *J* = 15.0, 8.7, 5.1 Hz, 2H). **13C NMR** (126 MHz, MeOD) δ 165.8 (C), 158.6 (C), 155.5 (C), 154.1 (CH), 139.4 (C), 131.7 (CH), 120.2 (CH), 98.9 (C), 62.9 (CH), 51.6 (CH_2), 44.1 (2 x CH_2), 40.1 (CH), 39.3 (2 x CH_3), 29.64 (2 x CH_2), 27.16(2 x CH_2), 24.59 (2 x CH_2). **HRMS** (ESI, *m*/*z*) calcd for $C_{21}H_{32}N_7O_1$ $[M + H]^+$: 398.2663; found, 398.2672.

Synthesis of (2*E*)-3-[4-Amino-1-(cyclopentylmethyl)-1*H*-pyrazolo[3,4-*d*] pyrimidin-3-yl]-1-(pyrrolidin-1-yl)prop-2-en-1-one (1A10). The crude product was purified by silica gel TLC plate using MeOH/DCM (5%) to afford the desired product (9.9 mg, 0.029 mmol, 84%). **1H NMR** (500 MHz, Methanol-d_4) δ 8.21 (s, 1H), 7.84 (d, *J* = 15.3 Hz, 1H), 7.22 (d, *J* = 15.3 Hz, 1H), 4.31 (d, *J* = 7.6 Hz, 2H), 3.75 (t, *J* = 6.9 Hz, 2H), 3.58 (t, *J* = 6.9 Hz, 2H), 2.58 (p, *J* = 7.4 Hz, 1H), 2.06 (p, *J* = 6.7 Hz, 2H), 1.99 – 1.93 (m, 2H), 1.71 – 1.65 (m, 4H), 1.60 – 1.56 (m, 2H), 1.36 (dd, *J* = 12.9, 6.0 Hz, 2H). **13C NMR** (126 MHz, MeOD) δ 167.2 (C), 160.0 (C), 156.9 (C), 155.5 (CH), 140.8 (C), 133.1 (CH), 121.6 (CH), 100.3 (C), 64.34 (CH_2),

53.0 (2 x CH_2), 41.5 (CH), 40.7 (2 x CH_2), 31.0 (2 x CH_2), 26.0 (2 x CH_2). **HRMS** (ESI, *m*/*z*) calcd for $C_{18}H_{25}N_6O_1$ $[M + H]^+$: 341.2084; found, 341.2084.

4. Synthesis and characterization of Compounds 2A1-10

General Synthesis of Library 2A Compounds 2A 1-10

(2E)-3-[1-(cyclopentylmethyl)-6-(methylamino)-1H-pyrazolo[3,4-d]pyrimidin-3-yl]prop-2-enoic acid **(12).** (0.029-0.053 mmol) was dissolved in anhydrous DMF (0.5 mL). To this, the appropriate secondary amine (4 eq.) and DIPEA (3 eq.) were then added. The resulting mixture was stirred for 5 min before adding HATU (1.2 eq.). The reaction vial was flushed with N_2, and the reaction stirred at r.t for 24 hours. The solvent was removed under vacuum and the residue partitioned between water and DCM. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The crude product was purified by silica gel TLC plate to afford the desired product.

Synthesis of (2E)-3-[1-(cyclopentylmethyl)-6-(methylamino)-1H-pyrazolo[3,4-d]pyrimidin-3-yl]-1-(piperidin-1-yl)prop-2-en-1-one (2A1). The crude product was purified by silica gel TLC plate using MeOH/DCM (5%) to afford the desired product (7.1 mg, 0.019 mmol, 66%). **1H NMR** (500 MHz, Methanol-d4) δ 9.00 (s, 1H), 7.62 (d, J = 15.8 Hz, 1H), 7.33 (d, J = 15.7 Hz, 1H), 4.56 (s, 1H), 4.22 (d, J = 7.6 Hz, 2H), 3.70 (dt, J = 20.2, 5.5 Hz, 4H), 2.99 (s, 3H), 2.58 (p, J = 7.4 Hz, 1H), 1.74 – 1.57 (m, 8H), 1.40 – 1.35 (m, 2H), 1.30 (dd, J = 11.9, 4.7 Hz, 4H). **13C NMR** (126 MHz, MeOD) δ 167.0 (C), 163.1 (C), 157.5 (CH), 154.6 (C), 142.5 (C), 133.8 (CH), 122.1 (CH), 106.7 (C), 51.9 (CH_2), 44.7 (2 x CH_2), 41.3 (CH), 31.1 (2 x CH_2), 28.5 (CH_3), 27.9 (2 x CH_2), 25.9 (2 x CH_2), 25.5 (CH_2). **HRMS** (ESI, m/z) calcd for C20H29N6O1 [M + H]+: 369.2397; found, 369.2413.

Synthesis of (2*E*)-3-[1-(cyclopentylmethyl)-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]-1-(morpholin-4-yl)prop-2-en-1-one (2A2). The crude product was purified by silica gel TLC plate using MeOH/DCM (3%) and then MeOH/DCM (4%) to afford the desired product (11 mg, 0.030 mmol, 74%).**1H NMR** (500 MHz, Methanol-d_4) δ 9.03 (s, 1H), 7.67 (d, *J* = 15.7 Hz, 1H), 7.31 (d, *J* = 15.7 Hz, 1H), 4.22 (d, *J* = 7.6 Hz, 2H), 3.79 – 3.71 (m, 8H), 2.99 (s, 3H), 2.58 (p, *J* = 7.4 Hz, 1H), 1.71 – 1.65 (m, 4H), 1.60 – 1.56 (m, 2H), 1.38 (q, *J* = 6.0, 5.1 Hz, 2H). **13C NMR** (126 MHz, DMSO) δ 164.2 (C), 161.4 (C), 155.8 (CH), 154.1 (C), 140.5 (C), 132.7 (CH), 120.5 (CH), 104.7 (C), 66.5 (2 x CH_2), 50.1 (CH_2), 45.7 (2 x CH_2), 42.1 (CH), 29.6 (2 x CH_2), 27.8 (CH_3), 24.5 (2 x CH_2). **HRMS** (ESI, *m*/*z*) calcd for $C_{19}H_{27}N_6O_2$ $[M + H]^+$: 371.2190; found, 371.2212.

Synthesis of (2*E*)-3-[1-(cyclopentylmethyl)-6-(methylamino)-1*H*-pyrazolo[3,4-*d*] pyrimidin-3-yl]-1-(4-methylpiperidin-1-yl)prop-2-en-1-one (2A3). The crude product was purified by silica gel TLC plate using MeOH/DCM (2 %) to afford the desired product (10.5 mg, 0.027 mol, 60 %).**1H NMR** (500 MHz, Methanol-d_4) δ 9.00 (s, 1H), 7.62 (d, *J* = 15.7 Hz, 1H), 7.33 (d, *J* = 15.7 Hz, 1H), 4.60 (d, *J* = 13.2 Hz, 1H), 4.26 (s, 1H), 4.22 (d, *J* = 7.6 Hz, 2H), 3.24 – 3.17 (m, 1H), 2.99 (s, 3H), 2.82 – 2.75 (m, 1H), 2.58 (p, *J* = 7.4 Hz, 1H), 1.84 – 1.74 (m, 2H), 1.72 – 1.64 (m, 5H), 1.61 – 1.56 (m, 2H), 1.40 – 1.35 (m, 2H), 1.22 – 1.12 (m, 2H), 0.99 (d, *J* = 6.5 Hz, 3H). **13C NMR** (126 MHz, MeOD) δ 167.0 (C), 163.1 (C), 157.5 (CH), 154.6 (C), 142.5 (C), 133.8 (CH), 122.1 (CH), 106.9 (C), 51.9 (CH_2),44.1 (2 x CH_2), 41.3 (CH), 35.1 (2 x CH_2), 32.3 (CH), 31.1 (2 x CH_2), 28.5 (CH_3), 25.9 (2 x CH_2), 22.0 (CH_3). **HRMS** (ESI, *m*/*z*) calcd for $C_{21}H_{31}N_6O_1$ $[M + H]^+$: 382.2554; found, 382.2571.

Synthesis of (2*E*)-3-[1-(cyclopentylmethyl)-6-(methylamino)-1*H*-pyrazolo[3,4-*d*] pyrimidin-3-yl]-1-[3-(dimethylamino)piperidin-1-yl]prop-2-en-1-one (2A4). The crude product was purified by silica gel TLC plate using MeOH/DCM (8 %) to afford the desired product (10 mg, 0.024 mol, 46 %).**1H NMR** (500 MHz, Methanol-d_4) δ 9.01 (s, 1H), 7.64 (d, *J* = 15.7 Hz, 1H), 7.33 (d, *J* = 15.8 Hz, 1H), 4.61 (d, *J* = 12.8 Hz, 1H), 4.51 (d, *J* = 13.0 Hz, 1H), 4.34 (d, *J* = 12.0 Hz, 1H), 4.22 (d, *J* = 7.5 Hz, 2H), 4.11 (d, *J* = 13.8 Hz, 1H), 3.09 – 3.01 (m, 1H), 2.99 (s, 3H), 2.65 – 2.54 (m, 5H), 2.49 – 2.44 (m, 2H), 2.14 – 2.08 (m, 1H), 1.93 (d, *J* = 22.3 Hz, 1H), 1.72 – 1.64 (m, 5H), 1.61 – 1.56 (m, 2H), 1.38 (dtd, *J* = 10.2, 5.5, 1.4 Hz, 2H). **13C NMR** (126 MHz, MeOD) δ 167.4 (C), 163.1 (C), 157.6 (CH), 154.6 (C), 142.3 (C), 134.4 (CH), 121.6 (CH), 106.9 (C), 62.6 (CH), 51.9 (CH_2), 47.5 (CH_2), 45.1 (CH_2), 42.1 (2 x CH_3), 41.3 (CH), 31.1 (2 x CH_2), 28.5 (CH_3), 27.8 (CH_2), 25.9 (2 x CH_2), 25.7 (CH_2). **HRMS** (ESI, *m*/*z*) calcd for $C_{22}H_{34}N_7O_1$ $[M + H]^+$: 412.2819; found, 412.2824.

Synthesis of (2*E*)-3-[1-(cyclopentylmethyl)-6-(methylamino)-1*H*-pyrazolo[3,4-*d*] pyrimidin-3-yl]-1-(4-hydroxypiperidin-1-yl)prop-2-en-1-one (2A5). The crude product was purified by silica gel TLC plate using MeOH/DCM (6 %) to afford the desired product (7.2 mg, 0.187 mol, 41 %). **1H NMR** (500 MHz, Methanol-d_4) δ 9.01 (s, 1H), 7.63 (d, *J* = 15.7 Hz, 1H), 7.35 (d, *J* = 15.7 Hz, 1H), 4.22 (d, *J* = 7.5 Hz, 2H), 4.17 (dd, *J* = 12.2, 6.1 Hz, 1H), 4.10 – 4.04 (m, 1H), 3.91 (tt, *J* = 8.2, 3.9 Hz, 1H), 3.52 – 3.45 (m, 1H), 3.36 – 3.32 (m, 1H), 2.99 (s, 3H), 2.58 (p, *J* = 7.3 Hz, 1H), 1.93 (d, *J* = 11.4 Hz, 2H), 1.68 (dddd, *J* = 16.9, 10.8, 8.3, 4.7 Hz, 4H), 1.62 – 1.48 (m, 4H), 1.41 – 1.35 (m, 2H). **13C NMR** (126 MHz, MeOD) δ 167.1 (C), 163.1(C), 157.5 (CH), 154.6 (C), 142.4 (C), 134.1 (CH), 121.9 (CH), 106.8 (C), 67.7 (CH), 52.4 (CH_2),44.2 (2 x CH_2), 41.3 (CH), 35.8 (2 x CH_2), 31.1 (2 x CH_2), 28.5 (CH_3), 25.9 (2 x CH_2). **HRMS** (ESI, *m*/*z*) calcd for $C_{20}H_{29}N_6O_2$ $[M + H]^+$: 385.2347; found, 385.2334.

Synthesis of (2*E*)-3-[1-(cyclopentylmethyl)-6-(methylamino)-1*H*-pyrazolo[3,4-*d*] pyrimidin-3-yl]-1-(4-phenylpiperidin-1-yl)prop-2-en-1-one (2A6). The crude product was purified by silica gel TLC plate using MeOH/DCM (6 %) to afford the desired product (10.5 mg, 0.0236 mol, 51 %). **1H NMR** (500 MHz, Methanol-d_4) δ 9.03 (s, 1H), 7.66 (d, *J* = 15.7 Hz, 1H), 7.39 (d, *J* = 15.7 Hz, 1H), 7.31 – 7.24 (m, 4H), 7.20 – 7.16 (m, 1H), 4.82 – 4.77 (m, 1H), 4.42 (d, *J* = 13.7 Hz, 1H), 4.22 (d, *J* = 7.6 Hz, 2H), 3.39 – 3.33 (m, 1H), 2.99 (s, 3H), 2.92 – 2.84 (m, 2H), 2.58 (p, *J* = 7.3 Hz, 1H), 2.02 – 1.92 (m, 2H), 1.79 – 1.65 (m, 6H), 1.63 – 1.56 (m, 2H), 1.42 – 1.35 (m, 2H). **13C NMR** (126 MHz, MeOD) δ 167.1 (C), 163.1 (C), 157.5 (CH), 154.6 (C), 146.7 (C), 142.5 (C), 134.0 (CH), 129.6 (2 x CH), 127.83 (2 x CH), 127.46 (CH), 122.06 (CH), 106.9 (C), 51.9 (CH_2), 43.9 (2 x CH_2), 41.3 (CH), 35.3 (CH), 34.3 (2 x CH_2), 31.1 (2 x CH_2), 28.5 (CH_3), 25.9 (2 x CH_2). **HRMS** (ESI, *m*/*z*) calcd for $C_{26}H_{33}N_6O_1$ $[M + H]^+$: 445.2710; found, 445.2711.

Synthesis of (2*E*)-3-[1-(cyclopentylmethyl)-6-(methylamino)-1*H*-pyrazolo[3,4-*d*] pyrimidin-3-yl]-1-(4-methoxypiperidin-1-yl)prop-2-en-1-one (2A7). The crude product was purified by silica gel TLC plate using MeOH/DCM (3 %) to afford the desired product (16. mg, 0.040 mol, 76 %). **1H NMR** (500 MHz, Methanol-d_4) δ 9.01 (s, 1H), 7.63 (d, *J* = 15.7 Hz, 1H), 7.34 (d, *J* = 15.7 Hz, 1H), 4.22 (d, *J* = 7.6 Hz, 2H), 4.01 – 3.93 (m, 2H), 3.54 (tt, *J* = 7.3, 3.4 Hz, 2H), 3.50 – 3.44 (m, 1H), 3.39 (s, 3H), 2.99 (s, 3H), 2.58 (p, *J* = 7.3 Hz, 1H), 1.96 (s, 2H), 1.73 – 1.64 (m, 5H), 1.62 – 1.56 (m, 3H), 1.37 (dt, *J* = 12.4, 6.9 Hz, 2H). **13C NMR** (126 MHz, MeOD) δ 167.1 (C), 163.1 (C), 157.5 (CH), 154.4 (C), 142.4 (C), 134.1 (CH), 121.9 (CH), 106.9 (C), 76.6 (CH), 56.1 (CH_3), 51.9 (CH_2), 44.4 (2 x CH_2), 41.3 (CH), 32.5 (2 x CH_2), 31.1 (2 x CH_2), 28.5 (CH_3), 25.9 (2 x CH_2). **HRMS** (ESI, *m*/*z*) calcd for $C_{21}H_{31}N_6O_2$ $[M + H]^+$: 399.2503; found, 399.2498.

Synthesis of (2*E*)-3-[1-(cyclopentylmethyl)-6-(methylamino)-1*H*-pyrazolo[3,4-*d*] pyrimidin-3-yl]-1-[4-(2-hydroxyethyl)piperidin-1-yl]prop-2-en-1-one (2A8). The crude product was purified by silica gel TLC plate using MeOH/DCM (6 %) to afford the desired product (14.6 mg, 0.035 mol, 77 %). **1H NMR** (500 MHz, Methanol-d_4) δ 9.00 (s, 1H), 7.61 (d, *J* = 15.7 Hz, 1H), 7.33 (d, *J* = 15.7 Hz, 1H), 4.62 (d, *J* = 13.1 Hz, 1H), 4.27 (d, *J* = 13.8 Hz, 1H), 4.21 (d, *J* = 7.6 Hz, 2H), 3.64 (t, *J* = 6.6 Hz, 2H), 3.25 – 3.18 (m, 1H), 2.99 (s, 3H), 2.79 (ddd, *J* = 15.8, 9.4, 3.1 Hz, 1H), 2.57 (p, *J* = 7.4 Hz, 1H), 1.90 – 1.81 (m, 2H), 1.79 (dt, *J* = 7.4, 3.9 Hz, 1H), 1.72 – 1.64 (m, 4H), 1.62 – 1.55 (m, 2H), 1.51 (t, *J* = 6.6 Hz, 2H), 1.41 – 1.33 (m, 2H), 1.26 – 1.16 (m, 2H). **13C NMR** (126 MHz, MeOD) δ 167.0 (C), 163.1 (C), 157.5 (CH), 154.6 (C), 142.4 (C), 133.8 (CH), 122.1 (CH), 106.8 (C), 60.2 (CH_2), 51.9 (CH_2), 44.0 (2 x CH_2), 41.3 (CH), 34.2 (CH_2), 33.9 (CH), 33.2 (2 x CH_2), 31.1 (2 x CH_2), 28.5 (CH_3), 25.9 (2 x CH_2). **HRMS** (ESI, *m*/*z*) calcd for $C_{22}H_{33}N_6O_1$ $[M + H]^+$: 413.2660; found, 413.2650.

Synthesis of (2*E*)-3-[1-(cyclopentylmethyl)-6-(methylamino)-1*H*-pyrazolo[3,4-*d*] pyrimidin-3-yl]-1-[4-(dimethylamino)piperidin-1-yl]prop-2-en-1-one (2A9). The crude product was purified by silica gel TLC plate using MeOH/DCM (8 %) to afford the desired product (12 mg, 0.030 mol, 55 %). **1H NMR** (500 MHz, Methanol-d_4) δ 9.02 (s, 1H), 7.65 (d, *J* = 15.7 Hz, 1H), 7.34 (d, *J* = 15.7 Hz, 1H), 4.46 (d, *J* = 13.9 Hz, 1H), 4.22 (d, *J* = 7.5 Hz, 2H), 3.36 – 3.32 (m, 1H), 3.28 – 3.23 (m, 1H), 2.99 (s, 3H), 2.92 – 2.86 (m, 1H), 2.84 – 2.80 (m, 1H), 2.78 (s, 6H), 2.61 – 2.54 (m, 1H), 2.20 – 2.12 (m, 2H), 1.71 – 1.65 (m, 6H), 1.60 – 1.56 (m, 2H), 1.40 – 1.34 (m, 2H). **13C NMR** (126 MHz, MeOD) δ 167.2 (C), 163.1 (C), 157.5 (CH), 154.6 (C), 142.3 (C), 134.6 (CH), 121.4 (CH), 106.8 (C), 64.3 (CH), 51.9 (CH_2), 45.5 (2 x CH_2), 41.3 (CH), 40.8 (2 x CH_3), 31.1 (2 x CH_2), 28.5 (CH_3), 27.7 (CH_2), 25.9(2 x CH_2). **HRMS** (ESI, *m*/*z*) calcd for $C_{22}H_{34}N_7O_1$ $[M + H]^+$: 412.2819; found, 412.2828.

Synthesis of (2*E*)-3-[1-(cyclopentylmethyl)-6-(methylamino)-1*H*-pyrazolo[3,4-*d*] pyrimidin-3-yl]-1-(pyrrolidin-1-yl)prop-2-en-1-one (2A10). The crude product was purified by silica gel TLC plate using MeOH/DCM (6 %) to afford the desired product (6.4 mg, 0.0180 mol, 40 %). **1H NMR** (500 MHz, Methanol-d_4) δ 8.99 (s, 1H), 7.66 (d, *J* = 15.9 Hz, 1H), 7.14 (d, *J* = 15.8 Hz, 1H), 4.22 (d, *J* = 7.6 Hz, 2H), 3.77 (t, *J* = 6.9 Hz, 2H), 3.56 (t, *J* = 6.9 Hz, 2H), 2.99 (s, 3H), 2.58 (p, *J* = 7.3 Hz, 1H), 2.06 (p, *J* = 6.9 Hz, 2H), 1.96 (p, *J* = 6.8 Hz, 2H), 1.69 (td, *J* = 9.4, 8.8, 4.8 Hz, 4H), 1.62 – 1.56 (m, 2H), 1.38 (q, *J* = 6.1, 5.2 Hz, 2H). **13C NMR** (126 MHz, MeOD) δ 166.4 (C) , 163.1 (C), 157.6 (CH), 154.5 (C), 142.3 (C), 133.3 (CH), 123.1 (CH), 106.9 (C), 51.9 (CH_2), 41.3 (CH), 31.1 (2 x CH_2), 28.5 (CH_3), 27.0 (2 x CH_2), 25.9 (2 x CH_2), 25.30 (2 x CH_2). **HRMS** (ESI, *m*/*z*) calcd for $C_{19}H_{27}N_6O_1$ $[M + H]^+$: 355.2241; found, 355.2244.

5. Synthesis and characterization of Compounds 2B1-11

General Synthesis of Intermediate Compounds 22a-k

3-iodo-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine **(7)** (0.182-0.461 mmol) was suspended in DMF (3.5 mL), and sodium hydride was added (1.4 eq., 60% dispersion in mineral oil), and the mixture stirred until gas evolution subsided. To this, the appropriate bromoalkane (1.5 eq.) was added, and the mixture was heated at 150 °C using microwave irradiation for 85 min. Then, the mixture was partitioned between EtOAc and water, and the organic layers separated. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The crude product was purified by silica gel flash chromatography.

Synthesis of 3-iodo-*N*-methyl-1-(propan-2-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (22a). The crude product was purified by silica gel flash chromatography EtOAc/Hexane (0-25 %) to obtain the pure product (35.3 mg, 0.111 mol, 61%).**1H NMR** (500 MHz, Chloroform-*d*) δ 8.37 (s, 1H), 5.40 – 5.36 (m, 1H), 4.98 (p, *J* = 6.8 Hz, 1H), 3.06 (d, *J* = 5.0 Hz, 3H), 1.53 (d, *J* = 6.7 Hz, 6H). **13C NMR** (126 MHz, $CDCl_3$) δ 162.1 (C), 154.9 (C), 153.9 (CH), 113.3 (C), 89.7 (C), 49.1 (CH_2), 28.7 (CH_3), 22.0 (CH_2). **LRMS** (*m*/*z*) $[M + H]^+$: 317.90.

Synthesis of 1-(2,2-dimethylpropyl)-3-iodo-*N*-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (22b). The crude product was purified by silica gel flash chromatography using MeOH/DCM (0-1 %) to obtain the pure product (42.2 mg, 0.122 mol, 48%). **1H NMR** (500 MHz, Chloroform-*d*) δ 8.38 (s, 1H), 5.34 (s, 1H), 4.08 (s, 2H), 3.05 (d, *J* = 5.0 Hz, 3H), 1.00 (s, 9H). **13C NMR** (126 MHz, CDCl3) δ 162.4 (C), 156.5 (C), 153.9 (CH), 112.8 (C), 89.8 (C), 58.1 (CH_2), 34.1 (C), 28.7 (CH_3), 28.1 (3 x CH_3). **LRMS** (*m*/*z*) $[M + H]^+$: 345.90.

Synthesis of 3-iodo-*N*-methyl-1-(3-methylbutyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (22c). The crude product was purified by silica gel flash chromatography using EtOAc/Hexane (0-30 %) to obtain the pure product (64.4 mg, 0.187 mol, 52%).**1H NMR** (500 MHz, DMSO-d_6) δ 8.43 (s, 1H), 7.61 (s, 1H), 4.21 (s, 2H), 2.85 (d, *J* = 4.7 Hz, 3H), 1.68 (q, *J* = 7.0 Hz, 2H), 1.43 (s, 1H), 0.91 (d, *J* = 6.6 Hz, 6H). **13C NMR** (126 MHz, DMSO) δ 162.0 (C), 154.5 (C), 153.7 (CH), 111.1 (C), 91.9 (C), 44.0 (CH_2) , 37.6 (CH_2), 27.8 (CH_3), 24.8 (CH), 22.1 (2 x CH_3).

Synthesis of 1-(2,2-dimethoxyethyl)-3-iodo-*N*-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (22d). The crude product was purified by silica gel flash chromatography using EtOAc/Hexane (0-40 %) to obtain the pure product (86.6 mg, 0.238 mol, 68%). **1H NMR** (500 MHz, Chloroform-*d*) δ 8.35 (s, 1H), 6.93 (s, 1H), 4.94 (t, *J* = 5.7 Hz, 1H), 4.40 (d, *J* = 5.7 Hz, 2H), 3.40 (s, 6H), 3.08 (d, *J* = 4.9 Hz, 3H). **13C NMR** (126 MHz, $CDCl_3$) δ 156.2 (C), 154.2 (C), 145.9 (CH), 112.9 (C), 101.1 (CH), 94.8 (C), 53.8 (2 x CH_3), 47.8 (CH_2), 28.6 (CH_3). **LRMS** (*m*/*z*) $[M + H]^+$: 363.90.

Synthesis of 1-(2,2-diethoxyethyl)-3-iodo-*N*-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (22e). The crude product was purified by silica gel flash chromatography using EtOAc/Hexane (0-40 %) to obtain the pure product (82.4mg, 0.211 mol, 78%). **1H NMR** (500 MHz, Chloroform-*d*) δ 8.35 (s, 1H), 6.87 (s, 1H), 5.03 (t, *J* = 5.7 Hz, 1H), 4.41 (d, *J* = 5.7 Hz, 2H), 3.76 (dq, *J* = 9.5, 7.1 Hz, 2H), 3.52 (dq, *J* = 9.4, 6.9 Hz, 2H), 3.08 (d, *J* = 4.9 Hz, 3H), 1.12 (td, *J* = 7.1, 0.8 Hz, 6H). **13C NMR** (126 MHz, CDCl3) δ 156.3 (C), 154.1 (C), 148.6 (CH), 124.6 (C), 99.7 (CH), 92.6 (C), 62.4 (2 x CH_2), 48.9 (CH_2), 28.6 (CH_3), 15.3 (2 x CH_3). **LRMS** (*m*/*z*) $[M + H]^+$: 391.90.

Synthesis of 3-iodo-*N*-methyl-1-[(oxolan-2-yl)methyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (22f). The crude product was purified by silica gel flash chromatography using EtOAc/Hexane (0-40 %) to obtain the pure product (90.7 mg, 0.2453 mol, 72%). **1H NMR** (500 MHz, Chloroform-*d*) δ 8.34 (s, 1H), 6.81 (s, 1H), 4.50 – 4.37 (m, 2H), 4.28 – 4.18 (m, 1H), 3.91 (ddd, *J* = 8.4, 7.1, 6.2 Hz, 1H), 3.78 (ddd, *J* = 8.4, 7.5, 6.2 Hz, 1H), 3.08 (d, *J* = 4.9 Hz, 3H), 2.07 – 2.00 (m, 1H), 1.99 – 1.89 (m, 2H), 1.80 – 1.72 (m, 1H). **13C NMR** (126 MHz, $CDCl_3$) δ 158.8 (C), 156.1 (C), 154.0 (CH), 112.3 (C), 92.1 (C), 70.8 (CH), 68.4 (CH_2), 50.9 (CH_2), 29.3 (CH_2), 28.6 (CH_3), 25.9 (CH_2). **LRMS** (*m*/*z*) $[M + H]^+$: 359.90.

