ELECTRONIC SUPPORTING INFORMATION

Discovery of pyrazolopyrimidines that selectively inhibit CSF-1R kinase by iterative design, synthesis and screening against Glioblastoma cells

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LIST OF CONTENTS

1. General synthetic experimental protocols

All the experiments detailed were carried out in closed reaction vessels; either in a Biotage® Initiator Third Generation microwave synthesiser, or they were carried out in an inert atmosphere under nitrogen. Chemicals and anhydrous solvents that were commercially available were purchased from a range of suppliers, including: Acros Organics, Alfa Aesar, Apollo Scientific, Fisher Scientific, Fluorochem, Manchester Organics, Matrix Scientific, Sigma Aldrich (Merck) 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 obtained 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. Compounds were purified using one of three methods. 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. Normal phase chromatography using a Biotage® Isolera automated purification machine using commercially available pre-loaded SNAP KP-,HP-silica and SFAR-silica columns was also used for some compounds. 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 recorded at ambient temperature on a 500 MHz Bruker Avance III spectrometer. Samples were dissolved in deuterated solvents commercially available from Sigma-Aldrich. 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 were confirmed where appropriate by DEPTQ90, 2D-HSQC and 2D-COSY spectra.

All compounds used in the biological screenings 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. All samples were made to a concentration of 1 mM in MeOH and were run using a 5-95% MeOH/H2O (+0.1% FA) gradient over 7 minutes on a Poroshell C18 Column. Data from this device was analyzed using Agilent data analysis software. Compounds for screening were all >90% purity by HPLC/ELSD analysis. High resolution mass spectra (HRMS) were obtained for the compounds that underwent further evaluation using a Bruker 3.0 T Apex II Mass Spectrometer. Data for these compounds are reported, along with the calculated value for the relative ion and the molecular formula.

2. Synthesis and characterization of scaffolds 4 and 8

Synthesis of 1H-pyrazolo[3,4-d]pyrimidin-4-amine (2). 5-Amino-1H-pyrazole-4-carbonitrile (1) (2.98 g, 27.5 mmol) was added to a 20 mL microwave vial equipped with a stirrer bar. Formamide (15 mL, 443 mmol, 16 eq.) was added. The vial was sealed and heated to 180 °C in a microwave while stirring for 75 minutes. Upon cooling, a white precipitate formed. The precipitate was collected by vacuum filtration and washed with water (2 x 25 mL). This was then dried overnight at 40 °C in a vacuum oven to give a white solid, 1H-pyrazolo[3,4-d]pyrimidin-4-amine (3.13 g, 23.2 mmol, 84 %). **1H NMR** (500 MHz, DMSO-d6) δ 13.31 (br. s., 3H), 8.13 (s, 1H), 8.06 (s, 1H). **13C NMR** (126 MHz, DMSO-d6) δ 157.9 (C), 155.8 (CH), 154.7 (C), 132.6 (CH), 99.5 (C). **LRMS** (ESI +ve) [M+H] 136.10.

Synthesis of 3-Iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine (3). 1H-pyrazolo[3,4-d]pyrimidin-4-amine (2.56 g, 19.8 mmol) was suspended in DMF (15 mL, 194.5 mmol) in a 20 mL microwave vial equipped with stirrer bar. To the vial, N-iodosuccinimde (5.11 g, 22.7 mmol, 1.2 eq.) was added. The vial was sealed and heated to 150 °C in a microwave while stirring, for 60 minutes. Upon removal from the microwave, ethanol (10 mL) was added to the mixture and the reaction was allowed to cool for 1 hr, yielding a pale-yellow precipitate. The precipitate was collected by vacuum filtration and washed with ethanol (3 x 15 mL). The product was then dried overnight at 40 °C in a vacuum oven, to give a sand coloured solid, 3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine (2.87 g, 10.9 mmol, 58 %). Repeated on a 3.54 mmol scale (147.5 mg, 0.570 mmol, 16%). **1H NMR** (500 MHz, DMSO-d6) δ 13.79 (br. S., 1H), 8.16 (s, 1H). **13C NMR** (126 MHz, DMSO-d6) δ 157.5 (C), 156.0 (CH), 155.0 (C), 102.5 (C), 89.7 (C) LRMS (ESI +ve) [M+H] 262.00.

Synthesis of 1-(cyclopentylmethyl)-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine (4). 3-Iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine (2.50 g, 9.6 mmol) was added to a 20 mL microwave vial equipped with stirrer bar and suspended in DMF (15 mL). To the mixture, sodium hydride (576.8 mg, 14.42 mmol, 60 % dispersion in mineral oil, 1.5 eq.) was added portion-wise and the sand-colored reaction mixture was stirred for 5 minutes at ambient temperature until the evolution of gas had subsided, yielding a brown reaction mixture. (Iodomethyl)cyclopentane (1.9 mL, 14.4 mmol, 1.5 eq.) was added dropwise to the stirring reaction over a minute. The vial was sealed, and the reaction was heated to 150 °C in a microwave while stirring for 60 minutes, giving a deep orange reaction mixture. EtOAc (25 mL) and water (25 mL) were added, and the organic layer was collected. The aqueous layer was washed with EtOAc (3 x 50 mL). The organic washes were combined, washed with brine (50 mL) and then dried over MgSO4. The dry organic phase was concentrated in vacuo to give the crude product, a pale-yellow solid. The crude product was purified by flash column chromatography on silica using a 0-5 % MeOH/DCM eluent gradient. The appropriate fractions by TLC were combined and concentrated to give the product, 1-(cyclopentylmethyl)-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine (1.86 g, 5.4 mmol, 56 %) as a pale orange crystalline solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.19 (s, 1H), 4.18 (d, J = 7.49

Hz, 2H), 2.43 (quin, J = 7.10 Hz, 1H), 1.53 - 1.62 (m, 4H), 1.43 - 1.52 (m, 2H), 1.20 - 1.30 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 158.2 (C), 156.4 (CH), 154.0 (C), 103.4 (C), 89.0 (C), 51.6 (CH2), 42.9 (CH), 30.0 (2 x CH2), 25.0 (2 x CH2). **LRMS** (ESI +ve) [M+H] 344.20.

Synthesis of 6-chloro-3-iodo-1H-pyrazolo[3,4-d]pyrimidine (6). 6-chloro-1H-pyrazolo[3,4-d]pyrimidine (5) (2.02 g, 13.1 mmol) was suspended in DMF (15 mL) in a 20 mL microwave vial equipped with stirrer bar. The suspension was stirred, and N-iodosuccinimide (3.84 g, 17.1 mmol, 1.3 eq) was added portion-wise. The vial was sealed and heated to 120 °C while stirring in a microwave reactor for 65 minutes. The resultant deep red reaction mixture was partitioned between EtOAc (50 mL) and water (50 mL). The organic layer was collected, and the aqueous layer was washed with EtOAc (3 x 50 mL). The organic layers were combined and washed with water (2 x 20 mL) and brine (50 mL). The organic fraction was then dried over $MgSO_4$ and concentrated in vacuo to give the crude product, a deep red solid. The crude product was purified by flash column chromatography on silica using a 0-10 % MeOH/DCM eluent gradient. The appropriate fractions by TLC were combined and concentrated under reduced pressure to yield the product, 6-chloro-3-iodo-1H-pyrazolo[3,4-d]pyrimidine (2.81 g, 10.0 mmol, 76 %) as a yellow solid. **1H NMR** (500 MHz, DMSO-d6) δ 14.63 (s, 1H), 9.01 (s, 1H). **13C NMR** (126 MHz, DMSO-d6) δ 157.8 (C), 155.6 (CH), 155.2 (C), 117.6 (C), 94.1 (C). **LRMS** (ESI +ve) [M+H] 281.00.

Synthesis of 3-iodo-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (7). 6-chloro-3-iodo-1H-pyrazolo[3,4-d]pyrimidine (1.04 g, 3.7 mmol) was added to a 20 mL microwave vial equipped with stirrer bar. The solid was suspended in THF (3 mL) and dissolved through sonication. The mixture was stirred and methylamine (12 mL, 24.0 mmol, 2 M in THF, 6.5 eq) was added dropwise over 5 minutes. The vial was sealed and heated to 150 °C while stirring in a microwave reactor for 60 minutes. The reaction vial was removed from the microwave and allowed to cool to ambient temperature, resulting the formation of a white precipitate, which was collected by vacuum filtration. The solid was washed with water (3 x 4 mL) and dried to yield the product, 3-iodo-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (636.6 mg, 2.32 mmol, 63 %) as a beige solid. Repeated on a 6.13 mmol scale (1.29 g, 4.59 mmol, 77 %). **1H NMR** (500 MHz, DMSO-d6) δ 13.39 (br. s., 1H), 8.45 (br. s., 1H), 7.53 (br. s., 1H), 2.83 (br. s., 3H). **13C NMR** (126 MHz, DMSO-d6) δ 162.2 (C), 157.1 (C), 156.9 (CH), 153.4 (C), 93.0 (C), 28.5 (CH3). **LRMS** (ESI +ve) [M+H] 275.90.