Synthesis of 1-[(1,3-dioxolan-2-yl)methyl]-3-iodo-*N*-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (22g). The crude product was purified by silica gel flash chromatography using EtOAc/Hexane (10-50 %) to obtain the pure product (89.2 mg, 0.247 mol, 70%). **1H NMR** (500 MHz, DMSO-d_6) δ 8.45 (s, 1H), 7.66 (s, 1H), 5.29 (s, 1H), 4.29 (d, *J* = 4.8 Hz, 2H), 3.93 (d, *J* = 6.4 Hz, 2H), 3.83 – 3.79 (m, 2H), 2.86 (d, *J* = 4.7 Hz, 3H). **13C NMR** (126 MHz, DMSO) δ 162.6 (C), 155.7 (C), 153.4 (CH), 111.6 (C), 100.7 (CH), 92.8 (C), 64.3 (2 x CH_2), 48.2 (CH_2), 27.8 (CH_3).

Synthesis of 1-(cyclohexylmethyl)-3-iodo-*N*-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (22h). The crude product was purified by silica gel flash chromatography using EtOAc/Hexane (0-20 %) to afford the desired product (100 mg, 0.519 mol, 76%). **1H NMR** (500 MHz, DMSO-d_6) δ 8.43 (s, 1H), 7.59 (s, 1H), 4.04 (d, *J* = 7.8 Hz, 2H), 2.85 (d, *J* = 4.7 Hz, 3H), 1.89 (d, *J* = 14.5 Hz, 1H), 1.67 – 1.48 (m, 6H), 1.19 – 1.12 (m, 3H), 0.97 (d, *J* = 12.2 Hz, 1H). 13**C NMR** (126 MHz, DMSO) δ 162.0 (C), 155.5 (C), 153.7 (CH), 111.5 (C), 91.9 (C), 51.6 (CH_2), 37.5 (CH), 30.0 (2 x CH_2), 27.8 (CH_3), 25.8 (CH_2), 25.0 (2 x CH_2).

Synthesis of 1-benzyl-3-iodo-*N*-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (22i). The crude product was purified by silica gel flash chromatography using EtOAc/Hexane (0-50 %) to afford the desired product (53.3 mg, 0.146 mol, 42%). **1H NMR** (500 MHz, DMSO-d_6) δ 8.46 (s, 1H), 7.69 (s, 1H), 7.35 – 7.31 (m, 2H), 7.30 – 7.26 (m, 3H), 5.40 (s, 2H), 2.88 (d, *J* = 4.7 Hz, 3H). **13C NMR** (126 MHz, DMSO) δ 162.2 (C), 155.3 (C), 153.9 (CH), 137.0 (C), 128.6 (2 x CH), 127.8 (2 x CH), 127.6 (CH), 111.6 (C), 92.5 (C), 49.3 (CH_2), 27.9 (CH_3).

Synthesis of 3-iodo-*N*-methyl-1-[(3-methylphenyl)methyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (22j). The crude product was purified by silica gel flash chromatography using EtOAc/Hexane (0-30 %) to afford the desired product (78.7 mg, 0.208 mol, 45%). **1H NMR** (500 MHz, DMSO-d_6) δ 8.46 (s, 1H), 7.69 (s, 1H), 7.21 (t, *J* = 7.6 Hz, 1H), 7.09 (q, *J* = 12.9 Hz, 3H), 5.35 (s, 2H), 2.88 (d, *J* = 4.7 Hz, 3H), 2.26 (s, 3H).**13C NMR** (126 MHz, DMSO) δ 162.2 (C), 155.2 (C), 153.8 (CH), 137.7 (C), 136.9 (C), 128.5 (2 x CH), 128.3 (CH), 125.0 (CH), 111.6 (C), 92.7 (C), 49.3 (CH_2), 27.9 (CH_3), 20.9 (CH_3).

Synthesis of 3-iodo-1-[(3-methoxyphenyl)methyl]-*N*-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (22k). The crude product was purified by silica gel flash chromatography using MeOH/DCM (0-1%); then repurified by silica gel TLC using EtOAc/Hexane (50 %) to afford the desired product (77.6 mg, 0.196 mol, 54%). **1H NMR** (500 MHz, Methanol-d_4) δ 8.39 (s, 1H), 7.21 (t, *J* = 7.9 Hz, 1H), 6.90 – 6.81 (m, 3H), 5.40 (s, 2H), 3.75 (s, 3H), 3.00 (s, 3H). **13C NMR** (126 MHz, MeOH) δ 163.9 (C), 161.3 (C), 157.0 (C), 155.1 (CH), 155.2 (C), 139.6 (CH), 130.7 (C), 121.1 (CH), 114.7 (CH), 114.3 (CH), 92.1 (C), 55.6 (CH_3), 51.0 (CH_2), 28.6 (CH_3). **LRMS** (*m*/*z*) $[M + H]^+$: 396.20

General Synthesis of Intermediate Compounds 23a-k

The appropriate intermediate compound **(22a-k)** (0.103-0.253 mmol) was dissolved in DMF/water/TEA (7.8 mL/0.78 mL/0. 78 mL). To this, TBAI (2 eq.), methyl acrylate (10 eq.) and $Pd(PPh_3)_2Cl_2$ (0.2 eq) were added. The reaction vial was flushed with N_2 for 10 minutes and then heated at 80 °C using a heating magnetic stirrer for 24 hours. After cooling to r.t, the mixture was extracted with DCM. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered through a plug of Celite ® and concentrated under vacuum. The crude product was purified by silica gel flash chromatography.

Synthesis of methyl (2*E*)-3-[6-(methylamino)-1-(propan-2-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoate (23a). The crude product was purified purified by silica gel flash chromatography using EtOAc/Hexane (0-50 %) to afford the desired product (13.3 mg, 0.048mmol, 50 %). **1H NMR** (500 MHz, Chloroform-*d*) δ 8.86 (s, 1H), 7.82 (d, *J* =

16.3 Hz, 1H), 6.61 (d, *J* = 16.3 Hz, 1H), 5.32 – 5.26 (m, 1H), 5.03 (p, *J* = 6.9 Hz, 1H), 3.83 (s, 3H), 3.07 (d, *J* = 5.0 Hz, 3H), 1.54 (d, *J* = 6.8 Hz, 6H). **13C NMR** (126 MHz, CDCl3) δ 167.2 (C), 161.5 (C), 155.5 (C), 153.0 (CH), 140.2 (C), 136.3 (CH), 120.4 (CH), 107.0 (C), 52.0 (CH), 48.7 (CH_3), 28.6 (CH_3), 21.8 (2 x CH_3).

Synthesis of methyl (2*E*)-3-[1-(2,2-dimethylpropyl)-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoate (23b). The crude product was purified by silica gel flash chromatography using EtOAc/Hexane (0-20 %) to afford the desired product (19.7 mg, 0.065 mmol, 63 %). **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 8.83 (s, 1H), 7.79 (d, *J* = 16.3 Hz, 1H), 6.63 (d, *J* = 16.3 Hz, 1H), 6.32 – 6.13 (m, 1H), 4.11 (s, 2H), 3.84 (s, 3H), 3.07 (d, *J* = 5.0 Hz, 3H), 1.02 (s, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 166.9 (C), 159.7 (C), 156.9 (C), 150.0 (CH), 141.1 (C), 135.2 (CH), 121.7 (CH), 106.1 (C), 58.0 (CH_2), 52.1 (CH_3), 34.1 (C), 28.5 (CH_3), 28.1 (3 x CH_3). **LRMS** (*m*/*z*) $[M + H]^+$: 304.0.

Synthesis of methyl (2*E*)-3-[6-(methylamino)-1-(3-methylbutyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoate (22c). The crude product was purified by silica gel flash chromatography using EtOAc/Hexane (0-40 %) to afford the desired product (48.2 mg, 0.159 mmol, 85 %). **1H NMR** (500 MHz, Chloroform-*d*) δ = 8.82 (s, 1H), 7.77 (d, *J*=16.3, 1H), 6.62 (d, *J*=16.3, 1H), 6.48 – 6.15 (m, 1H), 4.33 (t, *J*=7.2, 2H), 3.84 (s, 3H), 3.09 (d, *J*=5.0, 3H), 1.85 – 1.76 (m, 2H), 1.55 (dp, *J*=13.5, 6.7, 1H), 0.98 (d, *J*=6.6, 6H). **13C NMR** (126 MHz, $CDCl_3$) δ 166.8 (C), 158.7 (C), 156.0 (C), 153.0 (CH), 141.6 (C), 134.9 (CH), 121.5 (CH), 106.3 (C), 52.1 (CH_3), 45.3 (CH_2), 38.0 (CH_2), 28.5 (CH_3), 25.6 (CH), 22.4 (2 x CH_3).

Synthesis of methyl (2*E*)-3-[1-(2,2-dimethoxyethyl)-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoate (**23d).** The crude product was purified by silica gel flash chromatography using EtOAc/Hexane (0-70 %) to afford the desired product (48.3 mg, 0.150 mmol, 63 %). **1H NMR** (500 MHz, Chloroform-*d*) δ 8.84 (s, 1H), 7.77 (d, *J* = 16.3 Hz, 1H), 6.63 (d, *J* = 16.3 Hz, 1H), 5.96 (s, 1H), 4.96 (t, *J* = 5.7 Hz, 1H), 4.43 (d, *J* = 5.7 Hz, 2H), 3.84 (s, 3H), 3.40 (s, 6H), 3.07 (d, *J* = 4.9 Hz, 3H). **13C NMR** (126 MHz, CDCl3) δ 166.9 (C), 160.5 (C), 156.7 (C), 151.1 (CH), 141.6 (C), 135.3 (CH), 121.7 (CH), 106.4 (C), 101.3 (CH), 53.7 (2 x CH_3), 52.1 (CH_3), 47.6 (CH_2), 28.6 (CH_3). **LRMS** (*m*/*z*) $[M + H]^+$: 322.10.

Synthesis of methyl (2*E*)-3-[1-(2,2-diethoxyethyl)-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoate (23e). The crude product was purified by silica gel flash chromatography using EtOAc/Hexane (0-50 %) to afford the desired product (33.3 mg, 0.095 mmol, 45 %). **1H NMR** (500 MHz, Chloroform-*d*) δ 8.83 (s, 1H), 7.77 (dd, *J* = 16.2, 0.7 Hz, 1H), 6.63 (dd, *J* = 16.3, 0.7 Hz, 1H), 6.18 (d, *J* = 51.8 Hz, 1H), 5.06 (t, *J* = 5.7 Hz, 1H), 4.43 (d, *J* = 5.7 Hz, 2H), 3.84 (d, *J* = 0.7 Hz, 3H), 3.76 (dq, *J* = 9.5, 7.0 Hz, 2H), 3.52 (dq, *J* = 9.5, 7.0 Hz, 2H), 3.07 (d, *J* = 4.9 Hz, 3H), 1.12 (td, *J* = 7.1, 0.7 Hz, 6H). **13C NMR** (126 MHz, CDCl3) δ 166.9 (C), 160.0 (C), 156.7 (C), 141.6 (CH), 135.1 (C), 124.9 (CH), 121.9 (CH), 106.3 (C), 99.8 (CH), 62.3 (2 x CH_2) , 52.1 (CH_3), 48.7 (CH_2), 28.6 (CH_3), 15.3 (2 x CH_3). **LRMS** (*m*/*z*) $[M + H]^+$: 350.30.

Synthesis of methyl (2*E*)-3-[6-(methylamino)-1-[(oxolan-2-yl)methyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoate (23f). The crude product was purified by silica gel flash chromatography using EtOAc/Hexane (10-60 %) to afford the desired product (86 mg, 0.270 mmol, 99 %). **1H NMR** (500 MHz, Chloroform-*d*) δ 8.84 (s, 1H), 7.79 (d, J = 16.3 Hz, 1H), 6.62 (d, J = 16.2 Hz, 1H), 5.85 (s, 1H), 4.51 – 4.39 (m, 2H), 4.30 – 4.22 (m, 1H), 3.92 (ddd, J = 8.4, 7.2, 6.2 Hz, 1H), 3.83 (s, 3H), 3.77 (ddd, J = 8.3, 7.3, 6.3 Hz, 1H), 3.07 (d, J = 5.0 Hz, 3H), 2.05 – 1.86 (m, 3H), 1.78 (ddt, J = 12.4, 8.2, 6.2 Hz, 1H). **13C NMR** (126 MHz, CDCl3) δ 167.0 (C), 160.6 (C), 156.7 (C), 151.2(CH), 141.3 (C), 135.5 (CH), 121.4 (CH), 106.5 (C), 77.0 (CH), 68.4 (CH_2), 52.1 (CH_2), 50.5 (CH_3), 29.4 (CH_2), 28.6 (CH_3), 25.5(CH_2). **LRMS** (*m*/*z*) $[M + H]^+$: 318.20.

Synthesis of methyl (2*E*)-3-{1-[(1,3-dioxolan-2-yl)methyl]-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl}prop-2-enoate (23g). The crude product was purified by silica gel flash chromatography using EtOAc/Hexane (0-80 %); then repurified using MeOH/DCM (0-3%) to afford the desired product (57.8 mg, 0.181 mmol, 74 %). **1H NMR** (500 MHz, Chloroform-*d*) δ 8.85 (s, 1H), 7.80 (d, J = 16.3 Hz, 1H), 6.63 (d, J = 16.3 Hz, 1H), 6.05 (s, 1H), 5.41 (t, J = 4.6 Hz, 1H), 4.46 (d, J = 4.6 Hz, 2H), 4.04 – 4.00 (m, 2H), 3.93 – 3.90 (m, 2H), 3.83 (s, 3H), 3.08 (d, J = 4.9 Hz, 3H). **13C NMR** (126 MHz, CDCl3) δ 166.9 (C), 160.9 (C), 157.0 (C), 151.8 (CH), 141.7 (C), 135.6 (CH), 121.6 (CH), 106.4 (C), 101.6 (CH), 65.3 (2 x CH_2), 52.1 (CH_2), 49.2 (CH_3), 28.6 (CH_3). **LRMS** (*m*/*z*) $[M + H]^+$: 320.20.

Synthesis of methyl (2*E*)-3-[1-(cyclohexylmethyl)-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoate (23h). The crude product was purified by silica gel flash chromatography using EtOAc/Hexane (0-50 %) to afford the desired product (64.7 mg, 0.196 mmol, 78 %). **1H NMR** (500 MHz, Chloroform-*d*) δ 8.77 (s, 1H), 7.74 (d, *J* = 16.3 Hz, 1H), 6.62 (d, *J* = 16.4 Hz, 1H), 4.15 (d, *J* = 7.1 Hz, 2H), 3.85 (s, 3H), 3.10 (d, *J* = 4.8 Hz, 3H), 2.02 (dqd, *J* = 11.1, 7.4, 3.6 Hz, 1H), 1.76 – 1.56 (m, 5H), 1.26 – 1.19 (m, 3H), 1.05 (qd, *J* = 12.0, 3.4 Hz, 2H). **13C NMR** (126 MHz, $CDCl_3$) δ 166.5 (C), 162.8 (C), 156.2 (C), 151.6 (CH), 142.8 (C), 134.3 (CH), 123.0 (CH), 104.4 (C), 53.2 (CH_2), 52.3 (CH_3), 38.0 (CH), 30.8 (2 x CH_2), 28.5 (CH_3), 26.3 (CH_2), 25.7 (2 x CH_2). **LRMS** (*m*/*z*) $[M + H]^+$: 330.20.

Synthesis of methyl (2*E*)-3-[1-benzyl-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoate (23i). The crude product was purified by silica gel flash chromatography using EtOAc/Hexane (0-50 %) to afford the desired product (25.1 mg, 0.078 mmol, 52 %). **1H NMR** (500 MHz, Chloroform-*d*) δ 8.77 (s, 1H), 7.73 (dd, J = 16.4, 0.8 Hz, 1H), 7.40 – 7.31 (m, 5H), 6.61 (dd, J = 16.4, 0.9 Hz, 1H), 5.48 (s, 2H), 3.84 (d, J = 0.9 Hz, 3H), 3.16 – 3.08 (m, 3H). **13C NMR** (126 MHz, None) δ 166.4 (C), 159.3 (C), 155.9 (C), 150.9 (CH), 142.0 (C), 135.5 (C), 128.7 (2 x CH), 128.2 (2 x CH), 128.1 (CH), 122.2 (CH), 120.7 (CH), 106.0 (C), 51.9 (CH_3), 50.6 (CH_2), 28.3 (CH_3). **LRMS** (*m*/*z*) $[M + H]^+$: 324.20.

Synthesis of methyl (2*E*)-3-[6-(methylamino)-1-[(3-methylphenyl)methyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoate (23j). The crude product was purified by silica gel flash chromatography using EtOAc/Hexane (0-40 %) to afford the desired product (41 mg, 0.122 mmol, 62 %). **1H NMR** (500 MHz, Chloroform-*d*) δ 8.82 (s, 1H), 7.76 (d, *J* = 16.3 Hz, 1H), 7.23 – 7.15 (m, 3H), 7.12 – 7.09 (m, 1H), 6.61 (d, *J* = 16.3 Hz, 1H), 5.44 (s, 2H), 3.83 (s, 3H), 3.11 (d, *J* = 4.9 Hz, 3H), 2.33 (d, *J* = 0.7 Hz, 3H). **13C NMR** (126 MHz, $CDCl_3$) δ 166.6 (C), 161.2 (C), 156.1 (C), 153.1 (CH), 148.0 (C), 142.3 (C), 138.7 (C), 135.5 (CH), 134.6 (CH), 129.2 (CH), 128.9 (CH), 125.5 (CH), 122.5 (CH), 106.3 (C), 52.2 (CH_2), 50.9 (CH_3), 28.6 (CH_3), 21.5 (CH_3).

Synthesis of methyl (2*E*)-3-{1-[(3-methoxyphenyl)methyl]-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl}prop-2-enoate (23k). The crude product was purified by silica gel flash chromatography using EtOAc/Hexane (0-50 %) to afford the desired product (43.6mg, 0.123 mmol, 63 %). **1H NMR** (500 MHz, DMSO-d_6) δ 9.25 – 9.15 (m, 1H), 7.63 (d, J = 16.3 Hz, 1H), 7.23 (t, J = 7.9 Hz, 1H), 6.90 – 6.78 (m, 4H), 5.42 (s, 2H), 3.75 (s, 3H), 3.71 (s, 3H), 2.88 (d, J = 4.6 Hz, 3H). **13C NMR** (126 MHz, DMSO) δ 166.4 (C), 161.7 (C), 159.3 (C), 155.9 (C), 154.1 (CH), 149.7 (C), 140.1 (C), 138.3 (CH), 135.1 (CH), 129.8 (CH), 120.7 (CH), 119.8 (CH), 113.6 (CH), 112.9 (C), 55.0 (CH_3), 51.7 (CH_3), 49.3 (CH_2), 27.8 (CH_3).

General Synthesis of Intermediate Compounds 24a-k

The appropriate intermediate compound **(23a-k)** (0.046-0.427 mmol) was suspended in THF (10 mL) and 6M NaOH aqueous solution (10 eq.) was added. The reaction was heated at 60°C using a heating magnetic stirrer for 24 hours. Then, the solvent was evaporated under vacuum; the residue obtained was dissolved in water/EtOAc (1:1) and acidified (pH 4) with 1M HCl. The product was extracted with EtOAc, and the combined organic layers were dried under vacuum. The product was allowed to dry in an oven at 40 °C overnight and use without further purification.

Synthesis of (2*E*)-3-[6-(methylamino)-1-(propan-2-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoic acid (24a). Product yield: 16.6 mg, 0.064mmol, 99 %. **^{1}H NMR** (500 MHz, Chloroform-*d*) δ = 9.15 – 9.05 (m, 1H), 7.52 (d, *J*=16.3, 2H), 6.72 (d, *J*=16.2, 1H), 4.93 (s, 1H), 2.87 (d, *J*=4.8, 3H), 1.45 (d, *J*=6.7, 6H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 167.7 (C), 161.2 (C), 154.9 (C), 153.7 (CH), 139.9 (C), 133.6 (CH), 123.5 (CH), 105.5 (C), 47.7 (CH), 27.8 (CH_3), 21.5 (2 x CH_3).

Synthesis of (2*E*)-3-[1-(2,2-dimethylpropyl)-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoic acid (24b). Product yield: 12.9 mg, 0.044 mol, 77%. **^{1}H NMR** (500 MHz, Chloroform-d) δ 8.93 (d, J = 13.5 Hz, 1H), 8.00 – 7.84 (m, 1H), 6.83 – 6.64 (m, 1H), 4.13 (d, J = 7.2 Hz, 2H), 3.07 (d, J = 4.6 Hz, 3H), 1.03 (d, J = 3.0 Hz, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 168.7 (C), 162.5 (C), 157.0 (C), 150.8 (CH), 140.7 (C), 137.0 (CH), 121.2 (CH), 107.1 (C), 58.0 (CH_2) , 34.0 (C), 29.9 (CH_3), 28.1 (3 x CH_3). **LRMS** (*m*/*z*) $[M + H]^+$: 290.10

Synthesis of (2*E*)-3-[6-(methylamino)-1-(3-methylbutyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoic acid (24c). Product yield: 23.1 mg, 0.080 mmol, 54%. **^{1}H NMR** (500 MHz, Methanol-d_4) δ 8.95 (s, 1H), 7.86 (s, 1H), 7.69 (d, *J* = 16.3 Hz, 1H), 6.69 (d, *J* = 16.3 Hz, 1H), 4.33 (t, *J* = 7.1 Hz, 2H), 3.00 (s, 3H), 1.79 (q, *J* = 6.9 Hz, 2H), 1.51 (dh, *J* = 13.4, 6.8 Hz, 1H), 0.98 (d, *J* = 6.6 Hz, 6H). **^{13}C NMR** (126 MHz, MeOD) δ 170.0 (C), 162.9 (C), 157.4 (C), 154.4 (CH), 142.0 (C), 136.1 (CH), 123.3 (CH), 106.9 (C), 45.6 (CH_2), 39.1 (CH_2), 28.5 (CH_3), 26.5 (CH), 22.7 (2 x CH_3).

Synthesis of (2*E*)-3-[1-(2,2-dimethoxyethyl)-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoic acid (24d). Product yield: 27.2 mg, 0.089 mmol, 59 %. **^{1}H NMR** (500 MHz, DMSO-d_6) δ = 9.14 – 9.10 (m, 1H), 7.60 (s, 1H), 7.52 (d, *J*=16.3, 1H), 6.75 (d, *J*=16.3, 1H), 4.89 (t, *J*=6.0, 1H), 4.32 (d, *J*=5.6, 2H), 3.29 (s, 6H), 2.87 (d, *J*=4.9, 3H). **^{13}C NMR** (126 MHz, DMSO) δ 167.5 (C), 161.5 (C), 156.1 (C), 153.9 (CH), 147.8 (C), 140.5 (CH), 133.5 (CH), 118.5 (C), 100.8 (CH), 53.0 (2 x CH_3), 46.8 (CH_2), 27.8 (CH_3). **LRMS** (*m*/*z*) $[M + H]^+$: 308.10.

Synthesis of (2*E*)-3-[1-(2,2-diethoxyethyl)-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoic acid (24e). Product yield: 30.4 mg, 0.091 mmol, 95 %. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 12.56 (s, 1H), 9.13 (s, 1H), 7.58 (dd, *J* = 16.3, 1.2 Hz, 1H), 6.72 (dd, *J* = 16.2, 1.3 Hz, 1H), 5.75 (d, *J* = 1.3 Hz, 1H), 4.99 (s, 1H), 4.35 – 4.27 (m, 2H), 3.67 (p, *J* = 7.5 Hz, 2H), 3.47 – 3.41 (m, 2H), 2.89 – 2.85 (m, 3H), 1.00 (t, J = 7.0 Hz, 6H). **^{13}C NMR** (126 MHz, DMSO) δ 167.2 (C), 162.0 (C), 155.7 (C), 153.9 (CH), 140.1 (C), 134.5 (CH), 122.1 (CH), 105.1 (C), 99.1 (CH), 62.1 (2 x CH_2), 54.9 (CH_2), 29.0 (CH_3), 15.1 (2 x CH_3). **LRMS** (*m*/*z*) $[M + H]^+$: 336.20.

Synthesis of (2*E*)-3-[6-(methylamino)-1-[(oxolan-2-yl)methyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoic acid (24f). Product yield: 64 mg, 0.211 mol, 78%. **^{1}H NMR** (500 MHz, DMSO-d_6) δ = 9.12 (s, 1H), 7.54 (d, *J*=16.3, 2H), 6.72 (d, *J*=16.2, 1H), 4.36 – 4.27 (m, 2H), 4.16 (q, *J*=8.1, 1H), 3.76 (q, *J*=7.0, 1H), 3.64 – 3.59 (m, 1H), 2.89 – 2.82 (m, 3H), 1.92 (ddt, *J*=11.4, 8.4, 6.0, 1H), 1.86 – 1.78 (m, 2H), 1.74 – 1.69 (m, 1H). **^{13}C NMR** (126 MHz, DMSO) δ 167.5 (C), 161.5 (C), 156.1 (C), 153.8 (CH), 140.1 (C), 133.9 (CH), 123.0 (CH), 104.9 (C), 76.3 (CH), 67.1 (CH_2), 49.6 (CH_2), 32.4 (CH_2), 28.6 (CH_3), 24.8 (CH_2). **LRMS** (*m*/*z*) $[M + H]^+$: 304.20.

Synthesis of (2*E*)-3-{1-[(1,3-dioxolan-2-yl)methyl]-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl}prop-2-enoic acid (24g). Product yield: 37.2 mg, 0.122 mol, 63%. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 12.48 (s, 1H), 9.14 (s, 1H), 6.72 (d, *J* = 16.3 Hz, 1H), 5.33 (s, 1H), 4.34 (d, *J* = 4.8 Hz, 2H), 3.93 (s, 2H), 3.82 (td, *J* = 6.7, 5.9, 2.7 Hz, 2H), 2.88 – 2.85 (m, 3H). *The protons of double-bound and amine are hidden in the peaks of residual PPh_3 in the aromatic region. **^{13}C NMR** (126 MHz, DMSO) δ 167.2 (C), 159.5 (C), 156.3 (C), 151.0 (CH), 140.3(C), 136.9 (CH), 122.4 (CH), 108.4 (C), 100.7 (CH), 64.3 (2 x CH_2), 52.6 (CH_2), 29.0 (CH_3).

Synthesis of (2*E*)-3-[1-(cyclohexylmethyl)-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoic acid (24h). Product yield: 37.2 mg, 0.118 mol, 61%. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 12.52 (s, 1H), 9.12 (s, 1H), 7.56 (d, *J* = 16.2 Hz, 2H), 6.70 (d, *J* = 16.3 Hz, 1H), 4.08 (d, *J* = 7.0 Hz, 2H), 2.89 – 2.84 (m, 3H), 1.96 – 1.89 (m, 1H), 1.65 (d, *J* = 8.7 Hz, 2H), 1.58 (s, 2H), 1.52 (d, *J* = 12.8 Hz, 2H), 1.15 (d, *J* = 9.0 Hz, 2H), 1.00 (t, *J* = 12.0 Hz, 2H). **^{13}C NMR** (126 MHz, DMSO) δ 167.4 (C), 161.5 (C), 156.1 (C), 153.8 (CH), 139.8 (C), 134.8 (CH), 122.2 (CH), 104.7 (C), 51.5 (CH_2), 38.1 (CH), 30.1 (2 x CH_2), 27.7 (CH_3), 25.8 (CH_2), 25.0 (2 x CH_2). **LRMS** (*m*/*z*) $[M + H]^+$: 316.20.

Synthesis of (2*E*)-3-[1-benzyl-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoic acid (24i). Product yield: 14.5 mg, 0.047 mol, 63%. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 12.64 (s, 1H), 9.14 (s, 1H), 7.63 (s, 1H), 7.52 (d, J = 16.2 Hz, 1H), 7.36 – 7.24 (m, 5H), 6.71 (d, J = 16.3 Hz, 1H), 5.45 (s, 2H), 2.88 (d, J = 4.7 Hz, 3H). **^{13}C NMR** (126 MHz, DMSO) δ 167.3 (C), 161.6 (C), 156.0 (C), 153.9 (CH), 143.8 (C), 140.0 (CH), 137.6 (C), 134.0 (CH), 128.6 (2 x CH), 127.8 (2 x CH), 127.6 (CH), 102.7 (C), 48.7 (CH_2), 27.8 (CH_3). **LRMS** (*m*/*z*) $[M + H]^+$: 310.20.

Synthesis of (2*E*)-3-[6-(methylamino)-1-[(3-methylphenyl)methyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoic acid (24j). Product yield: 131.2 mg, 0.406mmol, 95%. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 12.58 (s, 1H), 9.14 (s, 1H), 7.65 (s, 1H), 7.55 (d, *J* = 16.3 Hz, 1H), 7.21 (t, *J* = 7.6 Hz, 1H), 7.15 – 7.04 (m, 3H), 6.72 (d, *J* = 16.3 Hz, 1H), 5.40 (s, 2H), 2.88 (d, *J* = 4.7 Hz, 3H), 2.26 (s, 3H). **^{13}C NMR** (126 MHz, DMSO) δ 167.7 (C), 162.0 (C), 156.3 (C), 154.2 (CH), 140.8 (C), 138.2 (C), 137.2 (C), 135.0 (CH), 129.0 (CH), 128.8 (CH), 127.2 (CH), 125.1 (CH), 122.8 (CH), 105.5 (C), 49.8 (CH_2), 28.3 (CH_3), 21.4 (CH_3). **LRMS** (*m*/*z*) $[M + H]^+$: 324.20.

Synthesis of (2*E*)-3-{1-[(3-methoxyphenyl)methyl]-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl}prop-2-enoic acid (24k). Product yield: 33.2 mg, 0.098 mmol, 80%. **^{1}H NMR** (500 MHz, DMSO) δ 12.55 (s, 1H), 9.15 (s, 1H), 7.65 (s, 1H), 7.59 – 7.53 (m, 1H), 7.48 (s, 1H), 7.23 (t, J = 7.6 Hz, 1H), 6.84 (dd, J = 8.2, 2.6 Hz, 1H), 6.71 (dd, J = 16.0, 3.0 Hz, 1H), 5.41 (s, 2H), 3.71 (d, J = 4.0 Hz, 3H), 2.88 (d, J = 5.0 Hz, 3H). **^{13}C NMR** (126 MHz, DMSO) δ 167.2 (C), 161.6 (C), 159.3 (C), 155.9 (C), 154.0 (CH), 140.3 (C), 138.3 (C), 134.5 (CH), 129.7 (CH), 122.2 (CH), 119.8 (CH), 113.6 (CH), 112.8 (CH), 105.0 (C), 55.0 (CH_3), 49.3 (CH_2), 27.8 (CH_3).

General Synthesis of Compounds 2B1-11

The appropriate intermediate compound **(23a-k)** (0.045-0.211 mmol) was dissolved in anhydrous DMF (0.5 mL). To this, the appropriate secondary amine (4 eq.) and DIPEA (3 eq.) were then added. The resulting mixture was stirred for 5 min before adding HATU (1.2 eq.). The reaction vial was flushed with N_2, and the reaction stirred at r.t for 24 hours. The solvent was removed under vacuum and the residue partitioned between water and DCM. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The crude product was purified by silica gel TLC plate to afford the desired product.

Synthesis of (2*E*)-1-(4-methoxypiperidin-1-yl)-3-[6-(methylamino)-1-(propan-2-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-en-1-one (2B1). The crude product was purified by silica gel TLC plate using MeOH/DCM (8%) to afford the desired product (9.5 mg, 0.026 mmol, 44%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 9.00 (s, 1H), 7.64 (d, *J* = 15.7 Hz, 1H), 7.32 (d, J = 15.8 Hz, 1H), 5.04 (p, *J* = 6.8 Hz, 1H), 3.97 (d, *J* = 12.9 Hz, 2H), 3.54 (tt, *J* = 7.5, 3.5 Hz, 2H), 3.50 – 3.43 (m, 1H), 3.39 (s, 3H), 3.00 (s, 3H), 1.96 (s, 2H), 1.67 – 1.59 (m, 2H), 1.52 (d, *J* = 6.7 Hz, 6H). **^{13}C NMR** (126 MHz, MeOD) δ 167.2 (C), 162.9 (C), 156.6 (C), 154.5 (CH), 142.4 (C), 134.3 (CH), 121.6 (CH), 107.3 (C), 76.6 (CH), 56.1 (CH_3), 44.4 (CH), 40.8 (2 x CH_2), 31.4 (2 x CH_2), 28.5 (CH_3), 21.8 (2 x CH_3). **HRMS** (ESI, *m*/*z*) calcd for $C_{18}H_{27}N_6O_2$ $[M + H]^+$: 359.2190; found, 359.2201.

Synthesis of (2*E*)-3-[1-(2,2-dimethylpropyl)-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]-1-(4-methoxypiperidin-1-yl)prop-2-en-1-one (2B2). The crude product was purified by silica gel TLC plate using MeOH/DCM (5 %) to afford the desired product (10.3 mg, 0.027 mmol, 60%).
^{1}H NMR (500 MHz, Methanol-d_4) δ 9.02 (s, 1H), 7.64 (d, *J* = 15.8 Hz, 1H), 7.33 (d, *J* = 15.8 Hz, 1H), 4.11 (s, 2H), 3.97 (d, *J* = 12.6 Hz, 2H), 3.58 – 3.52 (m, 2H), 3.50 – 3.44 (m, 1H), 3.39 (s, 3H), 2.99 (s, 3H), 1.96 (s, 2H), 1.62 (d, *J* = 9.5 Hz, 2H), 1.01 (s, 9H). **^{13}C NMR** (126 MHz, MeOD) δ 157.7 (C), 153.6 (C), 148.7 (C), 145.0 (CH), 132.8 (C), 124.8 (CH), 118.8 (CH), 113.0 (C), 67.2 (CH), 48.8 (CH_2), 46.6 (CH_3), 34.9 (2 x CH_2), 31.2 (2 x CH_2), 25.3(CH_3), 23.1(CH), 19.0 (3 x CH_3). **HRMS** (ESI, *m*/*z*) calcd for $C_{20}H_{31}N_6O_2$ $[M + H]^+$: 387.2503; found, 387.2514.

Synthesis of (2*E*)-1-(4-methoxypiperidin-1-yl)-3-[6-(methylamino)-1-(3-methylbutyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-en-1-one (2B3). The crude product was purified by silica gel TLC plate using MeOH/DCM (3.5 %) to afford the desired product (21.2 mg, 0.055 mmol, 74 %). **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 8.82 (d, J = 4.3 Hz, 1H), 7.76 – 7.68 (m, 1H), 7.19 – 7.14 (m, 1H), 5.51 (s, 1H), 4.30 (q, *J* = 6.1, 4.6 Hz, 2H), 3.96 (s, 1H), 3.86 (s, 1H), 3.53 – 3.44 (m, 3H), 3.39 – 3.32 (m, 3H), 3.08 – 3.02 (m, 3H), 1.89 (ddd, *J* = 14.3, 7.6, 3.9 Hz, 2H), 1.79 (q, *J* = 7.1 Hz, 2H), 1.65 (dp, *J* = 12.1, 4.0 Hz, 2H), 1.54 (td, *J* = 10.7, 8.1, 4.0 Hz, 1H), 1.02 – 0.92 (m, 6H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 164.8 (C), 161.0 (C), 155.7 (C), 152.1 (CH), 140.9 (C), 132.3 (CH), 120.1 (CH), 106.7 (C), 75.1 (CH), 55.7 (CH_3), 44.7 (CH_2), 43.0 (2 x CH_2), 38.0 (CH_2), 31.4 (2 x CH_2), 28.3 (CH_3), 25.4 (CH), 22.2 (2 x CH_3). **HRMS** (ESI, *m*/*z*) calcd for $C_{20}H_{31}N_6O_2$ $[M + H]^+$: 387.2503; found, 387.2504.

Synthesis of (2*E*)-3-[1-(2,2-dimethoxyethyl)-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]-1-(4-methoxypiperidin-1-yl)prop-2-en-1-one (2B4). The crude product was purified by silica gel TLC using MeOH/DCM (5 %); then repurified using EtOAc (100 %) to afford the desired product (21.2 mg, 0.052 mmol, 59 %). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 8.99 (s, 1H), 7.64 – 7.57 (m, 1H), 7.37 – 7.30 (m, 1H), 4.91 (td, J = 5.8, 1.9 Hz, 1H), 4.38 (d, J = 5.5 Hz, 2H), 3.97 (dd, J = 13.4, 6.9 Hz, 2H), 3.54 (tt, J = 6.8, 3.4 Hz, 2H), 3.50 – 3.43 (m, 1H), 3.38 (dd, J = 4.3, 1.6 Hz, 9H), 3.35 (d, J = 1.5 Hz, 1H), 2.99 (d, J = 2.1 Hz, 3H), 1.95 (s, 2H), 1.62 (dd, J = 20.7, 11.0 Hz, 2H). **^{13}C NMR** (126 MHz, MeOD) δ 167.0 (C), 163.1 (C), 157.9 (C), 154.6 (CH), 143.0 (C), 133.9 (CH), 122.2 (CH), 106.9 (C), 102.9 (CH), 76.6 (CH), 56.1 (CH_3), 54.1 (2 x CH_3), 49.8 (CH_2), 44.4 (2 x CH_2), 31.4 (2 x CH_2), 28.5 (CH_3). **HRMS** (ESI, *m*/*z*) calcd for $C_{19}H_{29}N_6O_4$ $[M + H]^+$: 405.2245; found, 405.2276.

Synthesis of (2*E*)-3-[1-(2,2-diethoxyethyl)-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]-1-(4-methoxypiperidin-1-yl)prop-2-en-1-one (2B5). The crude product was purified by silica gel TLC using MeOH/DCM (5 %); then repurified using EtOAc (100 %) to afford the desired product (22.2 mg, 0.051 mmol, 58 %). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 9.00 (s, 1H), 7.62 (dd, *J* = 15.8, 0.6 Hz, 1H), 7.34 (dd, *J* = 15.7, 0.9 Hz, 1H), 5.04 (t, *J* = 5.7 Hz, 1H), 4.38 (d, *J* = 5.7 Hz, 2H), 3.99 – 3.94 (m, 2H), 3.76 (dq, *J* = 9.6, 7.0 Hz, 2H), 3.57 – 3.46 (m, 5H), 3.39 (s, 3H), 3.00 (s, 3H), 1.95 (s, 2H), 1.67 – 1.56 (m, 2H), 1.08 (t, *J* = 7.0 Hz, 6H). **^{13}C NMR** (126 MHz, MeOD) δ 167.0 (C), 163.1 (C), 157.9 (C), 154.6 (CH), 142.9 (C), 133.9 (CH), 122.2 (CH), 106.9 (C), 101.3 (CH), 76.6 (CH), 63.5 (2 x CH_2), 56.1 (CH_3), 54.8 (CH_2), 44.4 (2 x CH_2), 31.4 (2 x CH_2), 28.5 (CH_3), 15.5 (2 x CH_3). **HRMS** (ESI, *m*/*z*) calcd for $C_{21}H_{33}N_6O_4$ $[M + H]^+$: 433.2558; found, 433.2549.

Synthesis of (2*E*)-1-(4-methoxypiperidin-1-yl)-3-[6-(methylamino)-1-[(oxolan-2-yl)methyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-en-1-one (2B6). The crude product was purified by silica gel TLC using MeOH/DCM (8 %); then repurified using EtOAc (100 %) to afford the desired product (36.2 mg, 0.090 mmol, 43 %). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 9.01 (s, 1H), 7.63 (dd, J = 15.8, 0.9 Hz, 1H), 7.35 (dd, J = 15.8, 1.3 Hz, 1H), 4.46 – 4.36 (m, 2H), 4.30 – 4.21 (m, 1H), 3.97 (d, J = 12.5 Hz, 2H), 3.89 (dt, J = 8.3, 6.7 Hz, 1H), 3.74 (ddd, J = 8.4, 7.2, 6.2 Hz, 1H), 3.54 (tt, J = 7.5, 3.5 Hz, 2H), 3.50 – 3.41 (m, 1H), 3.39 (d, J = 0.6 Hz, 3H), 2.99 (s, 3H), 2.07 – 1.77 (m, 6H), 1.67 – 1.56 (m, 2H). **^{13}C NMR** (126 MHz, MeOD) δ 167.1 (C), 163.1 (C), 157.9 (C), 154.6 (CH), 142.8 (C), 133.9 (CH), 122.1 (CH), 107.0 (C), 78.4 (CH), 76.6 (CH), 68.6 (CH_2), 56.1 (CH_3), 51.1 (CH_2), 44.4 (2 x CH_2), 31.4 (2 x CH_2), 30.0 (CH_2), 28.5 (CH_3), 26.3 (CH_2). **HRMS** (ESI, *m*/*z*) calcd for $C_{20}H_{29}N_6O_3$ $[M + H]^+$: 401.2296; found, 401.2295.

Synthesis of (2*E*)-3-{1-[(1,3-dioxolan-2-yl)methyl]-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl}-1-(4-methoxypiperidin-1-yl)prop-2-en-1-one (2B7). The crude product was purified by silica gel TLC using EtOAc (100 %); then repurified using MeOH/DCM (4 %) to afford the desired product (11.2 mg, 0.029 mmol, 25 %). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 9.01 (s, 1H), 7.62 (d, J = 15.7 Hz, 1H), 7.37 (d, J = 15.7 Hz, 1H), 5.36 (t, J = 4.5 Hz, 1H), 4.41 (d, J = 4.5 Hz, 2H), 4.01 – 3.95 (m, 4H), 3.89 – 3.85 (m, 2H), 3.60 – 3.50 (m, 2H), 3.50 – 3.43 (m, 1H), 3.39 (s, 3H), 3.00 (s, 3H), 1.99 – 1.90 (m, 2H), 1.62 (dd, J = 20.9, 12.3 Hz, 2H). **^{13}C NMR** (126 MHz, MeOD) δ 167.0 (C), 163.2 (C), 158.1 (C), 154.6 (CH), 143.0 (C), 133.7 (CH), 122.3 (CH), 106.9 (C), 102.7(CH), 76.63 (CH), 66.1 (2 x CH_2), 56.1 (CH_2, CH_3), 44.4 (2 x CH_2), 31.4 (2 x CH_2), 28.5 (CH_3). **HRMS** (ESI, *m*/*z*) calcd for $C_{19}H_{27}N_6O_4$ $[M + H]^+$: 403.2088; found, 403.2101.

Synthesis of (2*E*)-3-[1-(cyclohexylmethyl)-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]-1-(4-methoxypiperidin-1-yl)prop-2-en-1-one (2B8). The crude product was purified by silica gel TLC using MeOH/DCM (8 %) to afford the desired product (34.2 mg, 0.083 mmol, 72%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 9.03 – 8.99 (m, 1H), 7.67 – 7.59 (m, 1H), 7.38 – 7.30 (m, 1H), 4.15 – 4.11 (m, 2H), 3.98 (s, 2H), 3.55 (qd, *J* = 8.0, 7.3, 4.3 Hz, 2H), 3.46 (d, *J* = 14.1 Hz, 1H), 3.39 (dd, **J** = 3.9, 2.6 Hz, 3H), 3.01 – 2.98 (m, 3H), 2.82 (dd, *J* = 3.8, 2.5 Hz, 1H), 2.06 – 1.89 (m, 3H), 1.76 – 1.71 (m, 2H), 1.68 – 1.65 (m, 1H), 1.60 (d, *J* = 13.1 Hz, 3H), 1.28 – 1.20 (m, 3H), 1.06 (d, *J* = 12.3 Hz, 2H). **^{13}C NMR** (126 MHz, MeOD) δ 167.1 (C), 163.1 (C), 157.8 (C), 154.6 (CH), 142.5 (C), 134.0 (CH), 121.9 (CH), 106.8 (C), 76.6 (CH), 56.1 (CH_3), 53.3 (CH_2), 44.4 (2 x CH_2), 32.5 (CH), 31.8 (2 x CH_2), 31.4 (2 x CH_2), 28.5 (CH_3), 27.4 (CH_2), 26.7 (2 x CH_2). **HRMS** (ESI, *m*/*z*) calcd for $C_{22}H_{33}N_6O_2$ $[M + H]^+$: 413.2660; found, 413.2663.

Synthesis of (2*E*)-3-[1-benzyl-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]-1-(4-methoxypiperidin-1-yl)prop-2-en-1-one (2B9). The crude product was purified by silica gel TLC using MeOH/DCM (8 %) to afford the desired product (15.4 mg, 0.038 mmol, 82 %). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 9.02 (s, 1H), 7.61 (d, *J* = 15.7 Hz, 1H), 7.36 – 7.24 (m, 6H), 5.47 (s, 2H), 3.95 (s, 2H), 3.53 (dp, *J* = 7.5, 3.6 Hz, 2H), 3.49 – 3.42 (m, 1H), 3.38 (s, 3H), 3.01 (s, 3H), 1.91 (d, *J* = 18.4 Hz, 2H), 1.65 – 1.56 (m, 2H). **^{13}C NMR** (126 MHz, MeOD) δ 167.0 (C), 163.3 (C), 157.5 (C), 154.7 (CH), 143.4 (C), 138.1 (C), 133.8 (CH), 129.6 (2 x CH), 129.0 (2 x CH), 128.8 (CH), 122.3 (CH), 107.0 (C), 76.6 (CH), 56.1 (CH_3), 50.9 (CH_2), 44.4 (2 x CH_2), 31.4 (2 x CH_2), 28.5 (CH_3). **HRMS** (ESI, *m*/*z*) calcd for $C_{22}H_{27}N_6O_2$ $[M + H]^+$: 407.2190; found, 407.2195.

Synthesis of (2*E*)-1-(4-methoxypiperidin-1-yl)-3-[6-(methylamino)-1-[(3-methylphenyl)methyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-en-1-one (2B10). The crude product was purified by silica gel TLC using MeOH/DCM (8.5 %); then repurified using MeOH/DCM (5 %) to afford the desired product (28.1 mg, 0.067 mmol, 84 %). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 9.03 (s, 1H), 7.61 (d, *J* = 15.7 Hz, 1H), 7.34 (d, *J* = 15.7 Hz, 1H), 7.21 – 7.13 (m, 2H), 7.09 (t, *J* = 8.0 Hz, 2H), 5.43 (s, 2H), 3.99 – 3.93 (m, 2H), 3.53 (tt, *J* = 7.4, 3.6 Hz, 2H), 3.49 – 3.42 (m, 1H), 3.38 (s, 3H), 3.01 (s, 3H), 2.29 (s, 3H), 1.94 (s, 2H), 1.66 – 1.56 (m, 2H). **^{13}C NMR** (126 MHz, MeOD) δ 167.1 (C), 163.3 (C), 157.5 (C), 154.7 (CH), 143.0 (C), 139.5 (C), 138.0 (C), 133.8 (CH), 129.6 (CH), 129.6 (CH), 129.5 (CH), 126.1 (CH), 122.3 (CH), 107.0 (C), 76.6 (CH),

56.1 (CH_3), 50.7 (CH_2), 44.4 (2 x CH_2), 31.4 (2 x CH_2), 28.5 (CH_3), 21.4 (CH_3). **HRMS** (ESI, *m/z*) calcd for $C_{23}H_{29}N_6O_2$ [M + H]$^+$: 421.2347; found, 421.2345.

Synthesis of (2*E*)-3-{1-[(3-methoxyphenyl)methyl]-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl}-1-(4-methoxypiperidin-1-yl)prop-2-en-1-one (2B11). The crude product was purified by silica gel TLC using MeOH/DCM (8 %); then repurified using MeOH/DCM (5 %) to afford the desired product (28.1 mg, 0.067 mmol, 84 %). **^{1}H NMR** (500 MHz, Methanol-d_4) δ = 9.01 (s, 1H), 7.61 (dd, *J*=15.8, 1.0, 1H), 7.33 (dd, *J*=15.8, 1.0, 1H), 7.24 – 7.17 (m, 1H), 6.91 – 6.84 (m, 2H), 6.81 (ddt, *J*=8.3, 2.3, 1.0, 1H), 5.42 (s, 2H), 3.99 – 3.90 (m, 2H), 3.74 (d, *J*=1.0, 3H), 3.56 – 3.49 (m, 2H), 3.48 – 3.41 (m, 1H), 3.37 (d, *J*=1.0, 3H), 3.01 (d, *J*=1.0, 3H), 1.98 – 1.88 (m, 2H), 1.65 – 1.55 (m, 2H). **^{13}C NMR** (126 MHz, MeOD) δ 167.1 (C), 163.3 (C), 157.5 (C), 154.7 (CH), 143.0 (C), 139.5 (C), 138.0 (C), 133.8 (CH), 129.6 (CH), 129.6 (CH), 129.5 (CH), 126.1 (CH), 122.3 (CH), 107.0 (C), 76.6 (CH), 56.1 (CH_3), 50.9 (CH_2), 44.40 (2 x CH_2), 31.4 (2 x CH_2), 28.5 (CH_3), 21.4 (CH_3). **HRMS** (ESI, *m/z*) calcd for $C_{23}H_{29}N_6O_3$ [M + H]$^+$: 437.2296; found, 437.2298.

6. Synthesis and characterisation of Compounds 2C1-4

General Synthesis of Library 2C Compounds

Intermediate compound (2*E*)-3-[6-(methylamino)-1-[(3-methylphenyl)methyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoic acid **(24j)** (0.080 mmol) was dissolved in anhydrous DMF (0.5 mL). To this, the appropriate secondary amine (4 eq.) and DIPEA (3 eq.) were then added. The resulting mixture was stirred for 5 min before adding HATU (1.2 eq.). The reaction vial was flushed with N_2, and the reaction stirred at r.t for 24 hours. The solvent was removed under vacuum and the residue partitioned between water and DCM. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The crude product was purified by silica gel TLC plate to afford the desired product.

Synthesis of (2*E*)-1-(3-methoxypiperidin-1-yl)-3-[6-(methylamino)-1-[(3-methylphenyl)methyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-en-1-one (2C1). The crude product was purified by silica gel TLC plate using MeOH/DCM (5 %); then using MeOH/DCM (8 %) to afford the desired product (14 mg, 0.033 mmol, 42%). **^{1}H NMR** (500 MHz, Chloroform-*d*) δ = 8.83 (s, 1H), 7.76 – 7.68 (m, 1H), 7.20 (ddt, *J*=16.6, 12.3, 4.9, 4H), 7.09 (d, *J*=7.5, 1H), 6.06 (s, 1H), 5.44 (d, *J*=3.8, 2H), 4.15 (d, *J*=12.6, 1H), 3.75 (d, *J*=49.0, 1H), 3.41 (s, 3H), 3.38 (s, 2H), 3.31 (tt, *J*=7.3, 3.4, 1H), 3.10 (t, *J*=4.7, 3H), 2.32 (d, *J*=3.7, 3H), 1.99 (d, *J*=14.1, 1H), 1.86 (s, 1H), 1.65 (s, 1H), 1.52 (s, 1H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 165.1 (C), 160.1 (C), 156.1 (C), 150.4 (CH), 142.3 (C), 138.5 (C), 136.1 (C), 132.1 (CH), 129.0 (CH), 128.9 (CH), 128.7 (CH), 125.3 (CH), 121.4 (CH), 106.8 (C), 74.7 (CH), 56.3 (CH_3), 50.5 (CH_2), 45.8 (CH_2), 43.1 (CH_2), 30.5 (CH_2), 28.6 (CH_3), 23.6 (CH_2), 21.5 (CH_3). **HRMS** (ESI, *m/z*) calcd for $C_{23}H_{29}N_6O_2$ [M + H]$^+$: 421.2347; found, 421.2354.

Synthesis of (2*E*)-1-[4-(methoxymethyl)piperidin-1-yl]-3-[6-(methylamino)-1-[(3-methylphenyl)methyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-en-1-one (2C2). The crude product was purified by silica gel TLC plate using MeOH/DCM (5 %); then using MeOH/DCM (8 %) to afford the desired product (17.6 mg, 0.041 mmol, 51 %). **^{1}H NMR** (500 MHz, Methanol-d_4) δ 9.02 (s, 1H), 7.60 (d, *J* = 15.8 Hz, 1H), 7.33 (d, *J* = 15.8 Hz, 1H), 7.21 – 7.13 (m, 2H), 7.12 – 7.05 (m, 2H), 5.43 (s, 2H), 4.65 – 4.59 (m, 1H), 4.26 (d, *J* = 13.7 Hz, 1H), 3.33 (s, 3H), 3.27 (dd, *J* = 6.3, 1.3 Hz, 2H), 3.24 – 3.16 (m, 1H), 3.01 (s, 3H), 2.82 – 2.74 (m, 1H), 2.29 (s, 3H), 1.94 – 1.79 (m, 3H), 1.31 – 1.18 (m, 2H). **^{13}C NMR** (126 MHz, MeOD) δ 167.0 (C), 163.3 (C), 157.5 (C), 154.7 (CH), 143.0 (C), 139.5 (C), 138.0 (C), 133.7 (CH), 129.6 (CH), 129.6 (CH), 129.5 (CH), 126.1 (CH), 122.4 (CH), 107.0 (C), 78.3 (CH_2), 59.1 (CH_3), 50.9 (CH_2), 43.6 (2 x CH_2), 37.6 (CH), 31.0 (2 x CH_2), 28.5 (CH_3), 21.4 (CH_3). **HRMS** (ESI, *m/z*) calcd for $C_{24}H_{31}N_6O_2$ [M + H]$^+$: 435.2503; found, 435.2514. **LRMS** (*m/z*) [M + H]$^+$: 435.50.