Synthesis of 1-(cyclopentylmethyl)-3-iodo-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (8). 3-iodo-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (597.7 mg, 2.17 mmol) was added to a 20 mL microwave vial equipped with stirrer bar. The solid was dissolved in DMF (10 mL) and stirred at ambient temperature. Sodium hydride (130.9 mg, 3.25 mmol, 60% dispersion in mineral oil, 1.5 eq.) was added portion wise and the dark sand coloured reaction mixture was stirred for 5 minutes at ambient temperature until the evolution of gas had subsided, yielding a brown reaction mixture.

(Iodomethyl)cyclopentane (0.429 mL, 3.25 mmol, 1.5 eq.) was added dropwise to the stirring reaction over a minute. The vial was sealed, and the reaction was heated to 150 °C in a microwave while stirring for 85 minutes, giving a deep orange reaction mixture. EtOAc (15 mL) and water (15 mL) were added, and the organic layer was collected. The aqueous layer was washed with EtOAc (3 x 20 mL). The organic washes were combined, washed with water (2 x10 mL) and brine (30 mL) and then dried over MgSO4. The dry organic phase was concentrated in vacuo to give the crude product, a light brown residue. The crude product was purified by flash column chromatography on silica using a 0-5 % MeOH/DCM eluent gradient. The appropriate fractions by TLC were combined and concentrated to give the product, 1-(cylopentylmethyl)-3-iodo-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (497.5 mg, 1.39 mmol, 64 %). Repeated on a 4.62 mmol scale (1.15 g, 3.2 mmol, 69 %). **1H NMR** (500 MHz, DMSO-d6) δ 8.44 (s, 1H), 7.60 (s, 1H), 4.11 (d, J = 5.91 Hz, 2H), 2.85 (d, J = 3.23 Hz, 3H), 2.44 (quin, J = 7.11 Hz, 1H), 1.54 - 1.65 (m, 4H), 1.45 - 1.54 (m, 2H), 1.28 (d, J = 6.07 Hz, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 162.5 (C), 155.8 (C), 154.2 (CH), 112.0 (C), 92.3 (C), 50.7 (CH2), 32.1 (CH), 30.1 (2 x CH2), 27.8 (CH3), 24.9 (2 x CH2). **LRMS** (ESI +ve) [M+H] 358.00.

3. Synthesis and characterization of *N*-propargyl cyclic amines 10a-m

General Synthesis of Lipophilic *N*-propargylated cyclic amines. Amine (20 mmol, 2 eq.) was added to a 50 mL round-bottomed flask equipped with stirrer bar. The amine was suspended in diethyl ether (20 mL). The reaction was cooled to 0 °C while stirring. Propargyl bromide (10 mmol, 80 % in toluene) was added dropwise to the cold stirring reaction mixture. The flask was fitted with septum and flushed with nitrogen. The reaction was stirred at 0 °C for 30 minutes. The ice bath was removed, and the reaction was stirred at ambient temperature overnight under nitrogen. The reaction was quenched with water (10 mL) and was extracted with diethyl ether (4 x 40 mL). The organic fractions were combined, washed with water (10 mL), brine (40 mL) and dried over $MgSO_4$, before being concentrated under reduced pressure to give the crude product. The crude product was purified by flash column chromatography, using an eluent system of 0-15% MeOH in DCM. The appropriate fractions were combined and concentrated to give the desired products.

1-(prop-2-yn-1-yl)-piperidine (10a). Yield: 124.9 mg, 1.01 mmol, 17 %. **1H NMR** (500 MHz, CHLOROFORM-d) δ 3.27 (d, J = 2.52 Hz, 2H), 2.43 - 2.59 (m, 4H), 2.22 (s, 1H), 1.37 - 1.49 (m, 1H), 1.25 (s, 4H). **13C NMR** (126 MHz, CHLOROFORM-d) δ 79.4 (CH), 73.1 (C), 67.7 (CH2), 47.8 (2 x CH2), 40.9 (2 x CH2), 29.9 (CH2).

4-methyl-1-(prop-2-yn-1-yl)-piperidine (10b). Yield: 107.4 mg, 0.78 mmol, 14 %. **1H NMR** (500 MHz, CHLOROFORM-d) δ 3.27 - 3.30 (m, 3H), 3.01 - 3.08 (m, 0H), 2.85 (td, J = 2.70, 11.66 Hz, 2H), 2.22 (t, J = 2.44 Hz, 1H), 2.18 (dt, J = 2.36, 12.60 Hz, 2H), 1.65 (d, J = 13.08 Hz, 2H), 1.24 - 1.31 (m, 2H), 0.95 - 0.98 (m, 1H), 0.91 - 0.94 (m, 2H). **13C NMR** (126 MHz, CHLOROFORM-d) δ 79.5 (CH), 72.9 (C), 52.8 (CH2), 47.4 (2 x CH2), 34.4 (2 x CH2), 23.5 (CH3), 21.9 (CH).

1-(prop-2-yn-1-yl)-pyrrolidine (10c). Yield: 1.76 g, 16.1 mmol, 87 %. **1H NMR** (500 MHz, CHLOROFORM-d) δ 3.41 (d, J = 2.44 Hz, 2H), 2.59 - 2.65 (m, 4H), 2.20 (t, J = 2.44 Hz, 1H), 1.77 - 1.84 (m, 4H). **13C NMR** (126 MHz, CHLOROFORM-d) δ 79.6 (CH), 72.1 (C), 52.4 (CH2), 42.9 (2 x CH2), 23.7 (2 x CH2).

General Synthesis of Polar *N*-propargylated cyclic amines. Amine (6 mmol) was added to a 50 mL round-bottomed flask equipped with stirrer bar. The amine was suspended in THF (50 mL). Potassium carbonate (12 mmol, 2 eq.) was added, and the reaction mixture was stirred at ambient temperature for 5 minutes. Propargyl bromide (9 mmol, 80 % in Toluene, 1.5 eq.) was added dropwise to the stirring reaction mixture. The reaction was fitted with a condenser and stirred at reflux (68 °C) for 8 hours. The reaction was removed from the heat and allowed to cool while stirring overnight. The solvent was

removed in vacuo to give a residue. The residue was portioned between water (40 mL) and DCM (50 mL). The organic layer was collected, and the aqueous layer was washed with DCM (2 x 25 mL). The organic fractions were combined and dried over $MgSO_4$, before being concentrated under reduced pressure to give the crude product. The crude product was purified by flash column chromatography, using an eluent system of 0-20% MeOH in DCM. The appropriate fractions were combined and concentrated to give the desired products.

***N*-propargyl morpholine (10d).** Yield: 221 mg, 1.77 mmol, 31 %. **1H NMR** (500 MHz, CHLOROFORM-d) δ 3.73 (t, J = 4.70 Hz, 4H), 3.27 (d, J = 2.52 Hz, 2H), 2.55 (t, J = 4.70 Hz, 4H), 2.24 - 2.27 (m, 1H). **13C NMR** (126 MHz, CHLOROFORM-d) δ 78.5 (CH), 73.6 (C), 67.0 (CH2), 52.4 (2 x CH2), 47.3 (2 x CH2).

1-(prop-2-yn-1-yl)-piperidin-4-ol (10e). Yield: 12.1 mg, 0.09 mmol, 2 %. Repeated on a 12 mmol scale, yield: 817.2 mg, 5.87 mmol, 49 %. **1H NMR** (500 MHz, CHLOROFORM-d) δ 3.71 (quind, J = 4.40, 8.41 Hz, 1H), 3.31 (d, J = 2.44 Hz, 2H), 2.77 - 2.83 (m, 2H), 2.37 (dt, J = 2.76, 9.20 Hz, 2H), 2.24 (t, J = 2.44 Hz, 1H), 1.90 - 1.97 (m, 2H), 1.63 (dtd, J = 3.82, 9.26, 12.97 Hz, 2H), 1.41 (br. s., 1H). **13C NMR** (126 MHz, CHLOROFORM-d) δ 79.0 (CH), 73.0 (C), 67.5 (CH2), 49.8 (2 x CH2), 46.9 (2 x CH2), 34.4 (CH).

4-methoxy-(1-prop-2-yn-1-yl)-piperidine (10f). Yield: 389.5 mg, 2.54 mmol, 42 %. **1H NMR** (500 MHz, CHLOROFORM-d) δ 3.32 - 3.33 (m, 3H), 3.27 - 3.29 (m, 2H), 2.73 - 2.79 (m, 2H), 2.32 - 2.38 (m, 2H), 2.21 - 2.24 (m, 1H), 1.88 - 1.96 (m, 2H), 1.58 - 1.67 (m, 2H), 1.24 (s, 1H). **13C NMR** (126 MHz, CHLOROFORM-d) δ 98.5 (CH3), 79.1 (CH), 73.1 (C), 55.7 (CH2), 50.0 (2 x CH2), 47.0 (2 x CH2), 31.1 (CH).