Synthesis of (2*E*)-1-(4-ethoxypiperidin-1-yl)-3-[6-(methylamino)-1-[(3-methylphenyl)methyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-en-1-one (2C3). The crude product was purified by silica gel TLC plate using MeOH/DCM (5 %) to afford the desired product (19.4 mg, 0.045 mmol, 56%). **^{1}H NMR** (500 MHz, Methanol-d_4) δ = 9.03 (s, 1H), 7.61 (dd, *J*=15.7, 1.1, 1H), 7.34 (dd, *J*=15.7, 1.1, 1H), 7.17 (dd, *J*=15.8, 8.2, 2H), 7.09 (t, *J*=8.1, 2H), 5.43 (s, 2H), 4.00 (s, 2H), 3.63 (tt, *J*=7.7, 3.6, 1H), 3.57 (q, *J*=7.0, 2H), 3.51 (d, *J*=11.3, 1H), 3.45 – 3.38 (m, 1H), 3.02 (d, *J*=1.2, 3H), 2.29 (s, 3H), 1.92 (d, *J*=17.8, 2H), 1.60 (dd, *J*=20.8, 11.1, 2H), 1.20 (td, *J*=7.0, 1.2, 3H). **^{13}C NMR** (126 MHz, MeOD) δ 167.1 (C), 163.3 (C), 157.5 (C), 154.7 (CH), 143.0 (C), 139.5 (C), 138.0 (C), 133.8 (CH), 129.6 (CH), 129.6 (CH), 129.5 (CH), 126.1 (CH),

122.3 (CH), 107.0 (C), 75.0 (CH_2), 64.5 (CH_2), 50.9 (CH_2), 44.6 (2 x CH_2), 32.0 (2 x CH_2), 28.5 (CH_3), 21.4 (CH_3), 15.9 (CH_3). **HRMS** (ESI, *m*/*z*) calcd for $C_{24}H_{31}N_6O_2$ $[M + H]^+$: 435.2503; found, 435.2501.

Synthesis of (2*E*)-1-{1,4-dioxa-8-azaspiro[4.5]decan-8-yl}-3-[6-(methylamino)-1-[(3-methylphenyl)methyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-en-1-one (2C4). The crude product was purified by silica gel TLC plate using MeOH/DCM (3 %) to afford the desired product (20.9 mg, 0.047 mmol, 58 %). **^{1}H NMR** (500 MHz, Methanol-d_4) δ = 9.03 (s, 1H), 7.62 (d, *J*=15.7, 1H), 7.35 (d, *J*=15.8, 1H), 7.21 – 7.13 (m, 2H), 7.12 – 7.05 (m, 2H), 5.43 (s, 2H), 3.99 (s, 4H), 3.79 (p, *J*=5.6, 4H), 3.01 (s, 3H), 2.29 (s, 3H), 1.75 (dt, *J*=22.1, 5.6, 4H). **^{13}C NMR** (126 MHz, MeOD) δ 167.1 (C), 163.3 (C), 157.5 (C), 154.7 (CH), 142.9 (C), 139.5 (C), 137.9 (C), 133.9 (CH), 129.6 (CH), 129.6 (CH), 129.5 (CH), 126.1 (CH), 122.2 (CH), 107.8 (C), 107.0 (C), 65.5 (2 x CH_2), 50.9 (CH_2), 45.3 (CH_2), 41.8 (CH_2), 36.8 (CH_2), 35.8 (CH_2), 28.5 (CH_3), 21.4 (CH_3). **HRMS** (ESI, *m*/*z*) calcd for $C_{24}H_{29}N_6O_3$ $[M + H]^+$: 449.2296; found, 449.2301.

7. Synthesis and Characterization of Compounds 2D1-7

General Synthesis of Intermediate Compounds 27a-g

3-iodo-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine **(7)** (0.363-0.041 mmol) was suspended in DMF (3.5 mL), and sodium hydride was added (1.4 eq., 60% dispersion in mineral oil), and the mixture stirred until gas evolution subsided. To this, the appropriate bromoalkane (1.5 eq.) was added, and the mixture was heated at 150 ˚C using microwave irradiation for 85 min. Then, the mixture was partitioned between EtOAc and water, and the organic layers separated. The combined organic layers were washed with brine, dried over MgSO4, and concentrated under vacuum. The crude product was purified by silica gel flash chromatography.

Synthesis of 3-iodo-*N*-methyl-1-[(2-methylphenyl)methyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (27a). The crude product was purified by silica gel flash chromatography EtOAc/Hexane (0-35 %) to obtain the pure product (67.4 mg, 0.178 mol, 44 %). **^{1}H NMR** (500 MHz, DMSO-d_6) δ = 8.45 (s, 1H), 7.68 (s, 1H), 7.20 – 7.17 (m, 2H), 7.15 – 7.04 (m, 2H), 5.39 (s, 2H), 2.86 (d, *J*=4.2, 3H), 2.37 (d, *J*=10.0, 3H). **^{13}C NMR** (126 MHz, DMSO) δ 162.1 (C), 155.2 (C), 153.9 (CH), 136.0 (C), 134.9 (CH), 130.2(CH), 128.9 (CH), 127.7 (CH), 126.0 (C), 111.6 (C), 92.7 (C), 47.7 (CH_2), 27.3 (CH_3), 18.9 (CH_3).

Synthesis of 3-iodo-1-[(2-methoxyphenyl)methyl]-*N*-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (27b). The crud product was purified by silica gel flash chromatography EtOAc/Hexane (0-45 %) to obtain the pure product (103.8 mg, 0.263 mol, 66 %). **^{1}H NMR** (500 MHz, DMSO-d_6) δ = 8.50 (d, *J*=44.9, 1H), 7.58 (d, *J*=61.5, 1H), 7.30 – 7.23 (m, 1H), 7.02 (d, *J*=8.2, 1H), 6.88 – 6.83 (m, 2H), 5.37 (s, 2H), 3.80 (s, 3H), 2.88 – 2.82 (m, 3H). **^{13}C NMR** (126 MHz, DMSO) δ 162.2 (C), 156.5 (C), 155.6 (C), 153.8 (CH), 128.9 (CH), 128.4 (C), 127.4 (CH), 124.7 (C), 120.3 (CH), 110.9 (CH), 92.7 (C), 55.5 (CH_3), 44.2 (CH_2), 27.9 (CH_3).

Synthesis of 3-iodo-*N*-methyl-1-{[2-(trifluoromethyl)phenyl]methyl}-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (27c). The product was purified by silica gel flash chromatography EtOAc/Hexane (0-40 %) to obtain the pure product (92.9 mg, 0.214 mol, 54%). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.50 (s, 1H), 7.81 – 7.76 (m, 1H), 7.72 (s, 1H), 7.61 (t, *J* = 7.7 Hz, 1H), 7.52 (t, *J* = 7.6 Hz, 1H), 7.08 (d, *J* = 7.8 Hz, 1H), 5.59 (s, 2H), 2.80 (d, *J* = 4.6 Hz, 3H). **^{13}C NMR** (126 MHz, DMSO) δ 162.3 (C), 155.8 (C), 154.0 (CH), 135.0 (2 x C), 133.0 (CH), 129.4 (CH), 128.2 (CH), 126.0 (CH), 124.26 (q, $J_{C,F}$ =274.0, C), 111.7 (C), 93.6 (C), 45.7 (CH_2), 27.7 (CH_3). **LRMS** (*m*/*z*) $[M + H]^+$: 434.10

Synthesis of 3-iodo-*N*-methyl-1-[(4-methylphenyl)methyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (27d). The crude product was purified by silica gel flash chromatography EtOAc/Hexane (0-30 %) to obtain the pure product (85 mg, 0.224 mol, 56%). **^{1}H NMR** (500 MHz, DMSO-d_6) δ = 8.52 – 8.39 (m, 1H), 7.52 (s, 1H), 7.18 – 7.10 (m, 4H), 5.34 (s, 2H), 2.88 (d, *J*=4.7, 3H), 2.25 (s, 3H). **LRMS** (*m*/*z*) $[M + H]^+$: 380.20

Synthesis of 3-iodo-1-[(4-methoxyphenyl)methyl]-*N*-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (27e). The crude product was purified by silica gel flash chromatography EtOAc/Hexane (0-40 %) to obtain the pure product (40.9 mg, 0.103 mol, 28%). **^{1}H NMR** (500 MHz, Chloroform-*d*) δ = 8.32 (s, 1H), 7.36 – 7.32 (m, 2H), 6.88 – 6.84 (m, 2H), 5.39 (s,

2H), 3.78 (s, 3H), 3.13 (d, *J*=4.8, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 159.8 (C), 155.4 (C), 147.5 (C), 130.2 (CH), 130.1 (C), 127.4 (2 x CH), 114.9 (C), 113.0 (2 x CH), 93.2 (C), 55.4 (CH_3), 50.8 (CH_2), 28.6 (CH_3).

Synthesis of 3-iodo-*N*-methyl-1-{[4-(trifluoromethyl)phenyl]methyl}-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (27f). The crude product was purified by silica gel flash chromatography EtOAc/Hexane (0-40 %) to obtain the pure product (81.4 mg, 0.187 mol, 40%). **^{1}H NMR** (500 MHz, Chloroform-*d*) δ = 8.40 (d, *J*=8.1, 1H), 7.63 – 7.56 (m, 2H), 7.46 (d, *J*=8.2, 2H), 6.04 (s, 1H), 5.50 (d, *J*=3.5, 2H), 3.08 (t, *J*=3.9, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 160.9 (C), 155.9 (C), 152.0 (CH), 140.0 (C), 130.52 (q, $J_{C,F}$ =32.6, C), 128.6 (2 x CH), 125.9 (q, $J_{C,F}$ =3.9, 2 x CH), 124.1 (q, $J_{C,F}$ =272.1, C), 113.0 (C), 92.1 (C), 50.1 (CH_2), 28.7 (CH_3). **LRMS** (*m*/*z*) $[M + H]^+$: 434.10.

Synthesis of 3-iodo-*N*-methyl-1-{[3-(trifluoromethyl)phenyl]methyl}-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (27g). The crude product was purified by silica gel flash chromatography EtOAc/Hexane (0-40 %) to obtain the pure product (53.6 mg, 0.124 mmol, 30%). **^{1}H NMR** (500 MHz, DMSO-d_6) δ = 8.47 (s, 1H), 7.78 – 7.71 (m, 2H), 7.67 (d, *J*=7.7, 1H), 7.59 – 7.51 (m, 2H), 5.51 (s, 2H), 2.87 (d, *J*=4.6, 3H). **^{13}C NMR** (126 MHz, DMSO) δ 162.2 (C), 155.4 (C), 154.0 (C), 138.3 (CH), 132.0 (CH), 129.8 (CH), 129.06 (q, $J_{C,F}$ =31.5, C), 124.7 (CH), 124.5 (CH), 124.05 (q, $J_{C,F}$ =272.4, C), 111.7 (C), 93.2 (C), 48.9 (CH_2), 28.9 (CH_3).

General Synthesis of Intermediate Compounds 28 a-g

The appropriate intermediate compound **(27a-g)** (0.112-0.386 mmol) was dissolved in DMF/water/TEA (7.8 mL/0.78 mL/0. 78 mL). To this, TBAI (2 eq.), methyl acrylate (10 eq.) and $Pd(PPh_3)_2Cl_2$ (0.2 eq) were added. The reaction vial was flushed with N_2 for 10 minutes and then heated at 80 °C using a heating magnetic stirrer for 24 hours. After cooling to r.t, the mixture was extracted with DCM. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered through a plug of Celite ® and concentrated under vacuum. The crude product was purified by silica gel flash chromatography.

Synthesis of methyl (2*E*)-3-[6-(methylamino)-1-[(2-methylphenyl)methyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoate (28a). The crude product was purified by silica gel flash chromatography using EtOAc/Hexane (0-50 %) to afford the desired product (20.7 mg, 0.061 mmol, 36 %). **^{1}H NMR** (500 MHz, Chloroform-*d*) δ = 8.83 (s, 1H), 7.75 (d, *J*=16.4, 1H), 7.25 – 7.19 (m, 3H), 7.16 (qd, *J*=7.2, 2.1, 1H), 6.61 (d, *J*=16.3, 1H), 6.51 (s, 1H), 5.49 (s, 2H), 3.83 (s, 3H), 3.09 (d, *J*=4.9, 3H), 2.49 (d, *J*=6.5, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 166.7 (C), 159.2 (C), 156.2 (C), 149.4 (CH), 142.0 (C), 136.6 (CH), 134.9 (C), 133.9 (C), 130.7 (CH), 129.5 (CH), 128.5 (CH), 126.4 (CH), 122.1 (CH), 106.3 (C), 52.2 (CH_3), 48.4 (CH_2), 28.6 (CH_3), 19.6 (CH_3).

Synthesis of methyl (2*E*)-3-{1-[(2-methoxyphenyl)methyl]-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl}prop-2-enoate (28b). The crude product was by silica gel flash chromatography using EtOAc/Hexane (10-65 %) to afford the desired product (60.3 mg, 0.170 mmol, 82 %). **^{1}H NMR** (500 MHz, Chloroform-*d*) δ = 8.84 (s, 1H), 7.77 (d, *J*=16.3, 1H), 7.29 – 7.26 (m, 1H), 7.01 (d, *J*=7.2, 1H), 6.91 – 6.86 (m, 2H), 6.61 (d, *J*=16.3, 1H), 6.45 (d, *J*=16.4, 1H), 5.53 (s, 2H), 3.85 (s, 3H), 3.82 (s, 3H), 3.08 (d, *J*=4.9, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 166.8 (C), 159.6 (C), 157.2 (C), 156.5 (C), 149.4 (CH), 141.9 (C), 135.1 (CH), 129.4 (CH), 129.2 (C), 124.2 (CH), 121.9 (CH), 120.7 (CH), 110.7 (CH), 106.3 (C), 55.7 (CH_3), 52.1 (CH_3), 45.6 (CH_2), 28.6 (CH_3).

Synthesis of methyl (2*E*)-3-[6-(methylamino)-1-{[2-(trifluoromethyl)phenyl]methyl}-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoate (28c). The crude product was by silica gel flash chromatography using EtOAc/Hexane (0-40 %) to afford the desired product (31 mg, 0.079 mmol, 38 %). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 9.23 (s, 1H), 7.83 – 7.77 (m, 1H), 7.70 – 7.50 (m, 4H), 7.10 (s, 1H), 6.85 (d, *J* = 16.3 Hz, 1H), 5.65 (s, 2H), 3.76 (s, 3H), 2.81 (s, 3H). **^{13}C NMR** (126 MHz, DMSO) δ 166.3 (C), 161.7 (C), 156.3 (C), 154.2 (CH), 140.6 (C), 134.9 (CH), 133.0 (C), 129.3(CH), 128.2 (CH), 127.5 (CH), 126.0 (C), 125.3 (CH), 123.2 (C), 121.0 (CH), 104.3 (C), 51.7 (CH_3), 45.8 (CH_2), 27.6 (CH_3).

Synthesis of ethyl (2*E*)-3-[6-(methylamino)-1-[(4-methylphenyl)methyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoate (28d). The crude product was by silica gel flash chromatography using EtOAc/Hexane (0-40 %) to afford the desired product (16.6 mg, 0.049 mmol, 23 %). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 9.17 (s, 1H), 7.62 (d, *J* = 16.3 Hz, 2H), 7.18 (s, 2H), 7.13 (d, *J* = 7.5 Hz, 2H), 6.79 (d, *J* = 16.3 Hz, 1H), 5.40 (s, 2H), 3.75 (s, 3H), 2.88 (d, *J* = 4.8 Hz, 3H), 2.25 (s,

3H). **^{13}C NMR** (126 MHz, DMSO) δ 166.4 (C), 161.6 (C), 155.8 (C), 154.0 (CH), 140.0 (C), 136.9 (C), 135.1 (CH), 134.3 (CH), 129.1 (2 x CH), 127.8 (2 x CH), 120.5 (CH), 104.8 (C), 51.6 (CH_3), 49.2 (CH_2), 27.8 (CH_3), 20.6 (CH_3). **LRMS** (*m*/*z*) [M + H]$^+$: 338.30.

Synthesis of methyl (2*E*)-3-{1-[(4-methoxyphenyl)methyl]-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl}prop-2-enoate (28e). The crude product was purified by silica gel flash chromatography using EtOAc/Hexane (0-50 %) to afford the desired product (20 mg, 0.057 mmol, 51 %). **^{1}H NMR** (500 MHz, Chloroform-*d*) δ = 8.81 (s, 1H), 7.75 (d, *J*=16.3, 1H), 7.37 – 7.31 (m, 2H), 6.88 – 6.82 (m, 2H), 6.60 (d, *J*=16.3, 1H), 6.42 (d, *J*=36.9, 1H), 5.41 (s, 2H), 3.82 (s, 3H), 3.77 (s, 3H), 3.11 (d, *J*=4.9, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 166.8 (C), 159.7 (C), 156.0 (C),153.2 (C), 149.9 (CH), 141.9 (C), 135.0 (CH), 130.0 (2 x CH), 128.0 (C), 122.0 (CH), 114.3 (2 x CH), 106.5 (C), 55.4 (CH_3), 52.1 (CH_2), 50.3 (CH_3), 28.6 (CH_3).

Synthesis of methyl (2*E*)-3-[6-(methylamino)-1-{[4-(trifluoromethyl)phenyl]methyl}-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoate (28f). The crude product was by silica gel flash chromatography using EtOAc/Hexane (0-60 %); then using MeOH/DCM (0-2 %) to afford the desired product (25.3 mg, 0.065 mmol, 18 %). **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 8.86 (s, 1H), 7.75 (d, *J* = 16.4 Hz, 1H), 7.59 (d, *J* = 8.0 Hz, 2H), 7.50 – 7.44 (m, 2H), 6.64 (d, *J* = 16.3 Hz, 1H), 6.47 (d, *J* = 16.3 Hz, 1H), 5.53 (s, 2H), 3.83 (s, 3H), 3.09 (d, *J* = 4.9 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 166.7 (C), 159.6 (C), 156.3 (C), 149.9 (CH), 142.3 (C), 134.7 (CH), 132.3 (C), 130.6 (q, $J_{C,F}$=32.4, C), 128.7 (2 x CH), 125.9 (q, $J_{C,F}$=3.9, 2 x CH), 124.07 (q, $J_{C,F}$=272.2, C), 122.5 (CH), 106.7 (C), 52.2 (CH_3), 50.1 (CH_2), 28.6 (CH_3).

Synthesis of methyl (2*E*)-3-[6-(methylamino)-1-{[3-(trifluoromethyl)phenyl]methyl}-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoate (28g). The crude product was by silica gel flash chromatography using EtOAc/Hexane (0-40 %) to afford the desired product (29.5 mg, 0.075 mmol, 61 %). **^{1}H NMR** (500 MHz, Chloroform-*d*) δ = 8.84 (s, 1H), 7.74 (d, *J*=16.3, 1H), 7.71 (s, 1H), 7.57 (t, *J*=9.0, 2H), 7.47 (t, *J*=7.8, 1H), 6.96 (s, 1H), 6.63 (d, *J*=16.3, 1H), 5.52 (s, 2H), 3.84 (s, 3H), 3.11 (d, *J*=4.8, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 166.7 (C), 159.3 (C), 156.3 (C), 149.7 (CH), 142.3 (C), 136.6 (C), 134.7 (CH), 131.9 (CH), 131.3 (q, $J_{C,F}$=32.5, C), 129.5 (CH), 125.5 (CH), 125.3 (q, $J_{C,F}$=3.8, CH), 124.0 (q, $J_{C,F}$=272.4, C), 122.5 (CH), 106.3 (C), 52.2 (CH_3), 50.3 (CH_2), 28.5 (CH_3).

General Synthesis of Intermediate Compounds 29a-g

The appropriate intermediate compound **(28a-g)** (0.039-0.172 mmol) was suspended in THF (10 mL) and 6M NaOH aqueous solution (10 eq.) was added. The reaction was heated at 60°C using a heating magnetic stirrer for 24 hours. Then, the solvent was evaporated under vacuum; the residue obtained was dissolved in water/EtOAc (1:1) and acidified (pH 4) with 1M HCl. The product was extracted with EtOAc, and the combined organic layers were dried under vacuum. The product was allowed to dry in an oven at 40 °C overnight and use without further purification.

Synthesis of (2*E*)-3-[6-(methylamino)-1-[(2-methylphenyl)methyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoic (29a). Product yield: 28.1 mg, 0.0.87 mmol, 94 %. **^{1}H NMR** (500 MHz, DMSO-d_6) δ = 12.44 (s, 1H), 9.15 (s, 1H), 7.63 (s, 1H), 7.56 (dd, *J*=16.3, 2.4, 1H), 7.19 (q, *J*=5.2, 2H), 7.12 (s, 2H), 5.44 (s, 2H), 2.87 (d, *J*=5.0, 3H), 2.42 (s, 3H), 1.24 (q, *J*=4.9, 3.7, 1H). **^{13}C NMR** (126 MHz, DMSO) δ 167.2 (C), 161.6 (C), 155.8 (C), 154.0 (CH), 140.2 (C), 136.0 (CH), 134.8 (C), 134.5 (C), 130.2 (CH), 128.7 (CH), 127.7 (CH), 125.9 (CH), 122.1 (CH), 105.2 (C), 47.2 (CH_2), 29.0 (CH_3), 18.9 (CH_3).

Synthesis of (2*E*)-3-{1-[(2-methoxyphenyl)methyl]-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl}prop-2-enoic acid (29b). Product yield: 108 mg, 0.318 mmol, 100%. **^{1}H NMR** (500 MHz, DMSO-d_6) δ = 12.55 (s, 1H), 9.15 (s, 1H), 7.57 (s, 1H), 7.55 (d, *J*=16.2, 1H), 7.29 – 7.24 (m, 1H), 7.03 (d, *J*=8.2, 1H), 6.85 (t, *J*=6.8, 2H), 6.73 (dd, *J*=16.5, 2.8, 1H), 5.42 (s, 2H), 3.82 (s, 3H), 2.85 (s, 3H). **^{13}C NMR** (126 MHz, DMSO) δ 167.2 (C), 161.6 (C), 156.5 (C), 156.2 (C), 153.9 (CH), 140.3 (C), 134.4 (CH), 129.8 (2 x CH), 128.9 (C), 124.5 (CH), 122.4 (CH), 120.3 (CH), 110.9 (C), 55.5 (CH_3), 44.2 (CH_2), 29.4 (CH_3).

Synthesis of (2*E*)-3-[6-(methylamino)-1-{[2-(trifluoromethyl)phenyl]methyl}-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoic acid (29c). Product yield: 33.6 mg, 0.089 mmol, 100%. **^{1}H NMR** (500 MHz, DMSO-d_6) δ = 12.58 (s, 1H), 9.18 (s, 1H), 7.79 (dd, *J*=7.8, 1.4, 1H), 7.67 (s, 1H), 7.59 (d, *J*=3.4, 1H), 7.56 – 7.49 (m, 2H), 7.08 (s, 1H), 6.75 (d, *J*=16.3, 1H), 5.64 (s, 2H), 2.94 – 2.75 (m, 3H). **^{13}C NMR** (126 MHz, DMSO) δ 167.2 (C), 161.7 (C), 156.3 (C), 154.1 (CH), 140.9

(C), 134.9 (CH), 134.2 (CH), 133.0 (C) , 129.2 (CH) , 128.2 (CH), 126.2 (C), 126.0 (q, $J_{C,F}$ =5.8, CH), 124.3 (q, $J_{C,F}$ =273.9, C), 122.8 (CH), 104.7 (C), 45.8 (CH_2), 27.6 (CH_3).

Synthesis of (2*E*)-3-[6-(methylamino)-1-[(4-methylphenyl)methyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoic acid (29d). Product yield: 13 mg, 0.040 mmol, 100%. **^{1}H NMR** (500 MHz, DMSO-d_6) δ = 9.26 – 9.09 (m, 1H), 7.66 – 7.57 (m, 1H), 7.53 (d, *J*=16.2, 1H), 7.18 (s, 2H), 7.13 (d, *J*=7.5, 3H), 6.70 (d, *J*=16.3, 1H), 5.39 (s, 2H), 2.88 (d, *J*=4.8, 3H), 2.25 (s, 3H). **^{13}C NMR** (126 MHz, DMSO) δ 167.3 (C), 161.6 (C), 155.8 (C), 154.3 (CH), 140.3 (CH), 136.8 (C), 134.2 (CH), 133.8 (CH), 129.1 (2 x CH), 127.8 (2 x CH), 122.6 (CH), 105.2 (C), 49.1 (CH_2), 29.0 (CH_3), 20.6 (CH_3).

Synthesis of (2*E*)-3-{1-[(4-methoxyphenyl)methyl]-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl}prop-2-enoic acid (29e). Product yield: 31 mg, 0.091mmol,100%. **^{1}H NMR** (500 MHz, DMSO-d_6) δ = 12.45 (s, 1H), 9.13 (s, 1H), 7.63 (s, 1H), 7.55 (d, *J*=16.2, 1H), 7.26 (s, 2H), 6.91 – 6.85 (m, 2H), 6.70 (d, *J*=16.3, 1H), 5.37 (s, 2H), 3.71 (s, 3H), 2.89 (d, *J*=4.7, 3H). **^{13}C NMR** (126 MHz, DMSO) δ 167.2 (C), 161.6 (C), 158.8 (C), 155.6 (C), 153.9 (CH), 140.1 (C), 134.4 (CH), 129.3 (2 x CH), 128.3 (C), 122.2 (CH), 114.0 (2 x CH), 105.6 (C), 55.1 (CH_3), 48.9 (CH_2), 29.0 (CH_2).

Synthesis of (2*E*)-3-[6-(methylamino)-1-{[4-(trifluoromethyl)phenyl]methyl}-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoic acid (29f). Product yield: 18.1 mg, 0.048 mmol, 49 %. **^{1}H NMR** (601 MHz, DMSO-d_6) δ = 12.13 (s, 1H), 8.82 (s, 1H), 7.69 (dd, *J*=8.1, 6.3, 2H), 7.64 – 7.60 (m, 2H), 7.57 – 7.53 (m, 2H), 7.43 – 7.40 (m, 1H), 5.44 (s, 2H), 2.85 (dd, *J*=10.2, 4.9, 3H). **^{13}C NMR** (151 MHz, DMSO) δ 167.2 (C), 161.6 (C), 155.5 (C), 154.0 (CH), 145.2 (C), 142.2 (CH), 133.1 (CH), 132.4 (C), 132.0 (C), 128.2 (2 x CH), 125.4 (q, $J_{C,F}$ =4.2, 2 x CH), 125.1 (q, $J_{C,F}$ =271.9, C)106.2 (C), 48.2 (CH_2), 29.0 (CH_3).

Synthesis of (2*E*)-3-[6-(methylamino)-1-{[3-(trifluoromethyl)phenyl]methyl}-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-enoic acid (29g). Product yield: 31 mg, 0.082 mmol, 100 % was used without further purification. **^{1}H NMR** (500 MHz, Methanol-d_4) δ = 9.06 (s, 1H), 7.73 (d, *J*=9.5, 1H), 7.68 (s, 1H), 7.60 (dt, *J*=5.4, 3.0, 2H), 7.53 (t, *J*=7.7, 1H), 6.74 (d, *J*=16.3, 1H), 5.58 (s, 2H), 3.03 (s, 3H). **^{13}C NMR** (126 MHz, MeOD) δ 169.5 (C), 161.6 (C), 157.7 (C), 153.2 (CH), 143.4 (C), 139.0 (C), 135.7 (CH), 133.0 (CH), 130.6 (CH), 132.0 (q, $J_{C,F}$ =32.1, C), 125.8 (q, $J_{C,F}$ =3.9, CH),125.49 (q, $J_{C,F}$ =271.3, C),126.1 (CH), 124.1 (CH), 107.1 (C), 50.8 (CH_2), 28.5 (CH_3).

General Synthesis of Compounds 2D1-7

The appropriate intermediate compound **(29a-g)** (0.040-0.318 mmol) was dissolved in anhydrous DMF (0.5 mL). To this, the appropriate secondary amine (4 eq.) and DIPEA (3 eq.) were then added. The resulting mixture was stirred for 5 min before adding HATU (1.2 eq.). The reaction vial was flushed with N_2, and the reaction stirred at r.t for 24 hours. The solvent was removed under vacuum and the residue partitioned between water and DCM. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The crude product was purified by silica gel TLC plate to afford the desired product.

Synthesis of (2*E*)-1-(4-methoxypiperidin-1-yl)-3-[6-(methylamino)-1-[(2-methylphenyl)methyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-en-1-one (2D1). The product was purified by silica gel TLC plate using MeOH/DCM (6%); then using MeOH/DCM (8%) to afford the desired product (17.3 mg, 0.041 mmol, 47 %). **^{1}H NMR** (601 MHz, DMSO-d_6) δ 9.27 – 9.19 (m, 1H), 7.59 (s, 1H), 7.46 (d, *J* = 15.7 Hz, 1H), 7.33 (d, *J* = 15.7 Hz, 1H), 7.22 – 7.15 (m, 2H), 7.15 – 7.05 (m, 2H), 5.43 (s, 2H), 3.95 – 3.89 (m, 2H), 3.43 (tt, *J* = 8.0, 3.7 Hz, 2H), 3.27 (s, 3H), 3.23 (s, 1H), 2.87 (d, *J* = 5.0 Hz, 3H), 2.41 (s, 3H), 1.85 (s, 2H), 1.48 – 1.36 (m, 2H). **^{13}C NMR** (151 MHz, DMSO) δ 164.0 (C), 161.6 (C), 155.8 (C), 154.3 (CH), 141.2 (C), 135.9 (C), 134.9 (C), 132.2 (CH), 130.2 (CH), 128.6 (CH), 127.6 (CH), 126.0 (CH), 121.5 (CH), 104.7 (C), 75.0 (CH), 55.0 (CH_3), 47.1 (CH_2), 42.6 (2 x CH_2), 31.4 (2 x CH_2), 27.8 (CH_3), 18.9 (CH_3). **HRMS** (ESI, *m*/*z*) calcd for $C_{23}H_{29}N_6O_2$ $[M + H]^+$: 421.2347; found, 421.2335.

Synthesis of (2*E*)-3-{1-[(2-methoxyphenyl)methyl]-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl}-1-(4-methoxypiperidin-1-yl)prop-2-en-1-one (2D2). The crude product was purified by silica gel TLC plate using MeOH/DCM (4%); then using MeOH/DCM (2%) and finally MeOH/DCM (6%) to afford the desired product (39.1 mg, 0.090 mmol, 28 %).**^{1}H NMR** (601 MHz, DMSO-d_6) δ 9.23 (s, 1H), 7.56 (s, 1H), 7.47 (d, *J* = 15.7 Hz, 1H), 7.34 (d, *J* = 15.7 Hz, 1H), 7.26 (t, *J* = 7.8 Hz, 1H), 7.03 (d, *J* = 8.2 Hz, 1H), 6.86 – 6.74 (m, 2H), 5.41 (s, 2H), 3.93 (d, *J* = 13.4 Hz, 2H), 3.82 (s, 3H), 3.44 (tt, *J* = 8.1, 3.7 Hz, 2H), 3.27 (d, *J* = 1.3 Hz, 3H), 3.23 (d, *J* = 6.8 Hz, 1H), 2.88 – 2.81 (m, 3H), 1.86 (s, 2H),

1.42 (d, *J* = 35.8 Hz, 2H). **^{13}C NMR** (151 MHz, DMSO) δ 164.0 (C), 161.6 (C), 156.5 (C), 156.1 (C), 154.2 (CH), 141.2 (C), 132.2 (CH), 128.8 (2 x CH), 124.7 (C), 121.4 (CH), 120.32 (CH), 110.9 (CH), 105.0 (C), 75.0 (CH), 55.5 (CH_3), 55.0 (CH_3), 44.1 (CH_2), 42.6 (2 x CH_2), 31.4 (2 x CH_2), 27.8 (CH_3). **HRMS** (ESI, *m*/*z*) calcd for $C_{24}H_{29}N_6O_3$ $[M + H]^+$: 437.2296; found, 437.2294.

Synthesis of (2*E*)-1-(4-methoxypiperidin-1-yl)-3-[6-(methylamino)-1-{[2-(trifluoromethyl)phenyl]methyl}-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-en-1-one (2D3). The crude product was purified by silica gel TLC plate using MeOH/DCM (4 %); then using MeOH/DCM (7%) to afford the desired product (17.5 mg, 0.037 mmol, 45 %). **^{1}H NMR** (601 MHz, DMSO-d_6) δ = 9.26 (s, 1H), 7.80 – 7.78 (m, 1H), 7.63 (s, 1H), 7.58 (d, *J*=7.8, 1H), 7.51 (t, *J*=7.6, 1H), 7.48 (d, *J*=15.8, 1H), 7.38 (d, *J*=15.8, 1H), 7.05 (s, 1H), 5.63 (s, 2H), 3.92 (s, 2H), 3.44 (dq, *J*=7.9, 4.0, 2H), 3.27 (s, 3H), 3.24 (q, *J*=3.8, 1H), 2.80 (s, 3H), 1.86 (s, 2H), 1.43 (d, *J*=33.9, 2H). **^{13}C NMR** (126 MHz, DMSO) δ 163.9 (C), 161.7 (C), 156.3 (C), 154.4 (CH), 141.8 (C), 135.0 (CH), 133.0 (C), 132.0 (CH), 129.0 (CH), 128.1 (CH), 126.4 (C), 126.0(d, $J_{C,F}$ =5.5, CH) 124.3 (q, $J_{C,F}$=274.1, C) 122.0 (CH), 104.7 (C), 75.0 (CH), 55.0 (CH_3), 45.6 (CH_2), 42.6 (2 x CH_2), 31.4 (2 x CH_2), 27.6 (CH_3). **HRMS** (ESI, *m*/*z*) calcd for $C_{22}H_{26}F_3N_6O_2$ $[M + H]^+$: 475.2064; found, 475.2076.

Synthesis of (2*E*)-1-(4-methoxypiperidin-1-yl)-3-[6-(methylamino)-1-[(4-methylphenyl)methyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-en-1-one (2D4). The crude product was purified by silica gel TLC plate using MeOH/DCM (4 %) to afford the desired product (7.5 mg, 0.018 mmol, 45 %). **^{1}H NMR** (500 MHz, Methanol-d_4) δ = 9.02 (s, 1H), 7.61 (dd, *J*=15.7, 1.1, 1H), 7.33 (dd, *J*=15.7, 1.1, 1H), 7.21 (d, *J*=8.1, 2H), 7.12 (d, *J*=7.7, 2H), 5.42 (s, 2H), 3.95 (d, *J*=11.8, 2H), 3.56 – 3.50 (m, 2H), 3.48 – 3.42 (m, 1H), 3.38 (d, *J*=1.2, 3H), 3.01 (d, *J*=1.1, 3H), 2.29 (s, 3H), 1.94 (s, 2H), 1.62 (d, *J*=21.3, 2H). **^{13}C NMR** (126 MHz, MeOD) δ 167.07, 163.25, 157.47, 154.70, 142.92, 138.72, 135.06, 133.82, 130.21, 128.99, 122.21, 107.04, 76.6 (CH), 56.1 (CH_3), 50.7 (CH_2), 44.4 (2 x CH_2), 32.5 (CH_2), 31.4 (2 x CH_2), 28.5 (CH_3), 21.1 (CH_3). **HRMS** (ESI, *m*/*z*) calcd for $C_{23}H_{29}N_6O_2$ $[M + H]^+$: 421.2347; found, 421.2350.

Synthesis of (2*E*)-3-{1-[(4-methoxyphenyl)methyl]-6-(methylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl}-1-(4-methoxypiperidin-1-yl)prop-2-en-1-one (2D5). The crude product was purified by silica gel TLC plate using MeOH/DCM (5%); then using MeOH/DCM (8%) to afford the desired product (12.7 mg, 0.029 mmol, 32 %). **^{1}H NMR** (601 MHz, DMSO-d_6) δ 9.21 (s, 1H), 7.59 (s, 1H), 7.46 (d, *J* = 15.7 Hz, 1H), 7.32 (d, *J* = 15.7 Hz, 1H), 7.25 (s, 2H), 6.91 – 6.85 (m, 2H), 5.36 (s, 2H), 3.95 – 3.87 (m, 2H), 3.71 (s, 3H), 3.43 (tq, *J* = 8.0, 3.9 Hz, 2H), 3.27 (s, 3H), 3.24 (d, *J* = 8.0 Hz, 1H), 2.89 (d, *J* = 4.7 Hz, 3H), 1.85 (s, 3H), 1.42 (d, *J* = 29.1 Hz, 1H). **^{13}C NMR** (151 MHz, DMSO) δ 164.0 (C), 161.6 (C), 158.7(C) , 155.6 (C), 154.2 (CH), 141.1 (C), 132.2 (CH), 129.3 (2 x CH), 128.9 (C), 121.4 (CH), 113.9 (2 x CH), 104.9 (C), 75.0 (CH), 55.0 (CH_3), 55.0 (CH_3), 48.8 (CH_2), 42.6 (2 x CH_2), 31.4 (2 x CH_2), 27.8 (CH_3). **HRMS** (ESI, *m*/*z*) calcd for $C_{23}H_{29}N_6O_3$ $[M + H]^+$: 437.2296; found, 437.2307.

Synthesis of (2*E*)-1-(4-methoxypiperidin-1-yl)-3-[6-(methylamino)-1-{[4-(trifluoromethyl)phenyl]methyl}-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-en-1-one (2D6). The crude product was purified by silica gel TLC plate using MeOH/DCM (5%); then using MeOH/DCM (8%) to afford the desired product (3.1 mg, 0.007 mmol, 6 %). **^{1}H NMR** (601 MHz, Methanol-d_4) δ 9.04 (s, 1H), 7.63 (s, 1H), 7.62 – 7.59 (m, 2H), 7.49 (d, *J* = 8.0 Hz, 2H), 7.36 (d, *J* = 15.8 Hz, 1H), 5.57 (s, 2H), 3.96 (s, 2H), 3.54 (tt, *J* = 7.6, 3.8 Hz, 2H), 3.49 – 3.43 (m, 1H), 3.38 (s, 3H), 3.00 (s, 3H), 1.99 – 1.89 (m, 2H), 1.61 (q, *J* = 13.5, 8.9 Hz, 2H). **^{13}C NMR** (151 MHz, MeOD) δ 167.0 (C), 163.4 (C), 157.7 (C), 154.9 (CH), 143.4 (C), 142.5 (C), 133.7 (CH), 131.02 (q, $J_{C,F}$ = 31.9 Hz, C), 129.5 (2 x CH), 126.6 (q, $J_{C,F}$ = 3.6 Hz, 2 x CH), 124.7 (C), 122.5 (CH), 104.8 (C), 76.6 (CH), 56.1 (CH_3), 50.4 (CH_2) , 44.4 (2 x CH_2), 31.4 (2 x CH_2), 28.5 (CH_3). **HRMS** (ESI, *m*/*z*) calcd for $C_{23}H_{26}F_3N_6O_2$ $[M + H]^+$: 475.2064; found, 475.2084.

Synthesis of (2*E*)-1-(4-methoxypiperidin-1-yl)-3-[6-(methylamino)-1-{[3-(trifluoromethyl)phenyl]methyl}-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-en-1-one (2D7). The crude product was purified by silica gel TLC plate using MeOH/DCM (5%); then using MeOH/DCM (8%) to afford the desired product (13.1 mg, 0.028mmol, 34 %). **^{1}H NMR** (500 MHz, DMSO-d_6) δ = 9.24 (s, 1H), 7.76 (s, 1H), 7.68 – 7.64 (m, 2H), 7.58 (t, *J*=7.5, 2H), 7.46 (d, *J*=15.7, 1H), 7.35 (d, *J*=15.8, 1H), 5.55 (s, 2H), 3.92 (dd, *J*=13.9, 6.4, 2H), 3.43 (tt, *J*=7.9, 3.7, 2H), 3.27 (s, 3H), 3.24 (d, *J*=5.7, 1H), 2.87 (dq, *J*=8.8, 3.4, 3.0, 3H), 1.84 (d, *J*=15.2, 2H), 1.41 (ddd, *J*=30.4, 15.2, 6.3, 2H). **^{13}C NMR** (126 MHz, DMSO) δ 163.9 (C), 161.6 (C), 155.9 (C), 154.4 (CH), 141.5 (C), 138.2 (C), 132.0 (CH), 129.8 (CH), 129.19 (q, $J_{C,F,}$= 31.8 Hz, C) 124.6 (CH), 124.4 (CH), 124.4 (CH), 124.04 (q, $J_{C,F}$ = 272.3 Hz, C) 121.8 (CH), 104.8 (C), 75.0 (CH), 55.0 (CH_3), 48.8 (CH_2), 43.9 (2 x CH_2), 31.4 (2 x CH_2), 27.7 (CH_3). **HRMS** (ESI, *m*/*z*) calcd for $C_{22}H_{26}F_3N_6O_2$ $[M + H]^+$: 475.2064; found, 475.2079.

8. Synthesis and Characterization of Compounds 2E1-11

Synthesis of 6-Chloro-3-iodo-1-(3-(trifluoromethyl)benzyl)-1*H*-Pyrazolo[3,4-*d*]pyrimidine (30). To a solution of 6-chloro-3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidine **(6)** (200 mg, 0.71 mmol, 1.0 equiv) in THF (3.0 mL) was added 3-(trifluoromethyl)benzyl alcohol (276 mg, 1.57 mmol, 2.2 equiv) and PPh_3 (374 mg, 1.43 mmol, 2.0 equiv). The reaction vial was flushed with N_2 for 10 minutes and then added DIAD (288 mg, 1.43 mmol, 2.0 equiv) to the vial. The reaction was stirred at r.t overnight. After reaction, EtOAc and water were added to the mixture, and the organic layers separated. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated under vacuum. The crude product was purified by silica gel flash chromatography using EtOAc/Hexane (0−5%) (156 mg, 0.36 mmol, 50%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.77 (s, 1H), 7.67 (s, 1H), 7.58 (t, *J* = 8.5 Hz, 2H), 7.47 (t, *J* = 7.8 Hz, 1H), 5.65 (s, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 159.9 (C), 154.8 (C), 154.7 (CH), 136.0 (C), 131.9 (CH), 129.6 (CH), 125.6 (q, *J* = 3.8 Hz, 2 x CH), 125.4 (q, *J* = 3.8 Hz, 2 x C), 118.7 (C), 91.0 (C), 51.0 (CH_2). **LRMS** (*m*/*z*) $[M + H]^+$: 439.20.

General Synthesis of Compounds 31a-k

6-Chloro-3-iodo-1-(3-(trifluoromethyl)benzyl)-1*H*-Pyrazolo[3,4-*d*]pyrimidine **(30)** (100 mg, 0.23 mmol, 1.0 equiv) was dissolved in THF (3.0 ml). Different amines (0.69 mmol, 3.0 equiv) were added and then the mixture was heated at 150 °C using microwave radiation for 60 min. The precipitated was dried by vacuum filtration and washed with water, **31a-k** were obtained without further purification.

General Synthesis of Compounds 32a-k

To a solution of **31a-k** (1.0 equiv) in Dioxane (1.0 mL) was added PPh_3 (0.4 equiv), $Pd(OAc)_2$ (0.2 equiv), TEA (13.0 equiv) and methyl acrylate (12.5 equiv). The reaction vial was flushed with N_2 for 10 minutes and then heated at 100 °C for 24 hours. Upon cooling to r.t, the mixture was partitioned between water and DCM. The combined organic layers were washed with brine, dried over Na_2SO_4, concentrated under vacuum. The crude product was purified by silica gel flash chromatography (EtOAc/Hexane 0-16 %) to afford the desired products **32a-k** (79-97%).

General Synthesis of Compounds 33a-k

32a-k (1.0 equiv) were dissolved in THF (18 mL) and 6M NaOH aqueous solution (30.0 equiv) was added. The reaction was heated at 60°C for 24 hours. Then, the solvent was evaporated under reduced pressure, the residue was acidified (pH 2) with 1M HCl. The product was extracted with DCM, and the combined organic layers were dried over Na_2SO_4 and concentrated under vacuum. The crude products **33a-k** were obtained without further purification.

General Synthesis of Library 2E Compounds 2E 1-11

33a-k (1.0 equiv) were dissolved in anhydrous DMF (1.0 mL). To this, 4-methoxypiperidine (1.1 equiv) and DIPEA (2.0 equiv) were then added. The resulting mixture was stirred for 5 min before adding HATU (1.5 equiv). The reaction was stirred at r.t for 2 hours. EtOAc was added to the reaction solution. The resulting mixture was washed three times with brine. The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by silica gel flash chromatography (MeOH/DCM 0-2.5%) to afford the desired products **2E1-11**.

Synthesis of (*E*)-3-(6-amino-1-(3-(trifluoromethyl)benzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)-1-(4-methoxypiperidin-1-yl)prop-2-en-1-one (2E1). 34.6 mg, 0.0752 mmol, 55%. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.90 (s, 1H), 7.73 (d, *J* = 15.6 Hz, 1H), 7.62 (s, 1H), 7.54 (d, *J* = 7.5 Hz, 1H), 7.45 (dt, *J* = 15.3, 7.7 Hz, 2H), 7.21 (d, *J* = 15.6 Hz, 1H), 5.50 (s, 2H), 5.23 (s, 2H), 3.91 (d, *J* = 58.6 Hz, 2H), 3.49 (tt, *J* = 7.2, 3.5 Hz, 3H), 3.38 (s, 3H), 1.93 – 1.87 (m, 2H), 1.70 – 1.63 (m, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 164.8 (C), 161.7 (C), 156.2 (C), 153.6 (CH), 142.0 (C), 137.3 (C), 131.9 (CH), 131.4 (CH), 129.4 (CH), 125.0 (2 x CH), 124.9 (2 x C), 121.3 (CH), 107.9 (C), 75.3 (CH), 56.0 (CH_3), 49.8 (CH_2), 43.3 (CH_2), 39.5 (CH_2), 31.6 (CH_2), 30.3 (CH_2). **HRMS** (ESI, *m*/*z*) calcd for $C_{22}H_{24}F_3N_6O_2$ $[M + H]^+$: 461.1907; found, 461.1907.

Synthesis of (*E*)-3-(6-(dimethylamino)-1-(3-(trifluoromethyl)benzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)-1-(4-methoxypiperidin-1-yl)prop-2-en-1-one (2E2). 19.7 mg, 0.0404 mmol, 67%. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.90 (s, 1H), 7.73 (d, *J* = 15.6 Hz, 1H), 7.70 (s, 1H), 7.53 (t, *J* = 6.5 Hz, 2H), 7.43 (t, *J* = 7.7 Hz, 1H), 7.18 (d, *J* = 15.5 Hz, 1H), 5.50 (s, 2H), 3.91 (d, *J* = 61.8 Hz, 2H), 3.48 (tt, *J* = 7.3, 3.5 Hz, 3H), 3.38 (s, 3H), 3.27 (s, 6H), 1.89 (ddd, *J* = 14.2, 7.7, 3.7 Hz, 2H), 1.65 (ddt, *J* = 16.8, 7.6, 3.8 Hz, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 165.0 (C), 161.2 (C), 156.5 (C), 152.5 (CH), 141.8 (C), 137.6 (C), 132.6 (CH), 131.7 (CH), 129.3 (CH), 125.4 (2 x CH), 124.9 (C), 124.8 (C), 120.7 (CH), 106.0 (C), 75.4 (CH), 56.0 (CH_3), 50.0 (CH_2), 43.3 (CH_2), 39.5 (CH_2), 37.6 (2 x CH_3), 31.7 (CH_2), 30.2 (CH_2). **HRMS** (ESI, *m*/*z*) calcd for $C_{24}H_{28}F_3N_6O_2$ [M + H]$^+$: 489.2220; found, 489.2218.

Synthesis of (*E*)-3-(6-(ethylamino)-1-(3-(trifluoromethyl)benzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)-1-(4-methoxypiperidin-1-yl)prop-2-en-1-one (2E3). 29.0 mg, 0.0594 mmol, 47%. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.84 (s, 1H), 7.72 (d, *J* = 15.6 Hz, 1H), 7.69 (s, 1H), 7.53 (t, *J* = 8.1 Hz, 2H), 7.43 (t, *J* = 7.7 Hz, 1H), 7.18 (d, *J* = 15.6 Hz, 1H), 5.50 (s, 2H), 5.37 (s, 1H), 3.90 (d, *J* = 60.2 Hz, 2H), 3.58 – 3.42 (m, 5H), 3.38 (s, 3H), 1.89 (ddd, *J* = 14.1, 7.6, 3.7 Hz, 2H), 1.66 (ddd, *J* = 13.2, 8.3, 4.0 Hz, 2H), 1.27 (t, *J* = 7.2 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 164.9 (C), 161.2(C), 156.3 (C), 153.2 (CH), 141.8 (C), 137.5 (C), 132.3(CH), 131.7(CH), 129.3 (CH), 125.3 (2 x CH), 124.9 (2 x C), 120.9 (CH), 107.0 (C), 75.4 (CH), 56.0 (CH_3), 50.0 (CH_2), 43.2 (CH_2), 39.5 (CH_2), 36.6 (CH_2), 31.6 (CH_2), 30.3 (CH_2), 14.8 (CH_3). **HRMS** (ESI, m/z) calcd for $C_{24}H_{28}F_3N_6O_2$ [M + H]+: 489.2220; found, 489.2220.

Synthesis of (*E*)-1-(4-methoxypiperidin-1-yl)-3-(6-(propylamino)-1-(3-(trifluoromethyl)benzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)prop-2-en-1-one (2E4). 30.5 mg, 0.0607 mmol, 51%. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.83 (s, 1H), 7.71 (d, *J* = 15.6 Hz, 1H), 7.69 (s, 1H), 7.53 (t, *J* = 7.9 Hz, 2H), 7.43 (t, *J* = 7.7 Hz, 1H), 7.18 (d, *J* = 15.6 Hz, 1H), 5.50 (s, 2H), 5.40 (s, 1H), 3.91 (d, *J* = 59.7 Hz, 2H), 3.54 – 3.41 (m, 5H), 3.38 (s, 3H), 1.90 (ddd, *J* = 12.8, 7.4, 3.7 Hz, 2H), 1.67 (p, *J* = 7.2 Hz, 4H), 1.00 (t, *J* = 7.4 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 164.9 (C), 161.4 (C), 156.3 (C), 153.2 (CH), 141.8 (C), 137.5 (C), 132.3 (CH), 131.7 (CH), 129.3 (CH), 125.3 (2 x CH), 124.9 (2 x C), 120.9 (CH), 105.9 (C), 75.4 (CH), 56.0 (CH_3), 50.0 (CH_2), 43.6 (CH_2), 43.3 (CH_2), 39.5 (CH_2), 31.7 (CH_2), 30.3 (CH_2), 22.7 (CH_2), 11.6 (CH_3). **HRMS** (ESI, *m*/*z*) calcd for $C_{25}H_{30}F_3N_6O_2$ [M + H]$^+$: 503.2377; found, 503.2361.

Synthesis of (*E*)-3-(6-(butylamino)-1-(3-(trifluoromethyl)benzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)-1-(4-methoxypiperidin-1-yl)prop-2-en-1-one (2E5). 41.7 mg, 0.0808 mmol, 68%. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.83 (s, 1H), 7.71 (d, *J* = 15.5 Hz, 1H), 7.68 (s, 1H), 7.53 (t, *J* = 7.9 Hz, 2H), 7.43 (t, *J* = 7.8 Hz, 1H), 7.18 (d, *J* = 15.6 Hz, 1H), 5.49 (s, 2H), 5.41 (s, 1H), 3.90 (d, *J* = 60.5 Hz, 2H), 3.48 (qd, *J* = 9.2, 6.4 Hz, 5H), 3.37 (s, 3H), 1.89 (ddt, *J* = 14.7, 7.7, 3.9 Hz, 2H), 1.69 – 1.65 (m, 2H), 1.64 – 1.59 (m, 2H), 1.43 (h, *J* = 7.4 Hz, 2H), 0.96 (t, *J* = 7.3 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 164.9 (C), 161.4 (C), 156.3 (C), 153.1(CH), 141.8 (C), 137.5 (C), 132.3 (CH), 131.7 (CH), 129.3 (CH), 125.2 (2 x CH), 124.9 (2 x C), 120.9 (CH), 107.0 (C), 75.4 (CH), 56.0 (CH_3), 50.0 (CH_2), 43.2 (CH_2), 41.5 (CH_2), 39.5 (CH_2), 31.6 (2 x CH_2), 30.2 (CH_2), 20.3 (CH_2), 13.9 (CH_3). **HRMS** (ESI, *m*/*z*) calcd for $C_{26}H_{32}F_3N_6O_2$ [M + H]$^+$: 517.2533; found, 517.2521.

Synthesis of (*E*)-3-(6-(isopropylamino)-1-(3-(trifluoromethyl)benzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)-1-(4-methoxypiperidin-1-yl)prop-2-en-1-one (2E6). 22.5 mg, 0.0448 mmol, 36%. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.83 (s, 1H), 7.71 (d, *J* = 15.6 Hz, 1H), 7.68 (s, 1H), 7.53 (t, *J* = 6.8 Hz, 2H), 7.43 (t, *J* = 7.7 Hz, 1H), 7.18 (d, *J* = 15.6 Hz, 1H), 5.49 (s, 2H), 5.25 (s, 1H), 4.21 (h, *J* = 6.5 Hz, 1H), 3.91 (d, *J* = 60.1 Hz, 2H), 3.54 – 3.43 (m, 3H), 3.38 (s, 3H), 1.93 – 1.85 (m, 2H), 1.69 – 1.64 (m, 2H), 1.28 (d, *J* = 6.6 Hz, 6H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 164.9 (C), 160.6 (C), 156.3 (C), 153.2 (CH), 141.8 (C), 137.5 (C), 132.3 (CH), 131.7 (CH), 129.3 (CH), 125.3 (2 x CH), 124.9 (2 x C), 120.9 (CH), 106.9 (C), 75.4 (CH), 56.0 (CH_3), 50.0 (CH_2), 43.3 (1 x CH, 1 x CH_2), 39.5 (CH_2), 31.7 (CH_2), 30.3 (CH_2), 22.7 (2 x CH_3). **HRMS** (ESI, *m*/*z*) calcd for $C_{25}H_{30}F_3N_6O_2$ [M + H]$^+$: 503.2377; found, 503.2387.