1-methyl-4-(prop-2-yn-1-yl)-piperazine (10g). Yield: 120.2 mg, 0.87 mmol, 13 %. Repeated on a 12 mmol scale, yield: 361 mg, 2.62 mmol, 22 %. **1H NMR** (500 MHz, CHLOROFORM-d) δ 3.30 (d, J = 2.44 Hz, 2H), 2.35 - 2.67 (m, 8H), 2.28 - 2.30 (m, 3H), 2.24 (t, J = 2.48 Hz, 1H). **13C NMR** (126 MHz, CHLOROFORM-d) δ 98.3 (CH3), 78.8 (CH), 73.1 (C), 54.8 (CH2), 51.7 (2 x CH2), 46.7 (CH2), 45.9 (CH2).

1-propargyl-4-hydroxymethylpiperidine (10h). Yield: 79 mg, 0.52 mmol, 8 %. **1H NMR** (500 MHz, CHLOROFORM-d) δ 3.50 (d, J = 6.46 Hz, 2H), 3.27 - 3.32 (m, 2H), 2.88 - 2.95 (m, 2H), 2.17 - 2.25 (m, 2H), 1.74 - 1.81 (m, 2H), 1.44 - 1.55 (m, 2H), 1.23 - 1.37 (m, 2H). **13C NMR**

(126 MHz, CHLOROFORM-d) δ 79.2 (CH), 73.1 (C), 68.0 (CH2), 52.2 (CH2), 47.3 (2 x CH2), 38.2 (2 x CH2), 28.9 (CH).

1-propargyl-4-hydroxyethylpiperidine (10i). Yield: 370 mg, 2.21 mmol, 37.5 %. **1H NMR** (500 MHz, CHLOROFORM-d) δ 4.32 - 4.37 (m, 1H), 3.70 (t, J = 6.66 Hz, 2H), 3.27 - 3.30 (m, 2H), 2.88 (td, J = 2.70, 11.59 Hz, 2H), 2.49 (t, J = 7.90 Hz, 1H), 2.22 - 2.23 (m, 2H), 2.19 (dt, J = 2.60, 11.70 Hz, 2H), 1.73 (td, J = 2.00, 12.22 Hz, 2H), 1.52 (q, J = 6.70 Hz, 2H). **13C NMR** (126 MHz, CHLOROFORM-d) δ 79.3 (CH), 73.0 (C), 60.7 (CH2), 52.7 (CH2), 47.4 (2 x CH2), 39.5 (2 x CH2), 32.4 (CH2), 32.0 (CH).

***N*,*N*-dimethyl-1-(prop-2-ynyl)piperidin-4-amine (10j).** Yield: 131.2 mg, 0.79 mmol, 8 %. Repeated on a 12 mmol scale, yield: 184.2 mg, 1.10 mmol, 10 %. **1H NMR** (500 MHz, CHLOROFORM-d) δ 3.29 (d, J = 2.44 Hz, 2H), 2.90 - 2.95 (m, 2H), 2.29 (s, 6H), 2.23 (t, J = 2.44 Hz, 1H), 2.12 - 2.22 (m, 3H), 1.80 - 1.87 (m, 2H), 1.57 (dq, J = 4.02, 12.14 Hz, 2H). **13C NMR** (126 MHz, CHLOROFORM-d) δ 78.9 (CH), 73.0 (C), 61.9 (CH2), 51.7 (2 x CH2), 46.9 (2 x CH2), 41.5 (CH), 28.0 (2 x CH3).

***N*,*N*-dimethyl-2-(4-prop-2-ynylpiperazin-1-yl)ethanamine (10k).** Yield: 15 mg, 0.07 mmol, 2 %. Repeated on a 12 mmol scale, yield: 505.1 mg, 2.59 mmol, 21 %. **1H NMR** (500 MHz, DMSO-d6) δ 3.22 (d, J = 2.44 Hz, 2H), 3.10 - 3.13 (m, 1H), 2.38 - 2.46 (m, 6H), 2.28 - 2.37 (m, 6H), 2.12 (s, 6H). **13C NMR** (126 MHz, DMSO-d6) δ 79.4 (CH), 75.5 (C), 56.6 (CH2), 55.8 (CH2), 52.9 (CH2), 51.2 (2 x CH2), 46.0 (2 x CH2), 45.5 (2 x CH3).

4-(methylcarbonyl)-1-(prop-2-yn-1-yl)-piperazine (10l). Yield: 446 mg, 2.68 mmol, 45 %. **1H NMR** (500 MHz, CHLOROFORM-d) δ 3.63 - 3.67 (m, 2H), 3.47 - 3.51 (m, 2H), 3.33 (d, J = 2.52 Hz, 2H), 2.54 (td, J = 5.19, 16.81 Hz, 4H), 2.25 - 2.27 (m, 1H), 2.09 (s, 3H), 1.73 (s, 1H). **13C NMR** (126 MHz, CHLOROFORM-d) δ 169.1 (C), 78.2 (CH), 73.8 (C), 51.9 (CH2), 51.5 (CH2), 47.0 (CH2), 46.3 (CH2), 41.4 (CH2), 21.5 (CH3).

2-(4-prop-2-ynylpiperazin-1-yl)ethanol (10m). Yield: 54 mg, 0.32 mmol, 6 %. Repeated on a 12 mmol scale, yield: 683.1 mg, 4.06 mmol, 22 %. **1H NMR** (500 MHz, CHLOROFORM-d) δ 3.60 - 3.65 (m, 4H), 3.30 - 3.32 (m, 2H), 2.62 (br. s., 3H), 2.54 - 2.59 (m, 4H), 2.24 - 2.27 (m, 2H), 1.25 (s, 1H). **13C NMR** (126 MHz, CHLOROFORM-d) δ 78.8 (CH), 73.4 (C), 59.3 (CH2), 57.8 (CH2), 52.8 (CH2), 51.9 (2 x CH2), 46.9 (2 x CH2).

4. Synthesis and characterization of compounds A1-13 and B1-13

General Synthesis of Final Compounds A1-13. 1-(cyclopentylmethyl)-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine (0.300 mmol) was added to a 5 mL microwave vial equipped with stirrer bar. N-propargylated cyclic amine (1.2 eq.), bis(triphenylphosphine)palladium dichloride (5 mol%), copper (I) iodide (10 mol%) and triethylamine (2.0 eq.) were added to the vial. The reagents were suspended in THF (5 ml) and the vial was sealed with septum cap. The vial was placed into a microwave reactor and heated to and stirred at 70 °C for 2 h under microwave irradiation, yielding a reaction mixture that was cooled whilst stirring. The reaction mixture was partitioned between water (10 ml) and EtOAc (15 ml). The organic phase was collected, and the aqueous layer was washed with EtOAc (4 x 20 ml). The organic layers were combined, dried over $MgSO_4$, and concentrated in vacuo to give the crude product. The crude product was purified by preparative-TLC on silica using an 8 % MeOH/DCM eluent system. The appropriate dual-active band by UV and $KMnO_4$ visualization was scraped off the plate and washed with 15 % MeOH/DCM solution. The filtrate was collected and concentrated to give the product.

1-(cyclopentylmethyl)-3-(3-(piperidin-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (A1). Yield: 41.2 mg, 0.122 mmol, 42 %, as a dark orange-brown oil. **1H NMR** (500 MHz, DMSO-d6) δ 8.21 (s, 1H), 4.19 (d, J = 7.49 Hz, 2H), 3.61 (br. s., 2H), 2.45 (quin, J = 7.30 Hz, 1H), 1.44 - 1.64 (m, 16H), 1.38 (br. s., 2H), 1.22 - 1.30 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 158.2 (C), 156.9 (CH), 153.5 (C), 100.9 (C), 90.4 (C), 76.4 (2 x C), 66.4 (CH2), 53.2 (CH2), 51.5 (2 x CH2), 48.1 (2 x CH2), 30.0 (CH2), 25.9 (CH), 25.0 (2 x CH2), 24.0 (2 x CH2). **LRMS** (ESI +ve) [M+H] 339.20.

1-(cyclopentylmethyl)-3-(3-morpholinoprop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (A2). Yield: 52.4 mg, 0.154 mmol, 52 %, as a light brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.21 (s, 1H), 4.19 (d, J = 7.49 Hz, 2H), 3.64 (s, 2H), 3.62 (t, J = 4.70 Hz, 4H), 2.53 (t, J = 4.60 Hz, 4H), 2.45 (sxt, J = 7.40 Hz, 1H), 1.43 - 1.64 (m, 6H), 1.19 - 1.30 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 157.7 (C), 156.4 (CH), 153.0 (C), 125.2 (C), 100.4 (C), 90.5 (C), 76.7 (2 x C), 66.0 (2 x CH2), 51.8 (2 x CH2), 51.0 (CH2), 47.2 (CH), 29.5 (2 x CH2), 24.5 (2 x CH2). **LRMS** (ESI +ve) [M+H] 341.10.