Synthesis of (*E*)-3-(6-(*tert*-butylamino)-1-(3-(trifluoromethyl)benzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)-1-(4-methoxypiperidin-1-yl)prop-2-en-1-one (2E7). 41.0 mg, 0.0794 mmol, 87%. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.81 (s, 1H), 7.71 (d, *J* = 15.6 Hz, 1H), 7.68 (s, 1H), 7.54 (d, *J* = 7.7 Hz, 2H), 7.43 (t, *J* = 7.7 Hz, 1H), 7.17 (d, *J* = 15.6 Hz, 1H), 5.48 (s, 2H), 5.19 (s, 1H), 3.91 (d, *J* = 61.9 Hz, 3H), 3.48 (tt, *J* = 7.2, 3.5 Hz, 3H), 3.38 (s, 3H), 1.90 (ddt, *J* = 14.1, 7.7, 3.8 Hz, 2H), 1.66 (tdd, *J* = 11.2, 7.8, 4.7 Hz, 4H), 1.53 (dt, *J* = 14.2, 7.4 Hz, 2H), 0.94 (t, *J* = 7.4 Hz, 6H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ

165.0 (C), 161.5 (C), 156.3 (C), 153.2 (CH), 141.8 (C), 137.6 (C), 132.4 (CH), 131.8 (CH), 129.3 (CH), 125.3 (2 x CH), 124.9 (2 x C), 120.8 (CH), 106.9 (C), 75.4 (CH), 56.0 (CH_3), 54.0 (CH), 50.0 (CH_2), 43.3 (CH_2), 39.5 (CH_2), 31.6 (CH_2), 30.3 (CH_2), 27.3 (2 x CH_2), 10.3 (2 x CH_3). **HRMS** (ESI, *m*/*z*) calcd for $C_{27}H_{34}F_3N_6O_2$ $[M + H]^+$: 531.2690; found, 531.2691.

Synthesis of (*E*)-1-(4-methoxypiperidin-1-yl)-3-(6-(pentan-3-ylamino)-1-(3-(trifluoromethyl)benzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)prop-2-en-1-one (2E8). 50.5 mg, 0.0952 mmol, 71%. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.80 (s, 1H), 7.71 (d, *J* = 15.6 Hz, 1H), 7.68 (s, 1H), 7.59 – 7.48 (m, 2H), 7.43 (t, *J* = 7.7 Hz, 1H), 7.17 (d, *J* = 15.6 Hz, 1H), 5.49 (s, 2H), 5.42 (s, 1H), 3.91 (d, *J* = 58.8 Hz, 2H), 3.48 (tt, *J* = 7.3, 3.6 Hz, 3H), 3.38 (s, 3H), 1.90 (ddt, *J* = 13.9, 7.8, 3.9 Hz, 2H), 1.70 – 1.62 (m, 2H), 1.48 (s, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 165.0 (C), 160.7 (C), 155.8 (C), 153.0 (CH), 141.7 (C), 137.6 (C), 132.4 (CH), 131.6 (CH), 129.4 (CH), 125.2 (2 x CH), 125.0 (C), 124.9 (C), 120.8 (CH), 106.6 (C), 75.4 (CH), 56.0 (CH_3), 51.4 (C), 50.2 (CH_2), 43.3 (CH_2), 39.5 (CH_2), 31.6 (CH_2), 30.3 (CH_2), 28.7 (3 x CH_3). **HRMS** (ESI, *m*/*z*) calcd for $C_{26}H_{32}F_3N_6O_2$ $[M + H]^+$: 517.2533; found, 517.2537.

Synthesis of (*E*)-1-(4-methoxypiperidin-1-yl)-3-(6-(piperidin-1-yl)-1-(3-(trifluoromethyl)benzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)prop-2-en-1-one (2E9). 40.0 mg, 0.0757 mmol, 58%. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 9.32 (s, 1H), 7.75 (s, 1H), 7.65 (dt, *J* = 7.1, 2.0 Hz, 1H), 7.58 – 7.53 (m, 2H), 7.47 (d, *J* = 15.7 Hz, 1H), 7.35 (d, *J* = 15.7 Hz, 1H), 5.55 (s, 2H), 3.95 – 3.89 (m, 2H), 3.86 (t, *J* = 5.5 Hz, 4H), 3.43 (tt, *J* = 7.9, 3.7 Hz, 2H), 3.27 (s, 3H), 3.26 – 3.19 (m, 1H), 1.85 (s, 2H), 1.64 (q, *J* = 6.2 Hz, 2H), 1.53 (tq, *J* = 8.4, 4.3 Hz, 4H), 1.42 (d, *J* = 29.4 Hz, 2H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 163.9 (C), 159.8 (C), 155.9 (C), 154.3 (CH), 141.5 (C), 138.2 (C), 132.0 (2 x CH), 129.8 (CH), 124.7 (2 x CH), 124.5 (2 x C), 122.0 (CH), 104.8 (C), 75.0 (CH), 55.1 (CH_3), 49.0 (CH_2), 44.8 (2 x CH_2), 42.7 (2 x CH_2), 31.4 (CH_2), 30.2 (CH_2), 25.3 (2 x CH_2), 24.3 (CH_2). **HRMS** (ESI, *m*/*z*) calcd for $C_{27}H_{32}F_3N_6O_2$ $[M + H]^+$: 529.2533; found, 529.2532.

Synthesis of (*E*)-1-(4-methoxypiperidin-1-yl)-3-(6-morpholino-1-(3-(trifluoromethyl)benzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)prop-2-en-1-one (2E10). 28.0 mg, 0.0528 mmol, 52%. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.90 (s, 1H), 7.73 (d, *J* = 15.6 Hz, 1H), 7.68 (s, 1H), 7.54 (d, *J* = 7.8 Hz, 1H), 7.50 (d, *J* = 7.7 Hz, 1H), 7.43 (t, *J* = 7.7 Hz, 1H), 7.20 (d, *J* = 15.6 Hz, 1H), 5.49 (s, 2H), 4.00 – 3.89 (m, 5H), 3.87 – 3.74 (m, 5H), 3.49 (tt, *J* = 7.2, 3.5 Hz, 3H), 3.38 (s, 3H), 1.90 (ddt, *J* = 11.4, 7.5, 3.7 Hz, 2H), 1.69 – 1.62 (m, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 164.9 (C), 160.5 (C), 156.2 (C), 152.7 (CH), 141.8 (C), 137.4 (C), 132.3 (CH), 131.6 (CH), 129.4 (CH), 125.4 (CH), 125.3 (CH), 125.0 (C), 124.9 (C), 121.0 (CH), 106.9 (C), 75.4 (CH), 66.9 (2 x CH_2), 56.0 (CH_3), 50.1 (CH_2), 44.8 (2 x CH_2), 43.3 (CH_2), 39.5 (CH_2), 31.7 (CH_2), 30.2 (CH_2). **HRMS** (ESI, *m*/*z*) calcd for $C_{26}H_{30}F_3N_6O_3$ $[M + H]^+$: 531.2326; found, 531.2334.

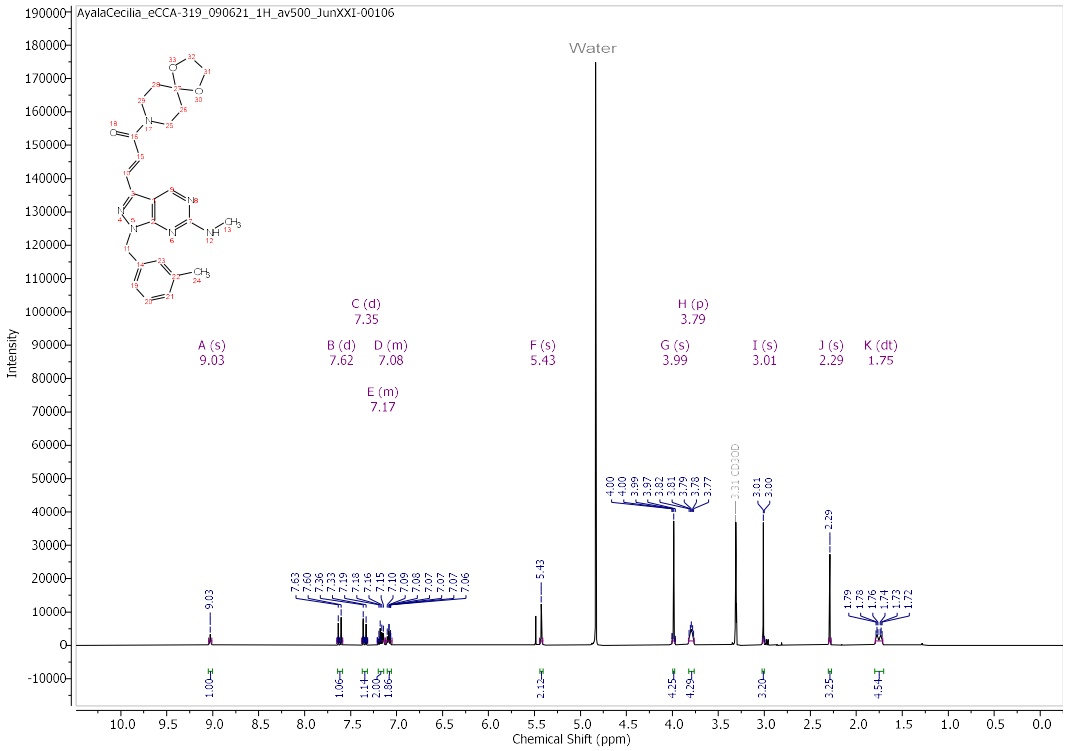
Synthesis of (*E*)-3-(6-(benzylamino)-1-(3-(trifluoromethyl)benzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)-1-(4-methoxypiperidin-1-yl)prop-2-en-1-one (2E11). 17.0 mg, 0.0309 mmol, 30%. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.85 (s, 1H), 7.72 (d, *J* = 15.5 Hz, 1H), 7.65 (s, 1H), 7.53 (d, *J* = 7.7 Hz, 1H), 7.45 (d, *J* = 7.7 Hz, 1H), 7.40 – 7.26 (m, 6H), 7.19 (d, *J* = 15.6 Hz, 1H), 5.80 (s, 1H), 5.48 (s, 2H), 4.70 (d, *J* = 5.9 Hz, 2H), 3.91 (d, *J* = 60.8 Hz, 2H), 3.48 (tt, *J* = 7.2, 3.5 Hz, 3H), 3.38 (s, 3H), 1.90 (ddt, *J* = 11.3, 7.3, 3.7 Hz, 2H), 1.70 – 1.62 (m, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 164.9 (C), 161.2 (C), 156.4 (C), 153.3 (CH), 141.9 (C), 138.9 (C), 137.4 (C), 132.2 (CH), 131.7 (CH), 129.4 (CH), 128.8 (2 x CH), 127.7 (2 x CH), 127.6 (CH), 125.2 (2 x CH), 124.9 (2 x C), 121.0 (CH), 105.0 (C), 75.4 (CH), 56.0 (CH_3), 50.0 (CH_2), 45.9 (CH_2), 43.2 (CH_2), 39.5 (CH_2), 31.6 (CH_2), 30.3 (CH_2). **HRMS** (ESI, *m*/*z*) calcd for $C_{29}H_{30}F_3N_6O_2$ $[M + H]^+$: 551.2377; found, 551.2387.

Synthesis and Characterization of Compound 2F1

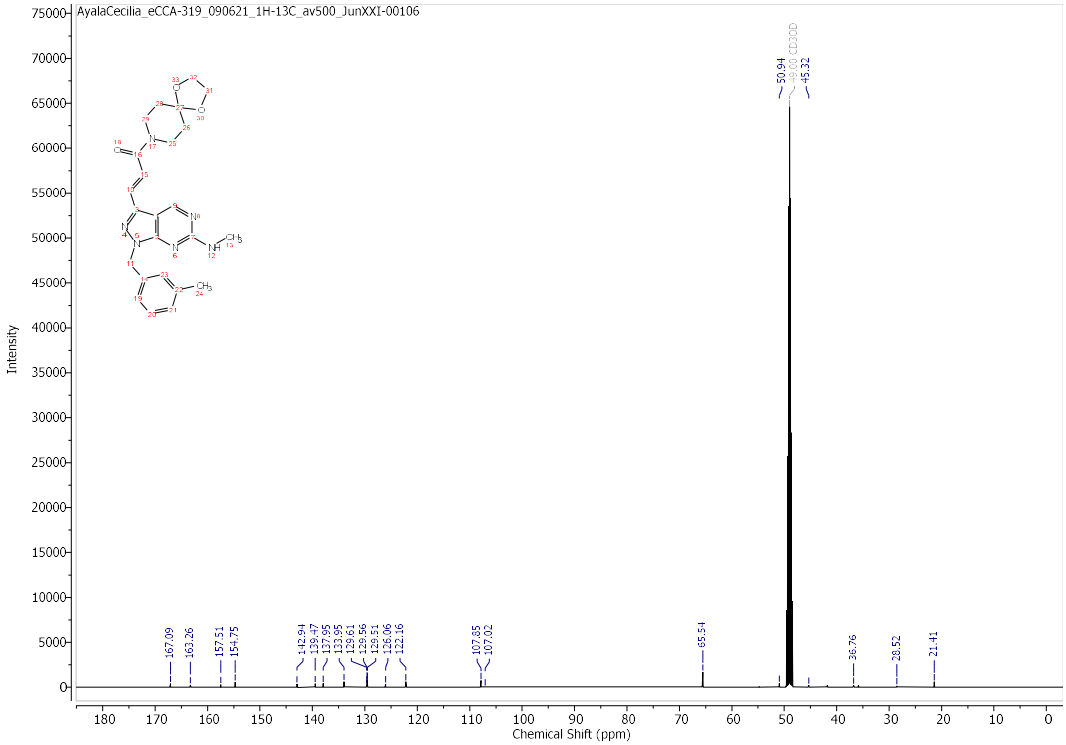
Synthesis of (2*E*)-1-{1,4-dioxa-8-azaspiro[4.5]decan-8-yl}-3-[6-(methylamino)-1-{[3-(trifluoromethyl)phenyl]methyl}-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl]prop-2-en-1-one (2F1). The intermediate compound **29g** (0.048 mmol) was dissolved in anhydrous DMF (0.5 mL). To this, the appropriate secondary amine (4 eq.) and DIPEA (3 eq.) were then added. The resulting mixture was stirred for 5 min before adding HATU (1.2 eq.). The reaction vial was flushed with N_2, and the reaction stirred at r.t for 24 hours. The solvent was removed under vacuum and the residue partitioned between water and DCM. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The crude product was purified by silica gel TLC plate to afford the desired product. The crude product was purified by silica gel TLC plate using MeOH/DCM (4%); then using MeOH/DCM (2%) to afford the desired product (13.5 mg, 0.027 mmol, 56 %). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 9.24 (s, 1H), 7.76 (s, 1H), 7.68 – 7.64 (m, 2H), 7.57 (t, *J* = 7.5 Hz, 2H), 7.48 (d, *J* = 15.7 Hz, 1H), 7.36 (d, *J* = 15.8 Hz, 1H), 5.55 (s, 2H), 3.92 (s, 4H), 3.75 – 3.61 (m, 4H), 2.88 (d, *J* = 4.7 Hz, 3H), 1.70 – 1.59 (m, 4H). **^{13}C NMR** (126 MHz, DMSO) δ 163.9 (C), 161.6 (C), 155.9 (C), 154.5 (CH), 141.5 (C), 138.2 (C), 132.1 (CH), 131.9 (CH), 129.8 (CH), 129.19 (q, $J_{C,F}$ = 31.7 Hz, C), 124.6 (CH), 124.4 (q, $J_{C,F}$ = 4.0 Hz, CH), 124.0 (q, $J_{C,F}$ = 272.3 Hz, C), 121.7 (CH), 106.3 (C), 104.8 (C), 63.8 (2 x CH_2), 48.8 (CH_2), 43.4 (CH_2), 35.5 (CH_2), 34.4(CH_2), 27.7 (CH_3). **HRMS** (ESI, *m*/*z*) calcd for $C_{24}H_{26}F_3N_6O_3[M + H]^+$: 503.2013; found, 503.2023.

9. NMR Spectrums of the Lead Compounds

Lead Compound 2C4 (a.k.a. eCCA319)

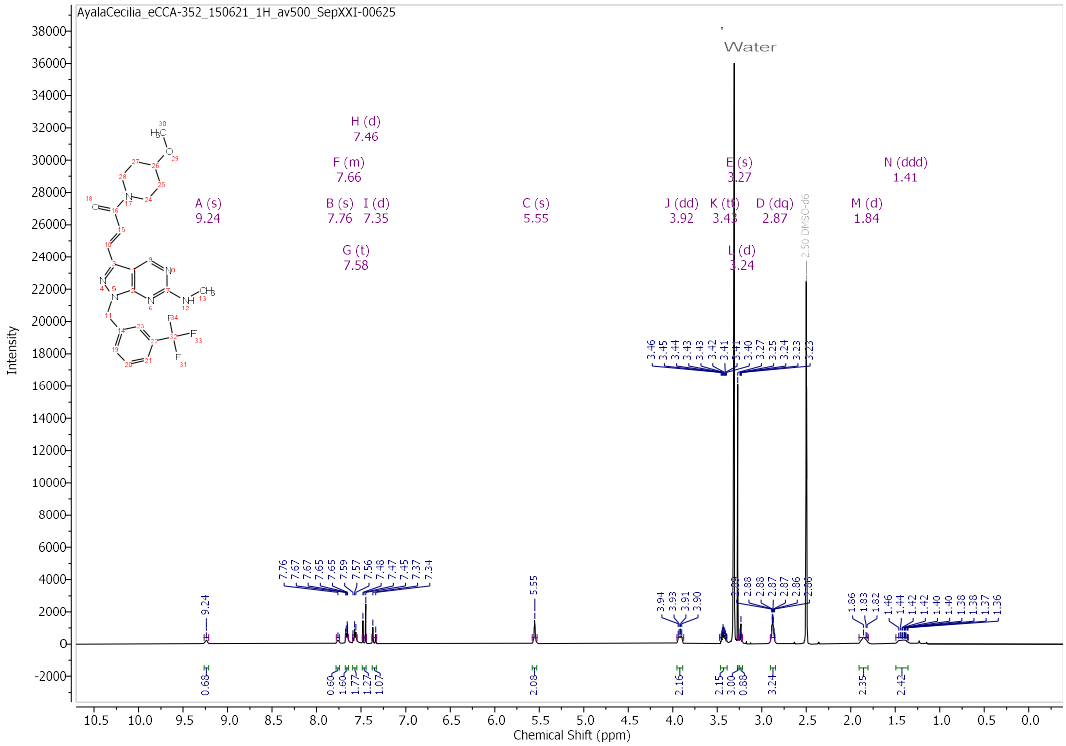


^{1}H NMR (500 MHz, Methanol-d_4) δ = 9.03 (s, 1H), 7.62 (d, *J*=15.7, 1H), 7.35 (d, *J*=15.8, 1H), 7.21 – 7.13 (m, 2H), 7.12 – 7.05 (m, 2H), 5.43 (s, 2H), 3.99 (s, 4H), 3.79 (p, *J*=5.6, 4H), 3.01 (s, 3H), 2.29 (s, 3H), 1.75 (dt, *J*=22.1, 5.6, 4H).

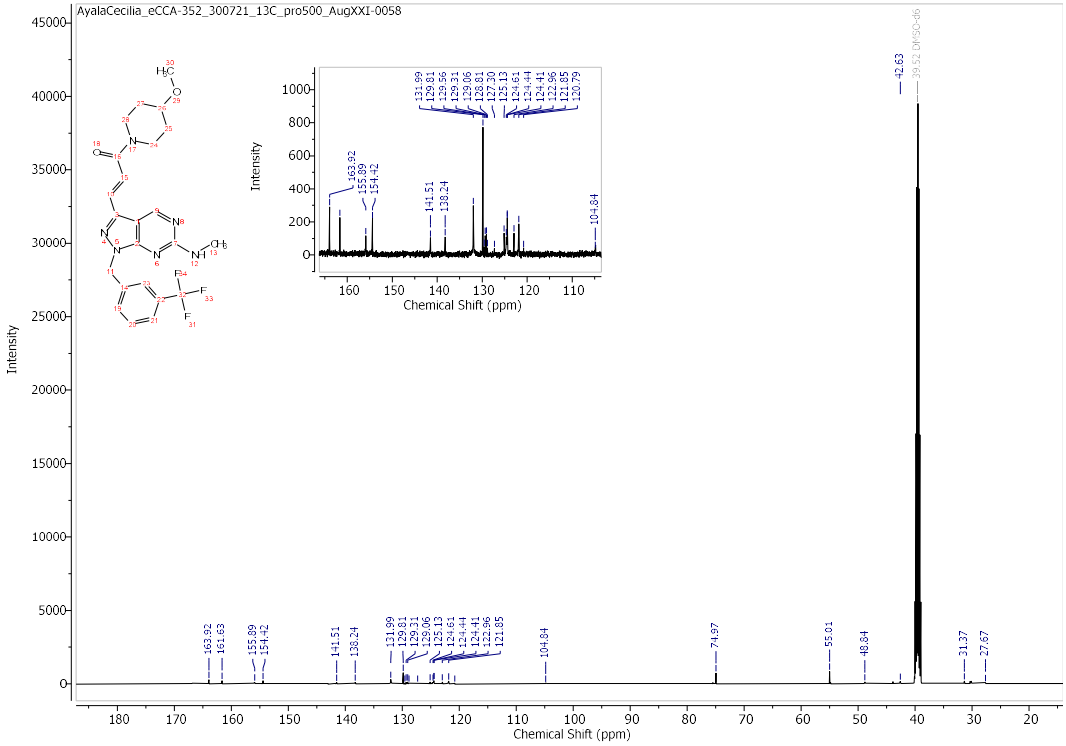


^{13}C NMR (126 MHz, MeOD) δ 167.1 (C), 163.3 (C), 157.5 (C), 154.7 (CH), 142.9 (C), 139.5 (C), 137.9 (C), 133.9 (CH), 129.6 (CH), 129.6 (CH), 129.5 (CH), 126.1 (CH), 122.2 (CH), 107.8 (C), 107.0 (C), 65.5 (2 x CH_2), 50.9 (CH_2), 45.3 (CH_2), 41.8 (CH_2), 36.8 (CH_2), 35.8 (CH_2), 28.5 (CH_3), 21.4 (CH_3). **HRMS** (ESI, *m/z*) calcd for $C_{24}H_{29}N_6O_3$ $[M + H]^+$: 449.2296; found, 449.2301.

Lead Compound 2D7 (a.k.a. eCCA352)

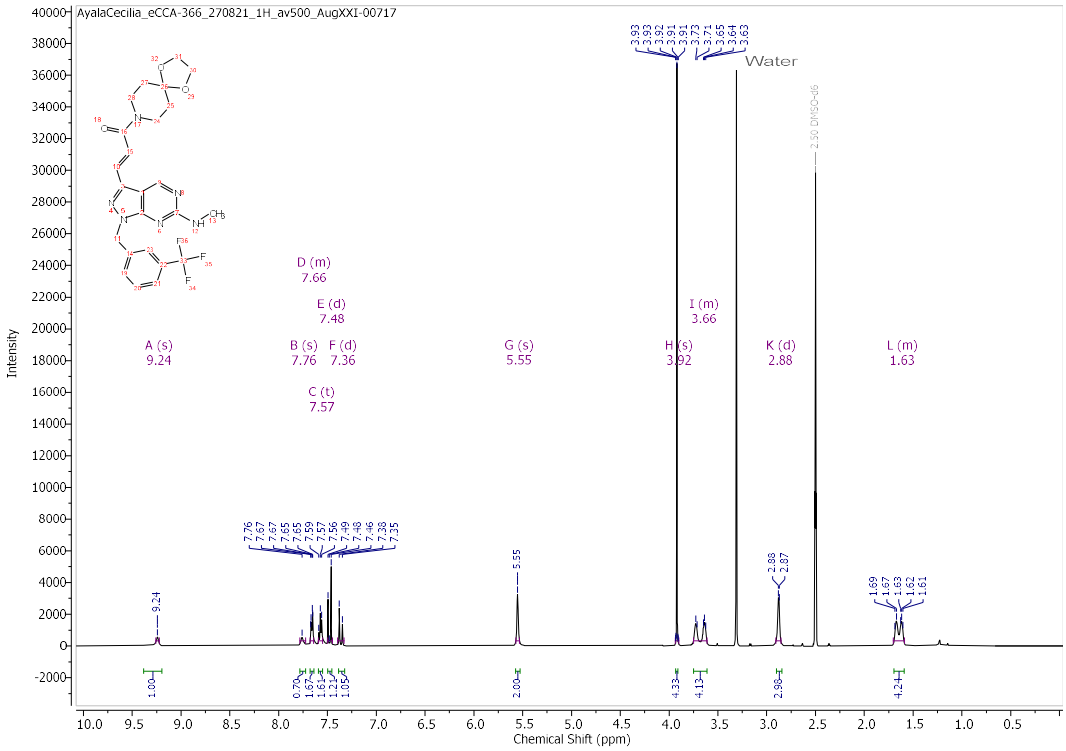


^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.24 (s, 1H), 7.76 (s, 1H), 7.68 – 7.64 (m, 2H), 7.58 (t, *J*=7.5, 2H), 7.46 (d, *J*=15.7, 1H), 7.35 (d, *J*=15.8, 1H), 5.55 (s, 2H), 3.92 (dd, *J*=13.9, 6.4, 2H), 3.43 (tt, *J*=7.9, 3.7, 2H), 3.27 (s, 3H), 3.24 (d, *J*=5.7, 1H), 2.87 (dq, *J*=8.8, 3.4, 3.0, 3H), 1.84 (d, *J*=15.2, 2H), 1.41 (ddd, *J*=30.4, 15.2, 6.3, 2H).

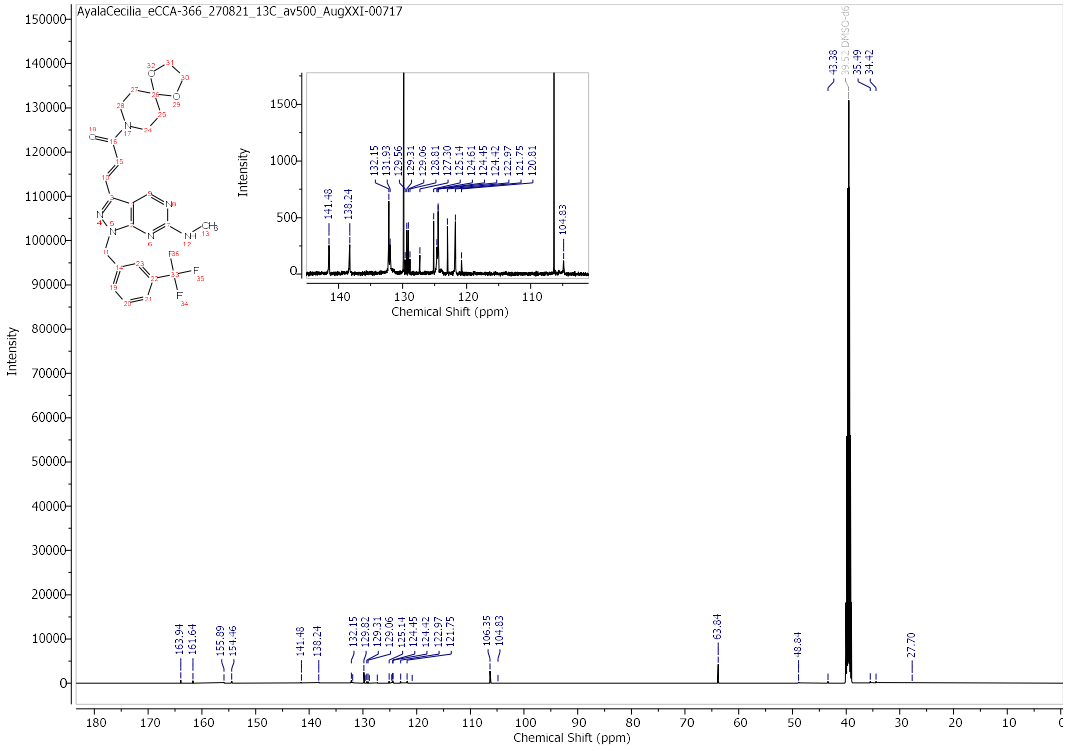


^{13}C NMR (126 MHz, DMSO) δ 163.9 (C), 161.6 (C), 155.9 (C), 154.4 (CH), 141.5 (C), 138.2 (C), 132.0 (CH), 129.8 (CH), 129.19 (q, $J_{C,F,}$= 31.8 Hz, C) 124.6 (CH), 124.4 (CH), 124.4 (CH), 124.04 (q, $J_{C,F}$ = 272.3 Hz, C) 121.8 (CH), 104.8 (C), 75.0 (CH), 55.0 (CH_3), 48.8 (CH_2), 43.9 (2 x CH_2), 31.4 (2 x CH_2), 27.7 (CH_3).

Lead Compound 2F1 (a.k.a. eCCA366)



^{1}H NMR (500 MHz, DMSO-d_6) δ 9.24 (s, 1H), 7.76 (s, 1H), 7.68 – 7.64 (m, 2H), 7.57 (t, *J* = 7.5 Hz, 2H), 7.48 (d, *J* = 15.7 Hz, 1H), 7.36 (d, *J* = 15.8 Hz, 1H), 5.55 (s, 2H), 3.92 (s, 4H), 3.75 – 3.61 (m, 4H), 2.88 (d, *J* = 4.7 Hz, 3H), 1.70 – 1.59 (m, 4H).



^{13}C NMR (126 MHz, DMSO) δ 163.9 (C), 161.6 (C), 155.9 (C), 154.5 (CH), 141.5 (C), 138.2 (C), 132.1 (CH), 131.9 (CH), 129.8 (CH), 129.19 (q, $J_{C,F}$ = 31.7 Hz, C), 124.6 (CH), 124.4 (q, $J_{C,F}$ = 4.0 Hz, CH), 124.0 (q, $J_{C,F}$ = 272.3 Hz, C), 121.7 (CH), 106.3 (C), 104.8 (C), 63.8 (2 x CH_2), 48.8 (CH_2), 43.4 (CH_2), 35.5 (CH_2), 34.4(CH_2), 27.7 (CH_3).

10. Biological protocols

Eight-Point Dose-Response Cell Viability Assay. Cells were plated in a 96-well plate at the appropriate concentration in 100 µL of suitable medium and additives (Table S1) and incubated for 48 h at 37 °C and 5 % CO_2. After 48 hours, media was replaced (95 µL) and cells were dosed (5 µL) with compounds in triplicate at final concentration at 30, 10, 3, 1, 0.3, 0.1, 0.03, 0.01 µM along with DMSO control (0.1 % v/v DMSO). Treated plates were incubated under standard conditions for 5 days. After 5 days, cell viability was evaluated using PrestoBlue™ and fluorescence data was collected, normalised and curves plotted using GraphPad ™.

Study of Cell Cycle Arrest. OE33 and FLO-1 cells were seeded in 6-well plates at 200,000 and 350,000 cells/well, respectively, in suitable medium and additives (Table S1) and incubated for 48 h at 37 °C and 5 % CO_2. After 48 hours, medium was replaced and cells were dosed with **2D7** in duplicate at final concentration of 3 µM along with DMSO control (0.1 % v/v DMSO). Treated plates were incubated under standard conditions for 72 hours. Then, the medium of OE33 cells was discarded, whilst the medium of FLO-1 was collected. Cells were trypsinised (500 µL), resuspended in fresh medium (2.5 mL) and placed into FACs tubes. Cells were centrifuged at 400 g for 5 minutes at 4 °C. The supernatant was discarded, the pellet washed with ice-cold PBS (3 mL), and centrifuged at 400 g for 5 minutes at 4 °C. Then, the pellet was resuspended in 300 µL ice-cold 50% FBS in deionized water. Cells were fixed by adding 900 µL ice-cold 70% EtOH in deionised water while vortexing gently and samples were stored at 4 °C overnight. The samples were centrifuged at 450 g for 5 minutes at 4°C and the supernatant was discarded. The pellet was further washed with ice-cold PBS (3 mL), centrifuged, and supernatant discarded. The pellet was resuspended in 500 µL ice-cold PI staining solution (50 µg/mL Propidium Iodide and 100 µg/mL RNase A) in PBS and the samples were incubated for 90 minutes in a HeraCell Incubator at 37 °C protected from light. The samples were kept on ice prior to analysis. Samples were analysed by flow cytometry using the BD LSR Fortessa X-20 Cell Analyser at the IGC FACS facility. Data was analysed using BD FACSDiva 8.0.1 Software to generate cell population data.

11. Supporting figures and tables

Table S1. Cell culture conditions and cell seeding.

Cell line	Medium	Additives	Seeding (Cells/well)
CP-A	Keratinocyte SFM	25 mg BPE, 2.5 µg rEGF	500
EPC-2	Keratinocyte SFM	25 mg BPE, 2.5 µg rEGF	1000
FLO-1	RPMI	10% FBS, 2 mM L-glutamine	1000
JH-EsoAd1	RPMI	10% FBS, 2 mM L-glutamine	1200
MFD-1	RPMI	10% FBS, 2 mM L-glutamine	1200
OACp4C	RPMI	10% FBS, 2 mM L-glutamine	1200
OE33	RPMI	10% FBS, 2 mM L-glutamine	500
SK-GT-4	RPMI	10% FBS, 2 mM L-glutamine	1000

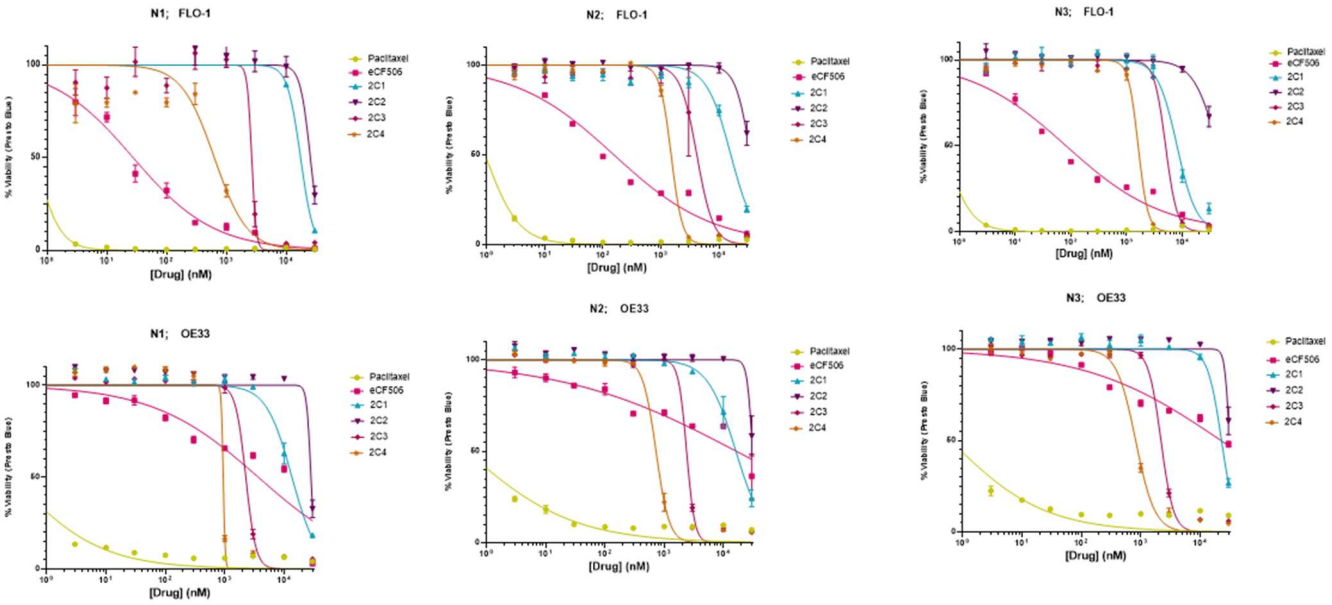


Figure S1. Dose Response Curves for Library 2C. FLO-1 and OE33 cells treated with 2C1, 2C2, 2C3 and 2C4 at 0.003- 30 µM doses, using Paclitaxel and eCF506 as positive controls. Cell viability data was obtained at day 5 using PrestoBlue reagent. Error bars: ± SD, n= 3.

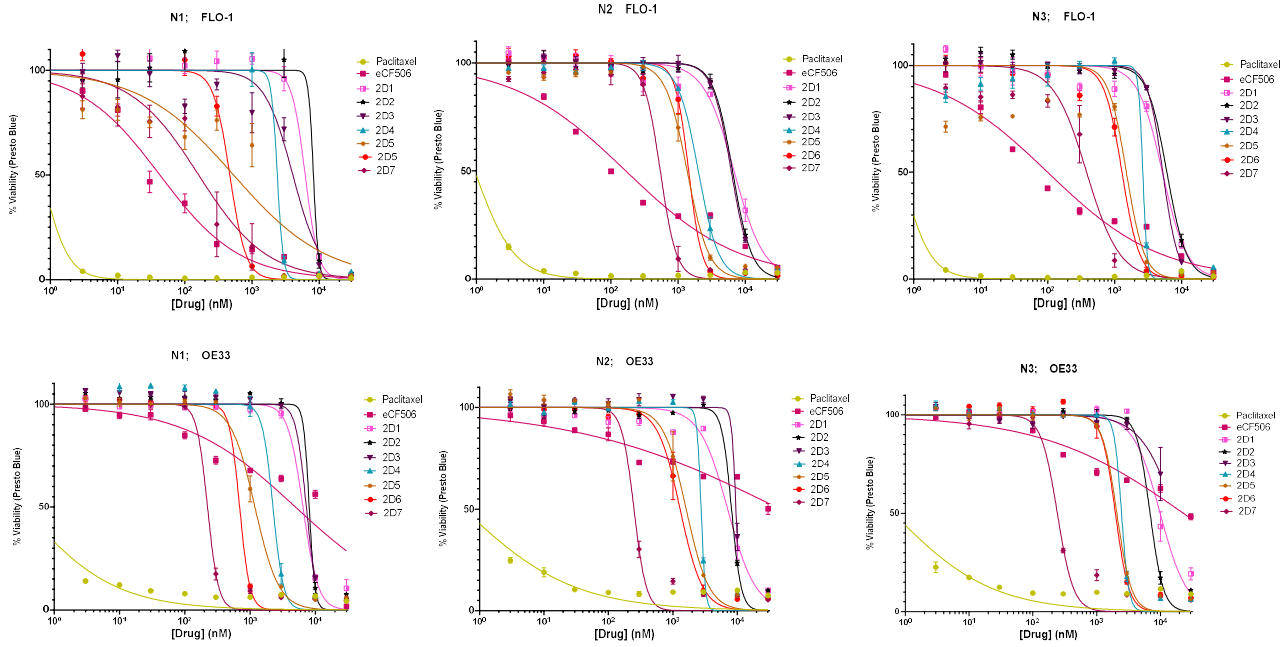


Figure S2. Dose Response Curves for Library 2D. FLO-1 and OE33 cells treated with 2D1-7 at 0.003- 30 µM doses, using Paclitaxel and eCF506 as positive controls. Cell viability data was obtained at day 5 using PrestoBlue reagent. Error bars: ± SD, n= 3.

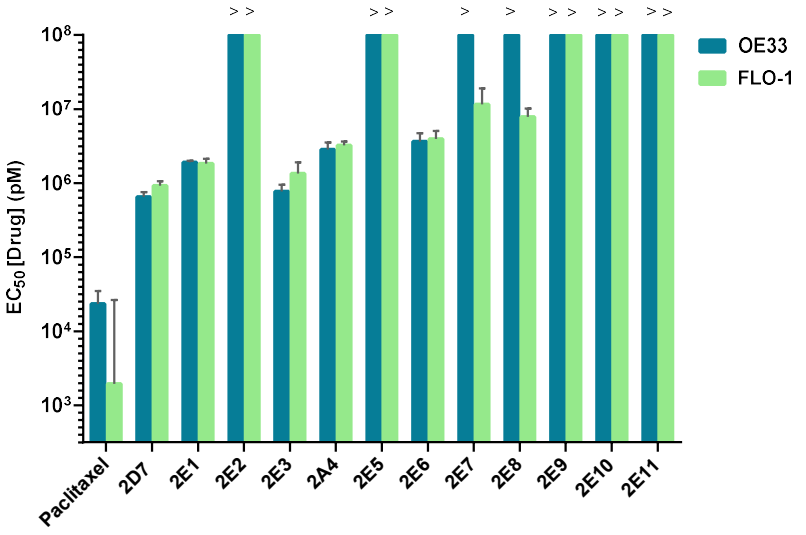


Figure S3. Cell screening of Library 2E. EC_{50} values calculated for **2E1-11** and positive control Paclitaxel and lead **2D7** against OE33 and FLO-1 cells. Dose range: 0.003- 30 µM. Cell viability was determined at day 5 using PrestoBlue reagent. Error bars: ± SD, n = 3. The most active compound of library 2E was **2E3** with EC_{50} values of 0.93 and 1.65 µM against OE33 and FLO-1 cells.

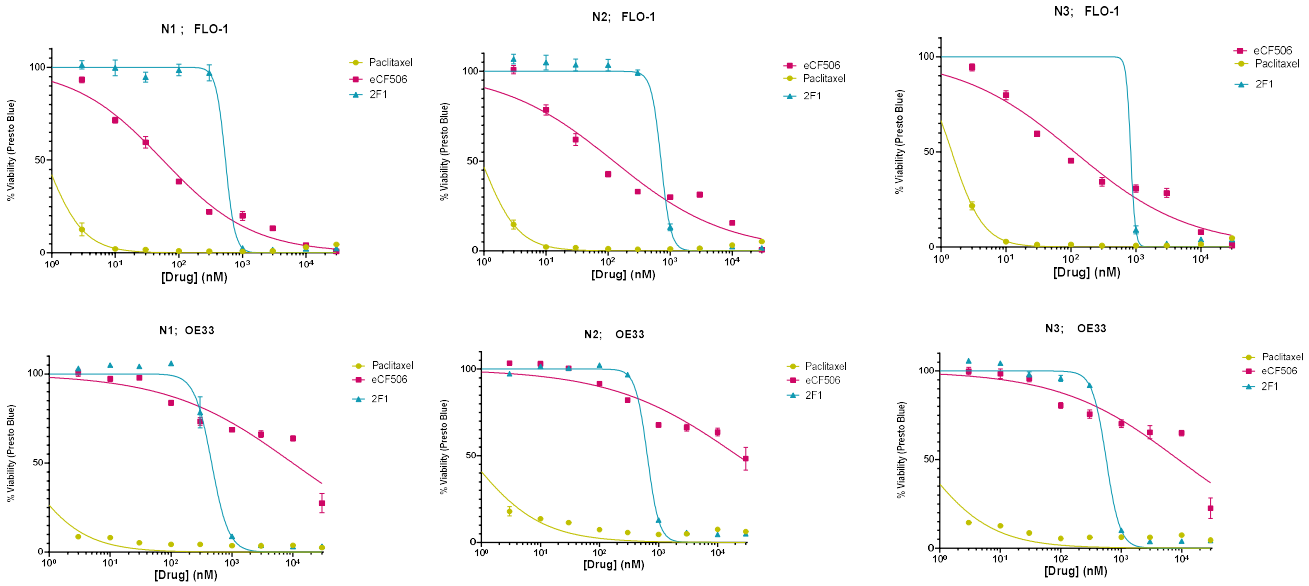


Figure S4. Dose Response Curves for Compound 2F. FLO-1 and OE33 cells treated with 2F1 at 0.003- 30 µM doses, using Paclitaxel and eCF506 as positive controls. Cell viability data was obtained at day 5 using PrestoBlue reagent. Error bars: ± SD, n= 3.

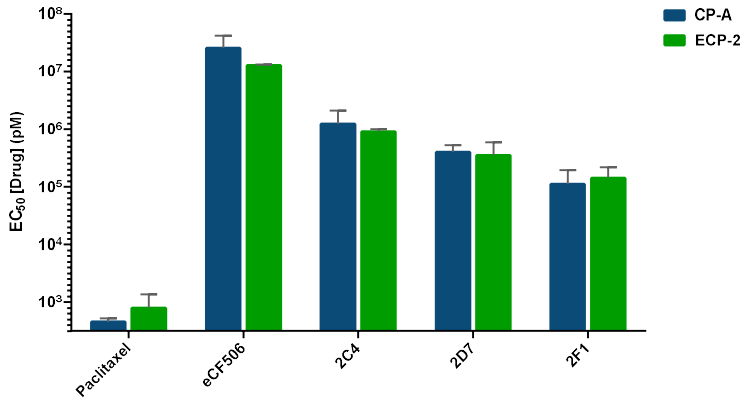


Figure S5. Screening of lead compounds in pre-malignant OC models. EC_{50} values calculated for **2C4**, **2D7**, **2F1** and positive controls eCF506 and Paclitaxel against pre-malignant CP-A and ECP-2 cells. Dose range: 0.003- 30 µM. Cell viability was determined at day 5 using PrestoBlue reagent. Error bars: ± SD, n = 3.

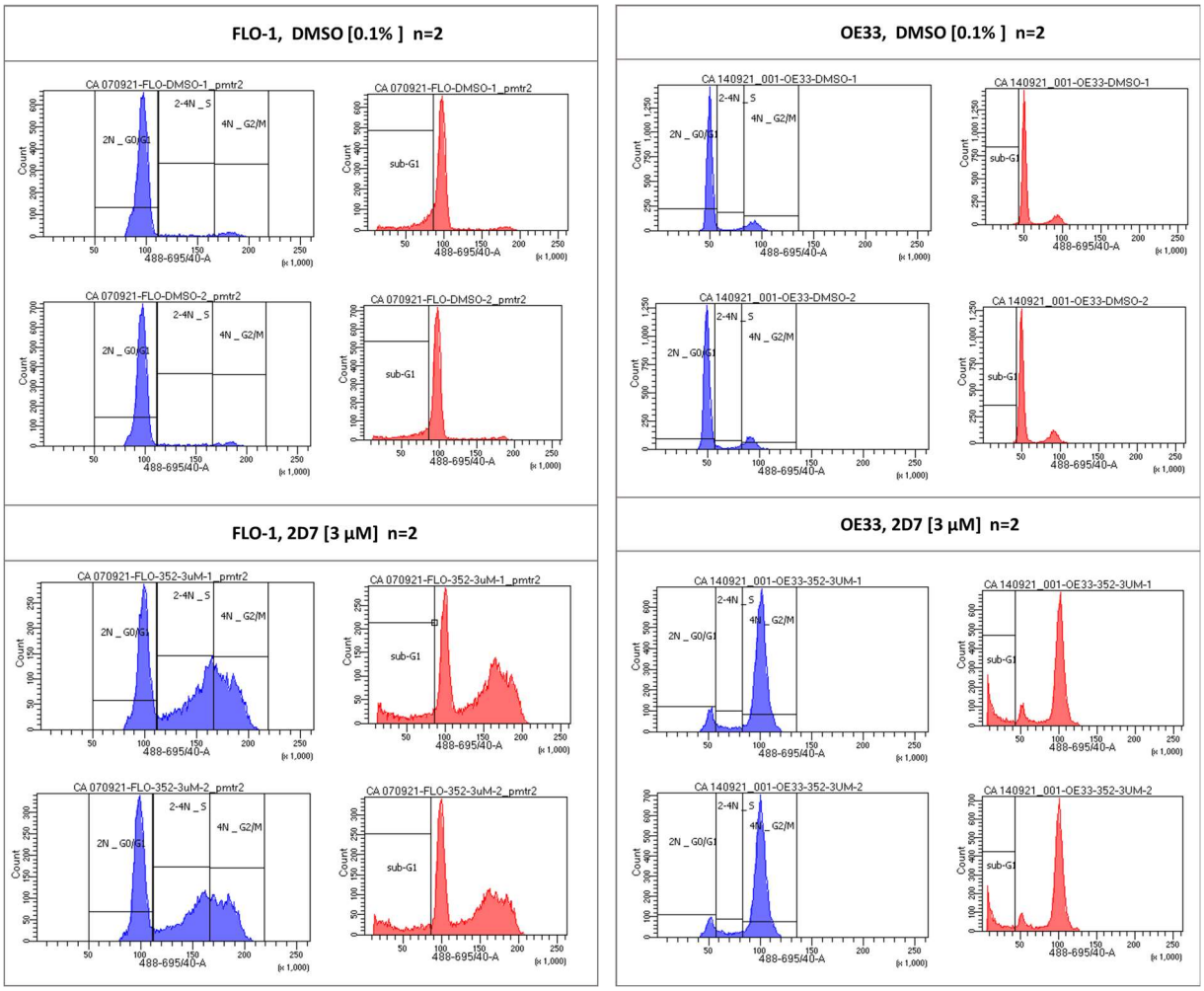


Figure S6. Cell Cycle Arrest Plots. Data from the cell count distribution by analysis from FLO-1 and OE33 cells treated with 2D7 at 3 µM for 72 hours. DMSO was used as negative control.

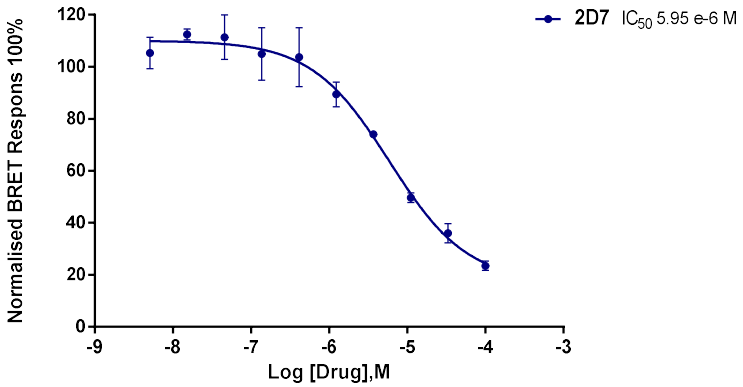


Figure S7. NanoBRET Target Engagement Assay. HEK293 cells were transfected with NanoLuc-Aurora A and treated with **2D7** and positive control CTx-0294885. Dose range of 2D7: 0.001-100 µM. AURKA target engagement was measured after 1 h of compound treatment. Error bars: ± SD, n = 2. Assay outsourced to Reaction Biology.

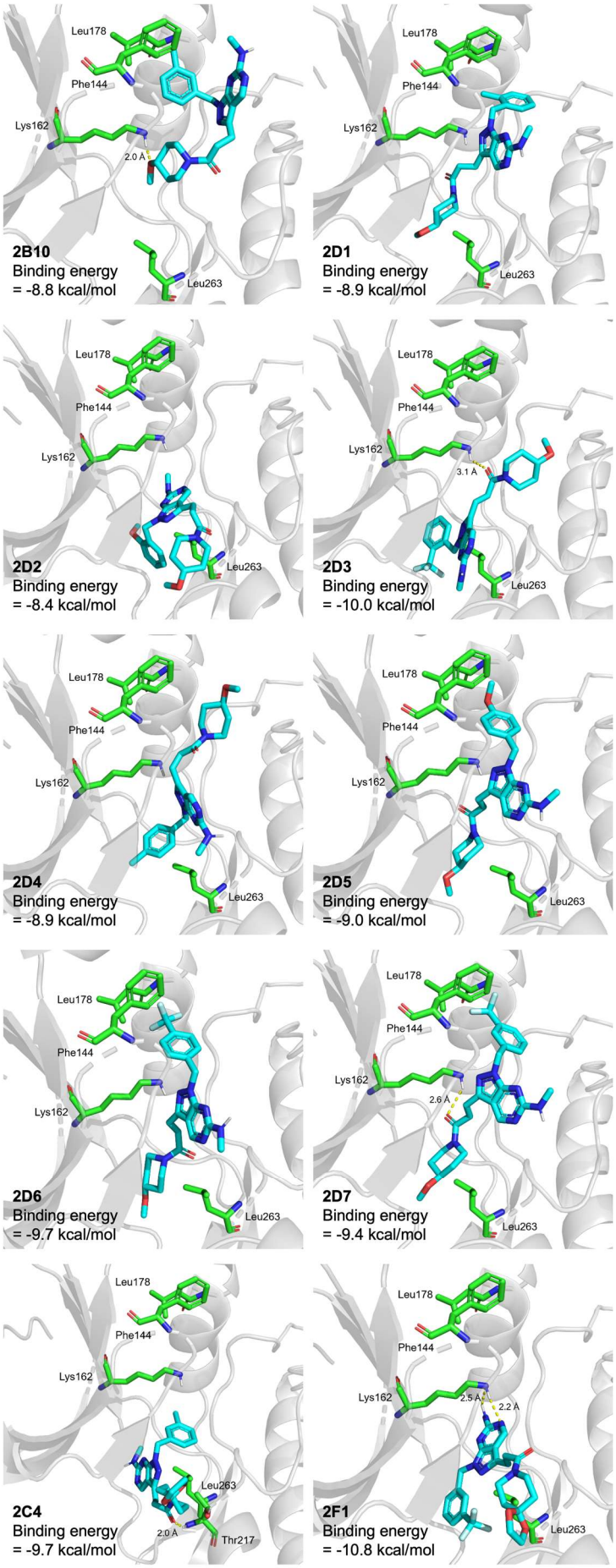


Figure S8. Predicted mode of binding of representative compounds. In silico docking of representative compounds in Aurora Kinase A (PDB:1MQ4). Key H bond interactions and their distances (Å) are shown as gold dashes. Binding energy of each compound is shown on the images. Images were created using PyMOL.

Table S2. Table shows the full results of the kinase profiling of lead compound **2D7** at single dose of 1 µM, in duplicate, against 340 wild-type protein kinases. The results are presented as the residual enzymatic activities (% of control).