1-(cyclopentylmethyl)-3-(3-(4-methylpiperidin-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (A3). Yield: 34.7 mg, 0.098 mmol, 30 %, as a light brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.22 (s, 1H), 4.20 (d, J = 7.49 Hz, 2H), 3.61 (s, 3H), 2.85 (d, J = 11.43 Hz, 2H), 2.42 - 2.49 (m, 1H), 2.18 (dt, J = 2.40, 11.53 Hz, 1H), 1.53 - 1.66 (m, 6H), 1.45 - 1.53 (m, 2H), 1.22 - 1.37 (m, 4H), 1.17 (dq, J = 3.74, 12.07 Hz, 2H), 0.90 (d, J = 6.54 Hz, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 157.7 (C), 156.4 (CH), 153.0 (C), 125.3 (C), 100.4 (C), 91.2

(CH2), 76.4 (2 x C), 52.1 (2 x CH2), 51.0 (2 x CH2), 47.4 (CH2), 33.8 (CH), 29.8 (CH), 29.5 (2 x CH2), 24.5 (2 x CH2), 21.8 (CH3). **LRMS** (ESI +ve) [M+H] 353.10.

1-(3-(4-amino-1-(cyclopentylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)prop-2-yn-1-yl)piperidin-4-ol (A4). Yield: 28.1 mg, 0.079 mmol, 27 %, as a golden residue. **1H NMR** (500 MHz, DMSO-d6) δ 8.21 (s, 1H), 8.00 (br. s, 2H), 4.55 (d, J = 4.02 Hz, 1H), 4.19 (d, J = 7.49 Hz, 2H), 3.60 (s, 2H), 3.46 (sxtd, J = 4.30, 8.28 Hz, 1H), 2.77 (td, J = 5.50, 11.35 Hz, 2H), 2.45 (quin, J = 7.31 Hz, 1H), 2.27 (dt, J = 2.40, 10.40 Hz, 2H), 1.74 (dd, J = 3.78, 14.98 Hz, 2H), 1.53 - 1.64 (m, 4H), 1.38 - 1.53 (m, 4H), 1.21 - 1.31 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 157.7 (C), 156.4 (CH), 153.0 (C), 125.3 (C), 100.4 (C), 91.2 (CH2), 76.4 (2 x C), 51.0 (2 x CH2), 49.7 (2 x CH2), 47.1 (CH2), 34.2 (2 x CH), 29.5 (2 x CH2), 24.5 (2 x CH2). **LRMS** (ESI +ve) [M+H] 355.00.

1-(cyclopentylmethyl)-3-(3-(4-methoxypiperidin-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (A5). Yield: 74 mg, 0.201 mmol, 65 %, golden brown oily solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.24 (br. s., 1H), 7.95 (br. s., 2H), 4.19 (d, J = 7.49 Hz, 2H), 3.62 (s, 2H), 3.28 - 3.33 (m, 3H), 3.13 - 3.20 (m, 1H), 2.68 - 2.81 (m, 2H), 2.41 - 2.48 (m, 1H), 2.26 - 2.38 (m, 2H), 1.81 - 1.91 (m, 2H), 1.38 - 1.67 (m, 8H), 1.22 - 1.31 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 157.7 (C), 156.4 (CH), 153.0 (C), 125.3 (C), 100.4 (C), 91.0 (CH2), 76.4 (2 x C), 75.1 (CH), 54.8 (CH2), 51.0 (2 x CH2), 49.4 (2 x CH2, Broad), 47.0 (CH), 30.5 (2 x CH2), 29.5 (2 x CH2), 24.5 (CH3). **LRMS** (ESI +ve) [M+H] 369.10.

(1-(3-(4-amino-1-(cyclopentylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)prop-2-yn-1-yl)piperidin-4-yl)methanol (A6). Yield: 44.1 mg, 0.120 mmol, 39 %, as a brown oil. **1H NMR** (500 MHz, DMSO-d6) δ 8.17 - 8.24 (m, 1H), 4.37 - 4.42 (m, 1H), 4.19 (d, J = 7.49 Hz, 2H), 3.59 - 3.63 (m, 2H), 3.20 - 3.26 (m, 2H), 2.41 - 2.48 (m, 1H), 2.11 - 2.18 (m, 2H), 2.06 (dt, J = 2.29, 11.51 Hz, 1H), 1.64 - 1.70 (m, 2H), 1.52 - 1.63 (m, 4H), 1.44 - 1.52 (m, 2H), 1.22 - 1.37 (m, 4H), 1.05 - 1.20 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 157.7 (C), 156.4 (CH), 153.0 (C), 125.3 (C), 100.4 (C), 91.2 (CH2), 76.4 (2 x C), 65.7 (CH), 52.0 (CH2), 51.0 (2 x CH2), 47.4 (2 x CH2), 37.9 (CH), 29.5 (2 x CH2), 28.6 (2 x CH2), 24.5 (CH2). **LRMS** (ESI +ve) [M+H] 369.00.

2-(1-(3-(4-amino-1-(cyclopentylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)prop-2-yn-1-yl)piperidin-4-yl)ethan-1-ol (A7). Yield: 28.9 mg, 0.076 mmol, 26 %, as a viscous golden oil. **1H NMR** (500 MHz, DMSO-d6) δ 8.21 (s, 1H), 4.28 - 4.32 (m, 2H), 4.19 (d, J = 7.57 Hz, 2H), 3.60 (s, 2H), 2.85 (d, J = 11.35 Hz, 1H), 2.69 - 2.74 (m, 1H), 2.45 (quin, J = 6.90 Hz, 1H), 2.16 (dt, J = 2.21, 11.51 Hz, 2H), 2.07 (dt, J = 2.05, 12.60 Hz, 1H), 1.65 (d, J = 12.69 Hz, 2H), 1.53 - 1.62 (m, 4H), 1.46 - 1.52 (m, 2H), 1.23 - 1.38 (m, 4H), 1.09 - 1.20 (m, 2H). **13C NMR** (126

MHz, DMSO-d6) δ 157.7 (C), 156.4 (CH), 153.0 (C), 125.3 (C), 100.4 (C), 91.2 (CH2), 76.4 (2 x C), 58.3 (CH2), 52.2 (2 x CH2), 52.0 (2 x CH2), 51.0 (CH2), 47.4 (CH2), 31.9 (CH), 31.6 (2 x CH2), 29.5 (2 x CH2), 24.5 (CH2). **LRMS** (ESI +ve) [M+H] 383.10.

1-(cyclopentylmethyl)-3-(3-(4-(dimethylamino)piperidin-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (A8). Yield: 23.5 mg, 0.062 mmol, 21 %, as a brown oily solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.21 (s, 1H), 7.87 (br. s, 2H), 4.19 (d, J = 7.49 Hz, 2H), 3.61 (s, 2H), 2.89 (d, J = 11.74 Hz, 2H), 2.45 (quin, J = 7.33 Hz, 1H), 2.19 (dt, J = 2.05, 12.10 Hz, 2H), 2.16 (s, 6H), 2.01 (tt, J = 3.90, 10.80 Hz, 1H), 1.75 (d, J = 12.06 Hz, 2H), 1.53 - 1.64 (m, 4H), 1.45 - 1.53 (m, 2H), 1.40 (dq, J = 3.90, 11.89 Hz, 2H), 1.20 - 1.31 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 157.7 (C), 156.4 (CH), 153.0 (C), 125.3 (C), 100.4 (C), 91.1 (CH2), 76.4 (2 x C), 61.2 (CH2), 51.3 (CH), 51.0 (2 x CH2), 47.0 (2 x CH2), 41.5 (CH), 29.5 (2 x CH2), 28.0 (2 x CH2), 24.5 (2 x CH3). **LRMS** (ESI +ve) [M+H] 382.10.

1-(cyclopentylmethyl)-3-(3-(4-(2-(dimethylamino)ethyl)piperazin-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (A9). Yield: 17.3 mg, 0.042 mmol, 14 %, as a golden solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.20 - 8.23 (m, 1H), 7.94 - 8.05 (m, 2H), 5.32 (s, 2H), 4.19 (d, J = 7.49 Hz, 2H), 3.10 (s, 6H), 2.94 - 3.03 (m, 2H), 2.56 - 2.65 (m, 4H), 2.44 (quin, J = 6.50 Hz, 1H), 1.52 - 1.64 (m, 4H), 1.42 - 1.52 (m, 2H), 1.27 (d, J = 5.91 Hz, 2H), 1.23 (t, J = 7.30 Hz, 6H). **13C NMR** (126 MHz, DMSO-d6) δ 157.7 (C), 156.9 (CH), 153.0 (C), 123.4 (C), 103.9 (C), 99.5 (CH2), 76.2 (2 x C), 63.0 (2 x CH2), 52.5 (2 x CH2), 51.5 (CH2), 51.3 (CH2), 46.4 (CH), 33.2 (2 x CH2), 30.0 (CH2), 25.0 (2 x CH2), 7.7 (2 x CH3). **LRMS** (ESI +ve) [M+H] 411.20.