			Residual Enzymatic Activity (%)		
#	Kinase Name	Kinase Family*	2D7	2D7	2D7, Mean
1	ABL1	TK	86	99	92
2	ABL2	TK	100	90	95
3	ACK1	TK	97	91	94
4	ACVR1	TKL	92	91	92
5	ACVR1B	TKL	113	97	105
6	ACVR2A	TKL	93	95	94
7	ACVR2B	TKL	123	116	119
8	ACVRL1	TKL	98	85	92
9	AKT1	AGC	100	101	101
10	AKT2	AGC	107	90	98
11	AKT3	AGC	100	93	96
12	ALK	TK	91	76	83
13	AMPKalpha1	CAMK	100	87	94
14	ARAF YDYD	TKL	92	97	95
15	ARK5	CAMK	100	89	94
16	ASK1	STE	85	84	84
17	AuroraA	OTHER	32	35	33
18	AuroraB	OTHER	85	77	81
19	AuroraC	OTHER	83	79	81
20	AXL	TK	110	96	103
21	BLK	TK	89	79	84
22	BMPR1A	TKL	94	92	93
23	BMPR1B	TKL	85	85	85
24	BMX	TK	111	100	106
25	BRAF	TKL	71	87	79
26	BRK	TK	95	97	96
27	BRSK1	CAMK	103	90	96
28	BRSK2	CAMK	106	99	102
29	BTK	TK	75	78	76
30	BUB1B	OTHER	98	93	96
31	CAMK1D	CAMK	91	79	85
32	CAMK2A	CAMK	100	90	95
33	CAMK2B	CAMK	114	79	97
34	CAMK2D	CAMK	89	96	93
35	CAMK2G	CAMK	105	90	97
36	CAMK4	CAMK	99	88	94
37	CAMKK1	OTHER	122	92	107
38	CAMKK2	OTHER	71	83	77
39	CDC42BPA	AGC	106	96	101

40	CDC42BPB	AGC	90	82	86
41	CDC7/DBF4	OTHER	107	100	103
42	CDK1/CycA2	CMGC	87	84	86
43	CDK1/CycB1	CMGC	93	80	86
44	CDK1/CycE1	CMGC	93	84	89
45	CDK10/CycQ	CMGC	104	95	99
46	CDK11B 357-795/CycK	CMGC	114	110	112
47	CDK12/CycK	CMGC	112	101	107
48	CDK13/CycK	CMGC	115	101	108
49	CDK16/CycY	CMGC	99	97	98
50	CDK17/p35NCK	CMGC	104	97	101
51	CDK18/CycY	CMGC	94	104	99
52	CDK19/CycC	CMGC	98	94	96
53	CDK2/CycA2	CMGC	101	98	100
54	CDK2/CycD1	CMGC	92	105	98
55	CDK2/CycE1	CMGC	97	103	100
56	CDK20/CycH	CMGC	96	91	94
57	CDK20/CycT1	CMGC	109	98	104
58	CDK3/CycC	CMGC	88	87	87
59	CDK3/CycE1	CMGC	97	88	93
60	CDK4/CycD1	CMGC	97	95	96
61	CDK4/CycD2	CMGC	108	94	101
62	CDK4/CycD3	CMGC	86	85	86
63	CDK5/p25NCK	CMGC	122	109	116
64	CDK5/p35NCK	CMGC	92	86	89
65	CDK6/CycD1	CMGC	95	77	86
66	CDK6/CycD2	CMGC	94	90	92
67	CDK6/CycD3	CMGC	98	77	87
68	CDK7/CycH/MAT1	CMGC	94	86	90
69	CDK8/CycC	CMGC	111	99	105
70	CDK9/CycK	CMGC	87	85	86
71	CDK9/CycT1	CMGC	88	92	90
72	CHK1	CAMK	93	90	91
73	CHK2	CAMK	118	100	109
74	CK1alpha1	CK1	99	101	100
75	CK1delta	CK1	98	105	102
76	CK1epsilon	CK1	98	105	101
77	CK1gamma1	CK1	107	99	103
78	CK1gamma2	CK1	102	109	106
79	CK1gamma3	CK1	107	110	108
80	CK2alpha1	OTHER	111	107	109
81	CK2alpha2	OTHER	112	107	109
82	CLK1	CMGC	87	88	88
83	CLK2	CMGC	94	92	93
84	CLK3	CMGC	113	98	105
85	CLK4	CMGC	94	103	98
86	COT	STE	122	102	112
87	CSF1R	TK	96	80	88

88	CSK	TK	114	82	98
89	DAPK1	CAMK	124	107	115
90	DAPK2	CAMK	98	93	96
91	DAPK3	CAMK	107	86	97
92	DCAMKL2	CAMK	84	92	88
93	DDR2	TK	99	100	99
94	DMPK	AGC	98	96	97
95	DNAPK	ATYPICAL	76	120	98
96	DYRK1A	CMGC	102	94	98
97	DYRK1B	CMGC	90	83	86
98	DYRK2	CMGC	96	94	95
99	DYRK3	CMGC	114	115	115
100	DYRK4	CMGC	117	108	112
101	EEF2K	ATYPICAL	99	89	94
102	EGFR	TK	111	106	108
103	EIF2AK2	OTHER	130	106	118
104	EIF2AK3	OTHER	103	96	99
105	EPHA1	TK	105	97	101
106	EPHA2	TK	113	94	104
107	EPHA3	TK	92	88	90
108	EPHA4	TK	80	80	80
109	EPHA5	TK	107	98	102
110	EPHA6	TK	93	91	92
111	EPHA7	TK	101	107	104
112	EPHA8	TK	107	101	104
113	EPHB1	TK	111	98	104
114	EPHB2	TK	92	91	92
115	EPHB3	TK	94	89	92
116	EPHB4	TK	83	73	78
117	ERBB2	TK	94	86	90
118	ERBB4	TK	106	82	94
119	ERK1	CMGC	105	103	104
120	ERK2	CMGC	95	81	88
121	ERK5	CMGC	109	96	103
122	ERK7	CMGC	101	88	95
123	FAK	TK	95	91	93
124	FER	TK	94	73	84
125	FES	TK	94	86	90
126	FGFR1	TK	95	86	90
127	FGFR2	TK	77	77	77
128	FGFR3	TK	88	80	84
129	FGFR4	TK	96	87	91
130	FGR	TK	105	74	90
131	FLT3	TK	89	85	87
132	FRK	TK	78	72	75
133	FYN	TK	96	87	91
134	GRK2	AGC	108	88	98
135	GRK3	AGC	103	94	99

136	GRK4	AGC	114	118	116
137	GRK5	AGC	119	129	124
138	GRK6	AGC	110	104	107
139	GRK7	AGC	113	100	107
140	GSG2	OTHER	83	96	90
141	GSK3alpha	CMGC	114	99	106
142	GSK3beta	CMGC	106	94	100
143	HCK	TK	91	77	84
144	HIPK1	CMGC	114	95	105
145	HIPK2	CMGC	97	101	99
146	HIPK3	CMGC	104	89	96
147	HIPK4	CMGC	111	104	108
148	HRI	OTHER	105	105	105
149	IGF1R	TK	101	88	94
150	IKKalpha	OTHER	98	93	95
151	IKKbeta	OTHER	109	94	101
152	IKKepsilon	OTHER	100	96	98
153	INSR	TK	110	85	97
154	INSRR	TK	93	95	94
155	IRAK1	TKL	112	99	106
156	IRAK4	TKL	100	94	97
157	ITK	TK	87	79	83
158	JAK1	TK	103	97	100
159	JAK2	TK	104	94	99
160	JAK3	TK	97	89	93
161	JNK1	CMGC	96	86	91
162	JNK2	CMGC	91	91	91
163	JNK3	CMGC	89	85	87
164	KIT	TK	83	81	82
165	LCK	TK	94	92	93
166	LIMK1	TKL	95	90	93
167	LIMK2	TKL	94	76	85
168	LKB1/MO25a/STRADa	CAMK	103	95	99
169	LRRK2	TKL	63	53	58
170	LTK	TK	108	87	97
171	LYN	TK	89	73	81
172	MAP3K1	STE	91	105	98
173	MAP3K10	STE	104	95	100
174	MAP3K11	STE	100	95	97
175	MAP3K7/MAP3K7IP1	STE	88	96	92
176	MAP3K9	STE	106	98	102
177	MAP4K2	STE	77	83	80
178	MAP4K4	STE	85	88	86
179	MAP4K5	STE	125	108	117
180	MAPKAPK2	CAMK	118	105	112
181	MAPKAPK3	CAMK	92	78	85
182	MAPKAPK5	CAMK	124	117	121
183	MARK1	CAMK	96	91	93

184	MARK2	CAMK	87	96	92
185	MARK3	CAMK	115	93	104
186	MARK4	CAMK	109	94	101
187	MASTL	AGC	99	91	95
188	MATK	TK	111	93	102
189	MEK1	STE	91	99	95
190	MEK2	STE	87	80	84
191	MEK5	STE	98	115	107
192	MEKK2	STE	100	106	103
193	MEKK3	STE	106	97	102
194	MELK	CAMK	112	97	104
195	MERTK	TK	90	85	88
196	MET	TK	93	90	91
197	MINK1	STE	90	79	85
198	MKK4	STE	106	96	101
199	MKK6 SDTD	STE	90	98	94
200	MKK7	STE	90	85	88
201	MKNK1	CAMK	93	101	97
202	MKNK2	CAMK	119	102	111
203	MLK4	TKL	120	98	109
204	MST1	STE	105	100	103
205	MST2	STE	114	86	100
206	MST3	STE	114	98	106
207	MST4	STE	90	91	90
208	MTOR	ATYPICAL	92	100	96
209	MUSK	TK	92	90	91
210	MYLK	CAMK	119	85	102
211	MYLK2	CAMK	93	97	95
212	MYLK3	CAMK	89	102	95
213	NDR1	AGC	112	100	106
214	NDR2	AGC	91	89	90
215	NEK1	OTHER	99	105	102
216	NEK11	OTHER	105	96	100
217	NEK2	OTHER	106	90	98
218	NEK3	OTHER	112	96	104
219	NEK4	OTHER	101	89	95
220	NEK6	OTHER	97	90	93
221	NEK7	OTHER	117	107	112
222	NEK9	OTHER	84	91	87
223	NIK	STE	111	99	105
224	NLK	CMGC	91	88	89
225	p38alpha	CMGC	89	89	89
226	p38beta	CMGC	86	89	87
227	p38delta	CMGC	106	98	102
228	p38gamma	CMGC	98	92	95
229	PAK1	STE	99	107	103
230	PAK2	STE	94	100	97
231	PAK3	STE	87	84	86

232	PAK4	STE	93	92	92
233	PAK6	STE	99	91	95
234	PAK7	STE	94	91	93
235	PASK	CAMK	86	109	97
236	PBK	OTHER	100	72	86
237	PDGFRalpha	TK	96	89	92
238	PDGFRbeta	TK	101	97	99
239	PDK1	AGC	96	98	97
240	PHKG1	CAMK	92	95	93
241	PHKG2	CAMK	87	76	81
242	PIM1	CAMK	107	102	105
243	PIM2	CAMK	113	91	102
244	PIM3	CAMK	87	89	88
245	PKA	AGC	101	95	98
246	PKCalpha	AGC	103	92	98
247	PKCbeta1	AGC	99	92	95
248	PKCbeta2	AGC	98	89	94
249	PKCdelta	AGC	100	98	99
250	PKCepsilon	AGC	99	98	99
251	PKCeta	AGC	122	96	109
252	PKCgamma	AGC	99	79	89
253	PKCiota	AGC	98	93	95
254	PKCmu	AGC	92	88	90
255	PKCnu	AGC	81	98	89
256	PKCtheta	AGC	100	95	97
257	PKCzeta	AGC	105	94	100
258	PKMzeta	AGC	87	105	96
259	PKN3	AGC	85	86	86
260	PLK1	OTHER	119	99	109
261	PLK3	OTHER	103	105	104
262	PRK1	AGC	86	91	89
263	PRK2	AGC	112	86	99
264	PRKD2	CAMK	98	86	92
265	PRKG1	AGC	101	91	96
266	PRKG2	AGC	108	93	101
267	PRKX	AGC	101	100	101
268	PYK2	TK	94	91	93
269	RAF1 YDYD	TKL	98	95	96
270	RET	TK	96	77	87
271	RIPK2	TKL	98	76	87
272	RIPK4	TKL	100	98	99
273	RIPK5	TKL	109	90	99
274	ROCK1	AGC	94	96	95
275	ROCK2	AGC	89	79	84
276	RON	TK	96	93	94
277	ROS	TK	82	77	79
278	RPS6KA1	AGC	103	91	97
279	RPS6KA2	AGC	88	83	86

280	RPS6KA3	AGC	108	109	109
281	RPS6KA4	AGC	90	81	86
282	RPS6KA5	AGC	101	98	99
283	RPS6KA6	AGC	94	92	93
284	S6K	AGC	102	92	97
285	S6Kbeta	AGC	97	94	95
286	SAK	OTHER	83	82	82
287	SGK1	AGC	97	87	92
288	SGK2	AGC	111	97	104
289	SGK3	AGC	90	101	96
290	SIK1	CAMK	97	89	93
291	SIK2	CAMK	106	95	101
292	SIK3	CAMK	100	98	99
293	SLK	STE	85	87	86
294	SNARK	CAMK	113	97	105
295	SNK	OTHER	108	94	101
296	SRC	TK	104	94	99
297	SRMS	TK	99	84	91
298	SRPK1	CMGC	106	99	102
299	SRPK2	CMGC	97	103	100
300	STK17A	CAMK	110	96	103
301	STK23	CAMK	124	104	114
302	STK25	STE	102	87	95
303	STK33	CAMK	96	89	93
304	STK39	STE	125	107	116
305	SYK	TK	105	96	100
306	TAOK2	STE	111	93	102
307	TAOK3	STE	101	95	98
308	TBK1	OTHER	126	103	114
309	TEC	TK	81	72	76
310	TGFBR1	TKL	89	84	86
311	TGFBR2	TKL	93	82	87
312	TIE2	TK	102	93	98
313	TLK1	AGC	93	90	92
314	TLK2	AGC	108	96	102
315	TNK1	TK	93	84	89
316	TRKA	TK	77	62	69
317	TRKB	TK	83	68	76
318	TRKC	TK	88	78	83
319	TSF1	OTHER	90	81	85
320	TSK2	CAMK	110	101	105
321	TSSK1	CAMK	103	77	90
322	TTBK1	CK1	116	91	103
323	TTBK2	CK1	102	108	105
324	TTK	OTHER	102	108	105
325	TXK	TK	110	108	109
326	TYK2	TK	91	91	91
327	TYRO3	TK	93	82	88

328	ULK2	OTHER	105	97	101
329	VEGFR1	TK	97	93	95
330	VEGFR2	TK	96	91	93
331	VEGFR3	TK	108	89	98
332	VRK1	CK1	105	96	100
333	VRK2	CK1	112	112	112
334	WEE1	OTHER	77	88	83
335	WNK1	OTHER	102	99	100
336	WNK2	OTHER	91	109	100
337	WNK3	OTHER	119	110	114
338	YES	TK	90	81	85
339	ZAK	TKL	103	101	102
340	ZAP70	TK	108	82	95

Table S3. EC50 values calculated for all the compound libraries, positive control Paclitaxel and eCF506 against OE33, FLO-1, SK-GT-4, OACp4C, MDF-1, JH-EsoAd1 cells, pre-malignant CP-A and ECP-2 cells. Dose range: 0.003- 30 µM. Cell viability was determined at day 5 using PrestoBlue reagent. Mean values and SD (n = 3) were also calculated. NC, value non calculated.

OE33 EC_{50} (µM)				MEAN	SD
Paclitaxel	0.00066	0.00039	0.00110	0.00071	0.00029
1A1	NC	>30	>30	>30	NC
1A2	>30	>30	>30	>30	NC
1A3	>30	>30	>30	>30	NC
1A4	>30	>30	>30	>30	NC
1A5	>30	>30	>30	>30	NC
1A6	>30	>30	>30	>30	NC
1A7	>30	>30	>30	>30	NC
1A8	>30	>30	>30	>30	NC
1A9	>30	>30	>30	>30	NC
1A10	>30	>30	>30	>30	NC
9	>30	>30	>30	>30	NC
10	>30	>30	>30	>30	NC

FLO-1 EC_{50} (µM)				MEAN	SD
Paclitaxel	0.00039	0.00170	0.00080	0.00096	0.00055
1A1	>30	>30	>30	>30	NC
1A2	>30	>30	>30	>30	NC
1A3	>30	>30	>30	>30	NC
1A4	>30	>30	>30	>30	NC
1A5	>30	>30	>30	>30	NC
1A6	>30	>30	>30	>30	NC
1A7	>30	>30	>30	>30	NC
1A8	>30	>30	>30	>30	NC
1A9	>30	>30	>30	>30	NC
1A10	>30	>30	>30	>30	NC
9	>30	>30	>30	>30	NC
10	>30	>30	>30	>30	NC

OE33 EC_{50} (µM)				MEAN	SD
Paclitaxel	0.00043	0.00053	0.00052	0.00050	0.00004
eCF506	6.99	18.79	23.005	16.262	6.778
2A1	>30	>30	>30	>30	NC
2A2	>30	>30	>30	>30	NC
2A3	>30	>30	>30	>30	NC
2A4	20.45	16.85	15.80	17.70	1.99
2A5	>30	>30	>30	>30	NC
2A6	>30	>30	>30	>30	NC
2A7	6.58	7.30	6.28	6.72	0.43
2A8	>30	>30	>30	>30	NC
2A9	15.84	23.03	6.00	14.96	6.98
2A10	>30	>30	>30	>30	NC
12	>30	>30	>30	>30	NC
13	>30	>30	>30	>30	NC

FLO-1 EC_{50} (µM)				MEAN	SD
Paclitaxel	0.00070	0.00091	0.00084	0.00081	0.00008
eCF506	0.13	0.14	0.36	0.21	0.10
2A1	11.13	11.49	7.28	9.97	1.91
2A2	>30	>30	>30	>30	NC
2A3	15.76	19.98	27.75	21.16	4.97
2A4	15.94	6.69	27.47	16.70	8.50
2A5	>30	>30	>30	>30	NC
2A6	>30	>30	>30	>30	NC
2A7	8.44	6.29	7.43	7.39	0.88
2A8	16.70	26.50	29.14	24.11	5.35
2A9	>30	>30	>30	>30	NA
2A10	6.81	16.16	26.28	16.41	7.95
12	>30	>30	>30	>30	NC
13	>30	>30	>30	>30	NC

OE33 EC_{50} (μM)				MEAN	SD
Paclitaxel	0.00075	0.00082	0.00057	0.00071	0.00011
eCF506	4.96	14.87	25.07	14.97	8.21
2B1	11.37	13.38	14.42	13.06	1.26
2B2	>30	>30	>30	>30	NC
2B3	19.40	21.52	18.28	19.74	1.34
2B4	>30	>30	>30	>30	NC
2B5	>30	>30	>30	>30	NC
2B6	>30	>30	>30	>30	NC
2B7	>30	>30	>30	>30	NC
2B8	5.41	5.55	8.58	6.51	1.46
2B9	6.54	7.41	8.51	7.49	0.80
2B10	1.48	2.13	1.89	1.83	0.27
2B11	2.76	2.87	3.04	2.89	0.11

FLO-1 EC_{50} (μM)				MEAN	SD
Paclitaxel	0.00089	0.00368	0.00218	0.00225	0.00114
eCF506	0.07	1.60	0.25	0.64	0.68
2B1	12.76	28.68	28.44	23.29	7.45
2B2	>30	>30	>30	>30	NC
2B3	11.49	28.01	16.77	18.76	6.89
2B4	>30	>30	>30	>30	NC
2B5	>30	>30	>30	>30	NC
2B6	>30	>30	>30	>30	NC
2B7	>30	>30	>30	>30	NC
2B8	4.20	8.44	8.82	7.15	2.09
2B9	5.77	9.01	9.36	8.05	1.62
2B10	1.94	2.90	2.77	2.54	0.42
2B11	3.23	5.82	4.85	4.63	1.07

OE33 EC_{50} (μM)				MEAN	SD
Paclitaxel	0.00026	0.00046	0.00062	0.00045	0.00015
eCF506	3.64	16.60	22.50	14.25	7.87
2C1	13.52	16.25	22.92	17.57	3.95
2C2	>30	>30	>30	>30	NC
2C3	2.26	2.39	2.19	2.28	0.08
2C4	0.94	0.73	0.82	0.83	0.09

FLO-1 EC_{50} (μM)				MEAN	SD
Paclitaxel	0.00063	0.00093	0.00052	0.00070	0.00017
eCF506	0.03	0.15	0.08	0.09	0.05
2C1	17.41	15.93	8.02	13.79	4.12
2C2	>30	>30	>30	>30	NC
2C3	2.65	3.11	5.00	3.59	1.02
2C4	0.63	1.49	1.61	1.24	0.44

OE33 EC_{50} (μM)				MEAN	SD
Paclitaxel	0.00032	0.00056	0.00065	0.00051	0.00014
eCF506	4.97	18.90	23.06	15.64	7.74
2D1	6.49	7.11	9.92	7.84	1.50
2D2	8.09	8.17	6.89	7.72	0.58
2D3	7.31	9.57	17.42	11.43	4.33
2D4	2.25	2.76	2.46	2.49	0.21
2D5	1.19	1.62	2.08	1.63	0.36
2D6	0.69	1.30	1.97	1.32	0.52
2D7	0.22	0.25	0.24	0.24	0.01

FLO-1 EC_{50} (μM)				MEAN	SD
Paclitaxel	0.00075	0.00094	0.00066	0.00078	0.00012
eCF506	0.04	0.17	0.10	0.10	0.05
2D1	6.20	6.78	5.20	6.06	0.65
2D2	8.23	6.45	6.06	6.91	0.94
2D3	4.06	6.16	5.27	5.16	0.86
2D4	2.38	2.00	2.65	2.34	0.27
2D5	0.53	1.36	1.51	1.13	0.43
2D6	0.47	1.44	1.29	1.07	0.43
2D7	0.16	0.54	0.38	0.36	0.16

OE33 EC_{50} (μM)				MEAN	SD
Paclitaxel	0.03669	0.03286	0.01082	0.02679	0.01140
2D7	0.49	0.79	0.69	0.66	0.12
2E1	1.77	1.64	1.86	1.76	0.09
2E2	> 30	> 30	> 30	> 30	NC
2E3	0.51	0.85	1.43	0.93	0.38
2E4	1.92	3.31	4.25	3.16	0.96
2E5	> 30	> 30	> 30	> 30	NC
2E6	2.68	5.99	3.40	4.02	1.42
2E7	> 30	> 30	> 30	> 30	NC
2E8	> 30	> 30	> 30	> 30	NC
2E9	> 30	> 30	> 30	> 30	NC
2E10	> 30	> 30	> 30	> 30	NC
2E11	> 30	> 30	> 30	> 30	NC

FLO-1 EC_{50} (μM)				MEAN	SD
Paclitaxel	0.02781	0.00175	0.00015	0.00990	0.01268
2D7	0.79	1.02	1.21	1.01	0.17
2E1	1.52	1.52	1.98	1.67	0.22
2E2	> 30	> 30	> 30	> 30	NC
2E3	1.21	1.32	2.41	1.65	0.54
2E4	3.70	3.19	3.20	3.36	0.24
2E5	> 30	> 30	> 30	> 30	NC
2E6	3.03	4.77	3.91	3.90	0.71
2E7	7.64	17.60	12.80	12.68	4.07
2E8	5.95	7.94	9.93	7.94	1.62
2E9	> 30	> 30	> 30	> 30	NC
2E10	> 30	> 30	> 30	> 30	NC
2E11	> 30	> 30	> 30	> 30	NC

OE33 EC_{50} (μM)				MEAN	SD
Paclitaxel	0.00031	0.00060	0.00047	0.00046	0.00012
eCF506	9.94	22.19	8.86	13.67	6.05
2F1	0.46	0.65	0.57	0.56	0.07

FLO-1 EC_{50} (μM)				MEAN	SD
Paclitaxel	0.00080	0.00092	0.00146	0.00106	0.00029
eCF506	0.06	0.12	0.11	0.10	0.03
2F1	0.54	0.71	0.83	0.70	0.12

CP-A EC_{50} (μM)				MEAN	SD
Paclitaxel	0.00051	0.00048	0.00040	0.00046	0.00005
eCF506	19.88	18.34	45.27	27.83	12.35
2C4	2.28	0.90	0.91	1.36	0.65
2D7	0.44	0.29	0.49	0.41	0.09
2F1	0.06	0.13	0.18	0.12	0.05

ECP-2 EC_{50} (μM)				MEAN	SD
Paclitaxel	0.00043	0.00120	0.00098	0.00087	0.00032
eCF506	12.27	13.32	12.74	12.77	0.43
2C4	0.91	0.82	1.00	0.91	0.07
2D7	0.27	0.25	0.64	0.39	0.18
2F1	0.09	0.16	0.20	0.15	0.05

SK-GT-4 EC_{50} (μM)				MEAN	SD
Paclitaxel	0.00097	0.01121	0.00490	0.00569	0.00422
eCF506	0.47	7.07	3.83	3.79	2.70
2C4	0.81	1.86	4.89	2.52	1.73
2D7	0.56	0.70	1.09	0.79	0.22
2F1	0.67	4.33	3.70	2.90	1.60

OACp4C EC_{50} (μM)				MEAN	SD
Paclitaxel	0.00360	0.00403	0.00382	0.00381	0.00017
eCF506	0.45	0.64	0.53	0.54	0.08
2C4	0.47	1.18	2.69	1.44	0.92
2D7	0.40	0.47	0.66	0.51	0.11
2F1	0.27	0.46	0.59	0.44	0.13

MDF-1 EC_{50} (μM)				MEAN	SD
Paclitaxel	0.00627	0.00776	0.00813	0.00739	0.00080
eCF506	0.09	0.20	0.17	0.15	0.05
2C4	2.30	6.05	6.98	5.11	2.02
2D7	0.97	1.49	5.24	2.57	1.90
2F1	0.85	1.05	0.87	0.92	0.09

JH-EsoAd1 EC_{50} (μM)				MEAN	SD
Paclitaxel	0.00131	0.00072	0.00134	0.00112	0.00028
eCF506	10.16	5.24	8.97	8.12	2.10
2C4	3.24	1.01	1.91	2.05	0.92
2D7	1.33	0.63	0.38	0.78	0.40
2F1	0.31	0.69	0.86	0.62	0.23

Table S4 Structural, Physicochemical and predicted PK properties of **2D7**

Molecular weight	474.48 $g \cdot mol^{-1}$
Num. rotable bonds	8
Num. H-bond acceptors	8
Num. H-bond donors	1
TPSA[a]	85.17 $Å^2$
cLogP[a]	3.46
Predicted solubility (pH = 7.4)[b]	>10 $\mu g \cdot mL^{-1}$
Papp (A-to-B, Caco-2)[c]	1.36×10^{-6} $cm \cdot s^{-1}$
Intestinal absorption (human, % absorbed)[c]	91.9%
f_u, plasma (human)[b]	0.052
CL_{int} (human)[b]	21.09 $\mu L \cdot min^{-1} \cdot mg^{-1}$

[a]SwissADME. [b]DruMAP. [c]pkCSM.