1-(4-(3-(4-amino-1-(cyclopentylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)prop-2-yn-1-yl)piperazin-1-yl)ethan-1-one (A10). Yield: 95.8 mg, 0.251 mmol, 86 %, as a golden brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.22 (br. S., 1H), 4.16 – 4.21 (m, 2H), 3.66 – 3.74 (m, 3H), 3.39 – 3.50 (m, 6H), 2.41 – 2.47 (m, 1H), 1.97 – 2.00 (m, 2H), 1.53 – 1.64 (m, 6H), 1.43 – 1.53 (m, 2H), 1.20 – 1.31 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 168.1 (C), 157.7 (C), 156.4 (CH), 153.0 (C), 125.2 (C), 90.3 (C), 76.7 (C), 74.5 (C), 69.1 (CH3), 51.5 (CH2), 51.0 (CH2), 46.9 (CH2), 46.3 (CH2), 45.4 (CH2), 40.6 (CH2), 29.5 (CH), 24.5 (2 x CH2), 21.1 (2 x CH2). **LRMS** (ESI +ve) [M+H] 382.10.

2-(4-(3-(4-amino-1-(cyclopentylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)prop-2-yn-1-yl)piperazin-1-yl)ethan-1-ol (A11). Yield: 57.7 mg, 0.150 mmol, 49 %, as a dark orange oil. **1H NMR** (500 MHz, DMSO-d6) δ 8.21 (s, 1H), 7.88 (br. s, 2H), 4.38 (br. s., 1H), 4.19 (d, J = 7.49 Hz, 2H), 4.08 (q, J = 5.20 Hz, 2H), 3.63 (s, 2H), 3.48 (q, J = 5.65 Hz, 2H), 3.17 (d, J = 4.89

Hz, 2H), 2.55 (br. s., 4H), 2.45 (quin, J = 7.50 Hz, 1H), 2.39 (br. s., 2H), 1.52 - 1.63 (m, 4H), 1.44 - 1.52 (m, 2H), 1.20 - 1.31 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 157.7 (C), 156.4 (CH), 153.0 (C), 125.2 (C), 100.4 (C), 90.8 (CH2), 76.6 (2 x C), 60.2 (CH2), 58.5 (CH2), 53.0 (CH2), 51.3 (CH2), 51.0 (2 x CH2), 48.6 (CH2), 46.9 (CH), 29.5 (2 x CH2), 24.5 (2 x CH2). **LRMS** (ESI +ve) [M+H] 384.20.

1-(cyclopentylmethyl)-3-(3-(4-methylpiperazin-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (A12). Yield: 63.5 mg, 0.180 mmol, 56 %, as a brown crystalline solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.21 (s, 1H), 7.88 (br. s, 1H), 6.50 (br. s, 1H), 4.19 (d, J = 7.49 Hz, 2H), 3.62 (s, 2H), 2.55 (br. s., 4H), 2.45 (quin, J = 7.30 Hz, 1H), 2.28 - 2.40 (m, 4H), 2.17 (s, 3H), 1.53 - 1.64 (m, 4H), 1.44 - 1.53 (m, 2H), 1.20 - 1.31 (m, 2H). **13C** NMR (126 MHz, DMSO-d6) δ 157.7 (C), 156.4 (CH), 153.0 (C), 125.2 (C), 100.4 (C), 90.8 (CH2), 76.5 (2 x C), 54.5 (2 x CH2), 51.3 (2 x CH2), 51.0 (CH3), 46.9 (CH2), 45.6 (CH), 29.5 (2 x CH2), 24.5 (2 x CH2). **LRMS** (ESI +ve) [M+H] 354.20.

1-(cyclopentylmethyl)-3-(3-(pyrrolidin-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (A13). Yield: 16.8 mg, 0.052 mmol, 17 %, as a golden oil. **1H NMR** (500 MHz, DMSO-d6) δ 8.21 (s, 1H), 8.06 (br. s, 1H), 6.53 (br. s, 1H), 4.18 (d, J = 7.57 Hz, 2H), 3.73 (s, 2H), 2.57 - 2.62 (m, 4H), 2.44 (quin, J = 7.60 Hz, 1H), 1.72 (td, J = 3.21, 6.82 Hz, 4H), 1.51 - 1.62 (m, 4H), 1.42 - 1.51 (m, 2H), 1.19 - 1.30 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 157.7 (C), 156.4 (CH), 153.0 (C), 125.3 (C), 100.4 (C), 91.4 (CH2), 75.9 (2 x C), 51.9 (2 x CH2), 51.0 (CH2), 43.0 (CH), 29.5 (2 x CH2), 24.5 (2 x CH2), 23.3 (2 x CH2). **LRMS** (ESI +ve) [M+H] 325.10.

General Synthesis of Final Compounds B1-13. 1-(cyclopentylmethyl)-3-iodo-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (0.300 mmol) was added to a 5 mL microwave vial equipped with stirrer bar. N-propargylated cyclic amine (1.2 eq.), bis(triphenylphosphine)palladium dichloride (5 mol%), copper (I) iodide (10 mol%) and triethylamine (2.0 eq.) were added to the vial. The reagents were suspended in THF (5 ml) and the vial was sealed with septum cap. The vial was placed into a microwave reactor and heated to and stirred at 70 °C for 2 h under microwave irradiation, yielding a reaction mixture, which was cooled whilst stirring for an hour. The reaction mixture was partitioned between water (10 ml) and EtOAc (20 ml). The organic phase was collected, and the aqueous layer was washed with EtOAc (3 x 25 ml). The organic layers were combined, dried over $MgSO_4$, and concentrated in vacuo to give the crude product. The crude product was purified by preparative-TLC on silica using an 8 % MeOH/DCM eluent system. The appropriate dual-active band by UV and KMnO4 visualization was scraped off the plate and washed with 15 % MeOH/DCM solution. The filtrate was collected and concentrated to give the product.

1-(cyclopentylmethyl)-N-methyl-3-(3-(piperidin-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B1). Yield: 47.9 mg, 0.136 mmol, 44 %, as a light brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.69 (br. s., 1H), 7.56 (br. s., 1H), 4.11 (d, J = 6.23 Hz, 2H), 3.55 (s, 2H), 3.36 - 3.38 (m, 1H), 2.85 (d, J = 3.86 Hz, 2H), 2.46 (quin, J = 7.20 Hz, 1H), 1.57 - 1.64 (m, 4H), 1.54 (td, J = 5.73, 11.23 Hz, 6H), 1.46 - 1.51 (m, 2H), 1.33 - 1.42 (m, 4H), 1.20 - 1.33 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 161.6 (C), 154.5 (CH), 152.7 (C), 127.6 (C), 126.7 (C), 90.4 (CH2), 89.5 (CH2), 76.0 (C), 74.9 (C), 52.6 (2 x CH2), 52.4 (CH2), 47.5 (CH2), 47.2 (CH), 29.6 (CH2), 25.4 (2 x CH2), 24.4 (2 x CH2), 23.5 (CH3). **LRMS** (ESI +ve) [M+H] 353.20.

1-(cyclopentylmethyl)-N-methyl-3-(3-morpholinoprop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B2). Yield: 28.9 mg, 0.082 mmol, 30%, as cream solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.71 (br. s., 1H), 7.57 (br. s., 1H), 4.11 (d, J = 5.83 Hz, 2H), 3.62 (t, J = 4.70 Hz, 4H), 3.60 (s, 2H), 3.58 (t, J = 4.70 Hz, 2H), 2.85 (d, J = 3.70 Hz, 2H), 2.54 (t, J = 4.40 Hz, 3H), 2.41 - 2.48 (m, 1H), 1.54 - 1.66 (m, 4H), 1.45 - 1.54 (m, 2H), 1.20 - 1.33 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 162.1 (C), 155.0 (2 x C), 153.2 (CH), 127.1 (C), 66.6 (2 x CH2), 66.5 (2 x C), 52.2 (2 x CH2), 52.1 (CH2), 47.5 (CH2). 47.2 (CH), 30.1 (4 x CH2), 24.9 (CH3). **LRMS** (ESI +ve) [M+H] 355.20.

1-(cyclopentylmethyl)-N-methyl-3-(3-(4-methylpiperidin-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B3). Yield: 43.1 mg, 0.118 mmol, 43 %, as a light brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.69 (s, 1H), 4.11 (d, J = 5.99 Hz, 2H), 3.57 (br. s., 2H), 3.38 (s, 1H), 2.85 (d, J = 3.47 Hz, 4H), 2.45 (quin, J = 7.30 Hz, 1H), 2.20 (t, J = 10.80 Hz, 2H), 2.09 (t, J = 10.96 Hz, 1H), 1.54 - 1.66 (m, 6H), 1.46 - 1.54 (m, 2H), 1.22 - 1.36 (m, 2H), 1.06 - 1.21 (m, 2H), 0.90 (d, J = 6.54 Hz, 3H), 0.87 (d, J = 6.54 Hz, 1H). **13C NMR** (126 MHz, DMSO-d6) δ 162.0 (C), 154.9 (2 x C), 153.2 (CH), 52.5 (2 x CH2), 52.4 (2 x CH2), 47.6 (CH2), 47.4 (CH), 34.3 (CH2), 30.3 (CH), 30.1 (4 x CH2), 24.9 (CH3), 22.2 (CH3) [Due to low sample concentration, several peaks are missing]. **LRMS** (ESI +ve) [M+H] 367.20.

1-(3-(1-(cyclopentylmethyl)-6-(methylamino)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)prop-2-yn-1-yl)piperidin-4-ol (B4). Yield: 39.7 mg, 0.108 mmol, 39 %, as a brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.70 (br. s., 1H), 7.39 - 7.66 (m, 1H), 4.51 - 4.57 (m, 1H), 4.11 (d, J = 6.38 Hz, 2H), 3.56 (s, 2H), 3.38 (s, 1H), 2.85 (d, J = 3.86 Hz, 2H), 2.74 - 2.80 (m, 2H), 2.42 - 2.48 (m, 1H), 2.24 - 2.33 (m, 2H), 2.12 - 2.21 (m, 1H), 1.67 - 1.78 (m, 2H), 1.54 - 1.64 (m, 4H), 1.45 - 1.53 (m, 2H), 1.33 - 1.45 (m, 2H), 1.21 - 1.32 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 161.6 (C), 160.7 (C), 154.5 (CH), 152.7 (C), 126.7 (C), 76.0 (2 x C), 75.4 (CH), 50.4 (CH2),

46.9 (2 x CH2), 46.2 (2 x CH2), 34.2 (CH), 29.6 (4 x CH2), 24.4 (CH3). **LRMS** (ESI +ve) [M+H] 369.10.

1-(cyclopentylmethyl)-3-(3-(4-methoxypiperidin-1-yl)prop-1-yn-1-yl)-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B5). Yield: 37.8 mg, 0.098 mmol, 31 %, as a brown oil. **1H NMR** (500 MHz, DMSO-d6) δ 8.71 (s, 1H), 4.11 (d, J = 6.23 Hz, 2H), 3.58 (s, 2H), 3.38 - 3.42 (m, 1H), 3.23 (s, 3H), 3.21 (s, 1H), 2.85 (d, J = 3.63 Hz, 2H), 2.72 - 2.80 (m, 1H), 2.60 - 2.67 (m, 1H), 2.41 - 2.48 (m, 1H), 2.29 - 2.38 (m, 2H), 2.18 - 2.25 (m, 1H), 1.78 - 1.90 (m, 2H), 1.54 - 1.65 (m, 4H), 1.37 - 1.53 (m, 4H), 1.20 - 1.34 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 161.7 (C), 154.5 (2 x C), 152.7 (CH), 126.7 (C), 89.5 (CH), 76.1 (C), 74.9 (C), 68.9 (CH2), 49.2 (2 x CH2), 46.9 (2 x CH2), 46.6 (CH2), 33.1 (CH), 30.4 (CH3), 29.6 (4 x CH2), 24.4 (CH3). **LRMS** (ESI +ve) [M+H] 383.20.

(1-(3-(1-(cyclopentylmethyl)-6-(methylamino)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)prop-2-yn-1-yl)piperidin-4-yl)methanol (B6). Yield: 40.8 mg, 0.106 mmol, 33 %, as a light brown oily residue. **1H NMR** (500 MHz, DMSO-d6) δ 8.70 (d, J = 9.06 Hz, 1H), 7.56 (br. s., 1H), 4.40 (t, J = 5.28 Hz, 1H), 4.11 (d, J = 5.99 Hz, 2H), 3.71 (s, 1H), 3.57 (s, 2H), 3.25 (t, J = 5.83 Hz, 2H), 2.83 - 2.90 (m, 3H), 2.45 (quin, J = 7.30 Hz, 1H), 2.18 (dt, J = 2.33, 11.53 Hz, 2H), 1.68 (d, J = 10.80 Hz, 2H), 1.54 - 1.64 (m, 4H), 1.43 - 1.54 (m, 2H), 1.22 - 1.37 (m, 4H), 1.16 (dq, J = 3.90, 12.12 Hz, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 161.6 (C), 154.5 (2 x C), 152.7 (CH), 126.7 (C), 76.0 (2 x C), 65.9 (CH2), 51.8 (CH2), 51.7 (CH), 50.2 (2 x CH2), 47.2 (2 x CH2), 37.9 (CH), 29.6 (CH2), 28.6 (4 x CH2), 24.4 (CH3). **LRMS** (ESI +ve) [M+H] 383.10.

2-(1-(3-(1-(cyclopentylmethyl)-6-(methylamino)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)prop-2-yn-1-yl)piperidin-4-yl)ethan-1-ol (B7). Yield: 33.5 mg, 0.084 mmol, 30 %, as a viscous golden oil. **1H NMR** (500 MHz, DMSO-d6) δ 8.69 (br. s., 1H), 7.45 - 7.64 (m, 1H), 4.31 (t, J = 5.12 Hz, 2H), 4.11 (d, J = 5.83 Hz, 2H), 3.56 (s, 2H), 3.43 (q, J = 5.40 Hz, 2H), 2.80 - 2.89 (m, 5H), 2.46 (quin, J = 7.80 Hz, 1H), 2.19 (dt, J = 1.97, 10.30 Hz, 2H), 1.67 (d, J = 11.74 Hz, 2H), 1.54 - 1.63 (m, 4H), 1.44 - 1.54 (m, 2H), 1.33 - 1.39 (m, 2H), 1.22 - 1.32 (m, 2H), 1.11 - 1.21 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 161.6 (C), 154.5 (2 x C), 152.6 (CH), 126.7 (C), 89.6 (CH2), 76.0 (C), 58.3 (2 x CH2), 54.8 (CH2), 52.1 (2 x CH2), 50.2 (CH2), 47.2 (CH2), 45.5 (CH), 31.9 (CH2), 31.5 (CH), 29.6 (4 x CH2), 24.4 (CH3). **LRMS** (ESI +ve) [M+H] 397.20.

1-(cyclopentylmethyl)-3-(3-(4-(dimethylamino)piperidin-1-yl)prop-1-yn-1-yl)-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B8). Yield: 40.2 mg, 0.102 mmol, 38 %, as a golden solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.71 (br. s., 1H), 7.52 - 7.62 (m, 1H), 4.11 (d, J = 4.73 Hz, 2H), 3.62 (s, 3H), 2.92 - 2.98 (m, 2H), 2.80 - 2.88 (m, 2H), 2.38 - 2.48 (m, 7H), 2.21 - 2.29

(m, 2H), 2.06 - 2.17 (m, 1H), 1.83 - 1.93 (m, 2H), 1.44 - 1.66 (m, 8H), 1.21 - 1.34 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 161.4 (C), 154.5 (2 x C), 153.0 (C), 126.6 (C), 89.2 (CH), 76.2 (2 x C), 61.7 (CH2), 54.9 (CH2), 50.5 (2 x CH2), 50.2 (CH), 46.5 (2 x CH2), 29.6 (2 x CH3), 29.5 (2 x CH2), 26.7 (2 x CH2), 24.0 (CH3). **LRMS** (ESI +ve) [M+H] 396.10.

1-(cyclopentylmethyl)-3-(3-(4-(2-(dimethylamino)ethyl)piperazin-1-yl)prop-1-yn-1-yl)-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B9). Yield: 48.3 mg, 0.114 mmol, 36 %, as a golden solid. **1H NMR** (500 MHz, METHANOL-d4) δ 8.66 - 8.70 (m, 1H), 4.18 (d, J = 7.57 Hz, 2H), 3.69 (s, 2H), 3.67 (s, 1H), 3.50 (q, J = 6.40 Hz, 2H), 3.21 (d, J = 1.97 Hz, 4H), 2.98 (s, 4H), 2.82 (s, 6H), 2.67 - 2.75 (m, 2H), 2.55 (quin, J = 7.50 Hz, 1H), 1.63 - 1.75 (m, 6H), 1.52 - 1.62 (m, 2H), 1.28 - 1.41 (m, 2H). **13C NMR** (126 MHz, DMSO) δ 161.6 (C), 154.5 (2 x C), 152.7 (CH), 126.7 (C), 79.4 (C), 76.2 (C), 75.7 (CH2), 54.9 (CH2), 52.8 (CH2), 52.7 (CH2), 52.3 (CH2), 51.1 (CH2), 50.9 (CH2), 50.2 (CH2), 49.5 (CH2), 46.7 (CH2), 45.9 (CH), 44.5 (CH2), 29.6 (2 x CH3), 24.5 (CH3). **LRMS** (ESI +ve) [M+H] 425.10.

1-(4-(3-(1-(cyclopentylmethyl)-6-(methylamino)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)prop-2-yn-1-yl)piperazin-1-yl)ethan-1-one (B10). Yield: 18.9 mg, 0.049 mmol, 18 %, as a brown oil. **1H NMR** (500 MHz, DMSO-d6) δ 8.76 (s, 1H), 4.11 (d, J = 5.20 Hz, 2H), 3.66 (s, 3H), 3.48 (s, 3H), 3.40 - 3.45 (m, 1H), 2.80 - 2.88 (m, 4H), 2.54 - 2.57 (m, 2H), 2.41 - 2.46 (m, 1H), 2.35 - 2.39 (m, 2H), 1.97 - 1.99 (m, 2H), 1.54 - 1.63 (m, 4H), 1.46 - 1.53 (m, 2H), 1.22 - 1.32 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 168.2 (C), 161.6 (C), 154.5 (2 x C), 152.8 (CH), 126.5 (C), 78.9 (C), 75.9 (C), 55.4 (CH2), 51.5 (CH2), 51.0 (CH2), 46.6 (CH2), 46.3 (CH2), 45.9 (CH), 45.5 (CH2), 29.6 (4 x CH2), 24.4 (CH3), 21.1 (CH3). **LRMS** (ESI +ve) [M+H] 396.20.

2-(4-(3-(1-(cyclopentylmethyl)-6-(methylamino)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)prop-2-yn-1-yl)piperazin-1-yl)ethan-1-ol (B11). Yield: 67.5 mg, 0.169 mmol, 50 %, as a brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.90 (s, 1H), 8.71 (br. s., 1H), 4.24 (d, J = 7.57 Hz, 1H), 4.11 (d, J = 5.99 Hz, 2H), 3.60 (s, 2H), 3.51 (br. s., 2H), 3.42 (s, 1H), 2.80 - 2.89 (m, 4H), 2.52 - 2.65 (m, 6H), 2.37 - 2.48 (m, 3H), 1.54 - 1.65 (m, 4H), 1.44 - 1.54 (m, 2H), 1.22 - 1.35 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 161.6 (C), 154.5 (2 x C), 152.7 (CH), 126.6 (C), 76.3 (2 x C), 69.0 (CH2), 59.9 (CH2), 52.9 (CH2), 50.2 (CH2), 46.6 (2 x CH2), 46.3 (CH2), 45.8 (CH), 29.5 (4 x CH2), 29.4 (CH2), 24.4 (CH3). **LRMS** (ESI +ve) [M+H] 398.10.

1-(cyclopentylmethyl)-N-methyl-3-(3-(4-methylpiperazin-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B12). Yield: 32.6 mg, 0.089 mmol, 39 %, as a red-brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.70 (s, 1H), 4.24 (d, J = 7.57 Hz, 1H), 4.11 (d, J = 6.23

Hz, 2H), 3.59 (s, 2H), 3.40 (s, 1H), 2.80 - 2.88 (m, 4H), 2.53 - 2.61 (m, 2H), 2.42 - 2.48 (m, 1H), 2.27 - 2.40 (m, 4H), 2.17 (s, 3H), 1.55 - 1.64 (m, 4H), 1.46 - 1.54 (m, 2H), 1.22 - 1.31 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 161.6 (C), 154.5 (2 x C), 152.7 (CH), 126.6 (C), 76.2 (2 x C), 54.5 (2 x CH2), 51.2 (2 x CH2), 50.2 (CH), 46.7 (CH2), 45.7 (CH2), 29.6 (4 x CH2), 24.4 (2 x CH3). **LRMS** (ESI +ve) [M+H] 368.20.

1-(cyclopentylmethyl)-N-methyl-3-(3-(pyrrolidin-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B13). Yield: 27.0 mg, 0.079 mmol, 23 %, as a light brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.69 (br. s., 1H), 7.55 (br. s., 1H), 4.11 (d, J = 6.23 Hz, 2H), 3.69 (s, 2H), 3.50 (s, 1H), 2.85 (d, J = 3.94 Hz, 2H), 2.58 - 2.62 (m, 2H), 2.45 (quin, J = 7.30 Hz, 1H), 1.73 (spt, J = 3.60 Hz, 4H), 1.67 - 1.71 (m, J = 3.00, 3.00, 7.25 Hz, 2H), 1.54 - 1.64 (m, 4H), 1.45 - 1.53 (m, 2H), 1.21 - 1.33 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 161.6 (C), 154.5 (2 x C), 152.7 (CH), 126.7 (C), 75.4 (2 x C), 68.3 (CH2), 51.8 (2 x CH2), 51.7 (CH2), 42.8 (2 x CH2), 42.5 (CH), 29.6 (2 x CH2), 24.4 (CH3), 23.3 (2 x CH2). **LRMS** (ESI +ve) [M+H] 339.00.

5. Synthesis and characterization of N-propargyl cyclic amines 10n-y

General Synthesis of Lipophilic N-propargylated cyclic amines. Amine (20 mmol, 2 eq.) was added to a 50 mL round-bottomed flask equipped with stirrer bar. The amine was suspended in diethyl ether (20 mL). The reaction was cooled to 0 °C while stirring. Propargyl bromide (10 mmol, 80 % in toluene) was added dropwise to the cold stirring reaction mixture. The flask was fitted with septum and flushed with nitrogen. The reaction was stirred at 0 °C for 30 minutes. The ice bath was removed, and the reaction was stirred at ambient temperature overnight under nitrogen. The reaction was quenched with water (10 mL) and was extracted with diethyl ether (4 x 40 mL). The organic fractions were combined, washed with water (10 mL), brine (40 mL) and dried over $MgSO_4$, before being concentrated under reduced pressure to give the crude product. The crude product was purified by flash column chromatography, using an eluent system of 0-15% MeOH in DCM. The appropriate fractions were combined and concentrated to give the desired products.

4-isopropyl-1-(prop-2-yn-1-yl)-piperidine (10s). Yield: 110.1 mg, 0.66 mmol, 8 %. **1H NMR** (500 MHz, DMSO-d6) δ 3.20 (d, J = 2.44 Hz, 2H), 3.08 (t, J = 2.40 Hz, 1H), 2.79 (td, J = 2.40, 11.27 Hz, 2H), 2.02 (dt, J = 2.40, 11.68 Hz, 2H), 1.59 (td, J = 2.00, 14.62 Hz, 2H), 1.38 (qd, J = 6.69, 13.34 Hz, 1H), 1.16 (dq, J = 3.82, 12.33 Hz, 2H), 0.88 - 0.97 (m, 1H), 0.84 (d, J = 6.78 Hz, 6H). **13C NMR** (126 MHz, DMSO-d6) δ 79.6 (CH), 75.3 (C), 52.1 (CH2), 46.4 (2 x CH2), 41.4 (2 x CH2), 31.9 (C), 28.7 (CH), 19.7 (2 x CH3).

1-(prop-2-yn-1-yl)-azepane (10u). Yield: 154.9 mg, 1.13 mmol, 4 %. **1H NMR** (500 MHz, CHLOROFORM-d) δ 3.38 (d, J = 2.44 Hz, 2H), 2.68 - 2.71 (m, 4H), 2.19 (t, J = 2.40 Hz, 1H), 1.65 - 1.72 (m, 4H), 1.59 - 1.63 (m, 4H). **13C NMR** (126 MHz, CHLOROFORM-d) δ 80.1 (CH), 72.0 (C), 55.1 (CH2), 48.0 (2 x CH2), 28.1 (2 x CH2), 26.7 (2 x CH2).

3-methyl-1-(prop-2-yn-1-yl)-piperidine (10y). Yield: 573.0 mg, 4.18 mmol, 41 %. **1H NMR** (500 MHz, CHLOROFORM-d) δ 3.29 (dd, J = 0.63, 2.44 Hz, 2H), 2.77 - 2.84 (m, 2H), 2.22 (dt, J = 0.55, 2.44 Hz, 1H), 2.09 (dt, J = 2.88, 11.41 Hz, 1H), 1.77 - 1.83 (m, 1H), 1.65 - 1.73 (m, 3H), 1.53 - 1.64 (m, 1H), 0.88 (d, J = 6.31 Hz, 3H), 0.79 - 0.87 (m, 1H). **13C NMR** (126 MHz, CHLOROFORM-d) δ 79.2 (CH), 72.8 (C), 60.5 (CH2), 52.6 (CH2), 47.3 (CH2), 32.5 (CH2), 31.2 (CH2), 25.5 (C), 19.0 (CH3)

General Synthesis of Polar N-propargylated cyclic amines. Amine (6 mmol) was added to a 50 mL round-bottomed flask equipped with stirrer bar. The amine was suspended in THF (50 mL). Potassium carbonate (12 mmol, 2 eq.) was added, and the reaction mixture was stirred at ambient temperature for 5 minutes. Propargyl bromide (9 mmol, 80 % in Toluene, 1.5 eq.) was added dropwise to the stirring reaction mixture. The reaction was fitted with a condenser and stirred at reflux (68 °C) for 8 hours. The reaction was removed from the heat and allowed to cool while stirring overnight. The solvent was removed in vacuo to give a residue. The residue was portioned between water (40 mL) and DCM (50 mL). The organic layer was collected, and the aqueous layer was washed with DCM (2 x 25 mL). The organic fractions were combined and dried over $MgSO_4$, before being concentrated under reduced pressure to give the crude product. The crude product was purified by flash column chromatography, using an eluent system of 0-20% MeOH in DCM. The appropriate fractions were combined and concentrated to give the desired products.

N,N-dimethyl-1-(prop-2-yn-1-yl)-piperidin-3-amine (10n). Yield: 266.7 mg, 1.60 mmol, 21 %. **1H NMR** (500 MHz, CHLOROFORM-d) δ 3.35 (d, J = 2.36 Hz, 2H), 3.08 (td, J = 1.80, 10.58 Hz, 1H), 2.78 (td, J = 3.66, 10.96 Hz, 1H), 2.69 (tt, J = 3.72, 10.58 Hz, 1H), 2.48 (s, 6H), 2.26 (t, J = 2.44 Hz, 1H), 2.27 (t, J = 10.32 Hz, 1H), 2.19 (dt, J = 2.96, 11.41 Hz, 1H), 1.98 - 2.04 (m, 1H), 1.84 (quind, J = 3.70, 13.56 Hz, 1H), 1.61 (ttd, J = 4.03, 11.97, 13.47 Hz, 1H), 1.35 (dq, J = 4.20, 12.10 Hz, 1H). **13C NMR** (126 MHz, CHLOROFORM-d) δ 78.4 (CH), 73.5 (C), 61.9 (CH2), 53.9 (CH2), 52.0 (CH2), 47.2 (CH2), 41.5 (CH2), 25.9 (CH), 24.0 (2 x CH3).

N-methyl-1-(prop-2-yn-1-yl)-piperidine-4-carboxamide (10o). Yield: 38.0 mg, 0.211 mmol, 3 %. **1H NMR** (500 MHz, DMSO-d6) δ 7.64 (q, J = 4.10 Hz, 1H), 3.23 (d, J = 2.44 Hz, 2H), 3.11 (t, J = 2.44 Hz, 1H), 2.77 (td, J = 2.50, 11.15 Hz, 2H), 2.55 (d, J = 4.57 Hz, 3H), 2.07 (dt, J = 2.68, 11.66 Hz, 2H), 1.99 (tt, J = 3.94, 11.70 Hz, 1H), 1.61 – 1.67 (m, 2H), 1.54 (dq, J = 4.02,

10.40 Hz, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 174.6 (C), 79.5 (CH), 75.5 (C), 51.2 (CH2), 46.4 (2 x CH2), 41.5 (2 x CH2), 28.4 (CH), 25.4 (CH3).

N-(1-(prop-2-yn-1-yl)-piperidin-4-yl)-acetamide (10p). Yield: 24.1 mg, 0.133 mmol, 2 %. **1H NMR** (500 MHz, DMSO-d6) δ 7.73 (d, J = 7.57 Hz, 1H), 3.39 - 3.49 (m, 1H), 3.22 (d, J = 2.44 Hz, 2H), 3.12 (t, J = 2.40 Hz, 1H), 2.73 (td, J = 3.20, 11.98 Hz, 2H), 2.13 (dt, J = 2.44, 11.63 Hz, 2H), 1.77 (s, 3H), 1.71 (dd, J = 2.99, 12.30 Hz, 2H), 1.35 (dq, J = 3.86, 11.77 Hz, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 168.3 (C), 79.6 (CH), 75.5 (C), 50.7 (CH2), 46.2 (2 x CH2), 45.6 (2 x CH2), 31.5 (CH), 22.7 (CH3).

1-(prop-2-yn-1-yl)-4-(pyrrolidin-1-yl)-piperidine (10q). Yield: 274.5 mg, 1.43 mmol, 21 %. **1H NMR** (500 MHz, DMSO-d6) δ 3.21 (d, J = 2.44 Hz, 2H), 3.10 (t, J = 2.44 Hz, 1H), 2.73 (td, J = 2.71, 11.53 Hz, 2H), 2.46 (br. s., 4H), 2.11 (dt, J = 2.33, 11.65 Hz, 2H), 1.90 (br. s., 1H), 1.81 (d, J = 12.53 Hz, 2H), 1.65 (spt, J = 3.20 Hz, 4H), 1.37 (dq, J = 2.92, 9.70 Hz, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 79.6 (CH), 75.4 (C), 60.8 (CH2), 50.9 (2 x CH2), 50.4 (2 x CH2), 46.1 (2 x CH2), 30.9 (2 x CH2), 22.3 (CH).

1'-(prop-2-yn-1-yl)-1,4'-bipiperidine (10r). Yield: 308.0 mg, 1.49 mmol, 24 %. **1H NMR** (500 MHz, DMSO-d6) δ 3.21 (d, J = 2.44 Hz, 2H), 3.10 (t, J = 2.44 Hz, 1H), 2.80 (td, J = 2.40, 11.59 Hz, 2H), 2.43 - 2.48 (m, 4H), 2.20 (br. s., 1H), 2.07 (dt, J = 2.17, 11.76 Hz, 2H), 1.70 (d, J = 11.59 Hz, 2H), 1.49 (t, J = 5.00 Hz, 4H), 1.43 (dd, J = 3.31, 11.98 Hz, 2H), 1.34 - 1.41 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 79.5 (CH), 75.4 (C), 61.7 (CH2), 51.3 (2 x CH2), 49.6 (2 x CH2), 46.1 (2 x CH2), 27.2 (2 x CH2), 25.7 (CH2), 24.3 (CH).

4-phenyl-1-(prop-2-yn-1-yl)-piperidine (10t). Yield: 238.5 mg, 1.20 mmol, 19 %. **1H NMR** (500 MHz, DMSO-d6) δ 7.26 - 7.30 (m, 2H), 7.21 - 7.25 (m, 2H), 7.18 (tt, J = 1.60, 7.10 Hz, 1H), 3.29 (d, J = 2.44 Hz, 2H), 3.14 (t, J = 2.44 Hz, 1H), 2.88 (td, J = 2.10, 11.11 Hz, 1H), 2.45 (tt, J = 3.75, 12.09 Hz, 1H), 2.23 (dt, J = 2.52, 11.66 Hz, 2H), 1.71 - 1.78 (m, 2H), 1.65 (dq, J = 3.70, 11.70 Hz, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 146.1 (C), 128.3 (CH), 126.7 (2 x CH), 126.0 (2 x CH), 79.6 (CH), 75.5 (C), 52.2 (CH2), 46.4 (2 x CH2), 41.3 (2 x CH2), 32.9 (CH).

Synthesis of Ethyl-(1-prop-2-yn-1-yl)-piperidin-4-carboxylate (10v). Ethyl isonipecotate (3.08 mL, 20.00 mmol) was added to a 100 mL round-bottomed flask equipped with a stirrer bar. The oil was suspended in DMF (50 mL) and was stirred to a homogeneous solution. Potassium carbonate (4.15 g, 30.00 mmol, 1.5 eq.) was added portion wise to the stirring mixture and the flask was filled with septum and flushed with nitrogen. Propargyl bromide (3.21 mL, 30.00 mmol, 1.5 eq., 80 % in toluene) was

added dropwise and the reaction was stirred overnight at ambient temperature under nitrogen to give a cream suspension. The reaction was poured into water (200 mL) and the organic layers were extracted with diethyl ether (4 x 100 mL). The organic layers were collected and combined, washed with water (20 mL) and brine (50 mL). The organic layers were dried over $MgSO_4$, filtered and concentrated, to give the product, ethyl-(1-prop-2-yn-1-yl)-piperidin-4-carboxylate (1.86 g, 9.53 mmol, 48 %). **1H NMR** (500 MHz, DMSO-d6) δ 4.05 (q, J = 7.09 Hz, 2H), 3.23 (d, J = 2.52 Hz, 2H), 3.11 (t, J = 2.44 Hz, 1H), 2.73 (td, J = 3.50, 11.74 Hz, 2H), 2.24 (tt, J = 4.00, 11.17 Hz, 1H), 2.14 (dt, J = 2.60, 11.39 Hz, 2H), 1.81 (qd, J = 3.70, 13.24 Hz, 2H), 1.55 (dq, J = 3.60, 13.00 Hz, 2H), 1.17 (t, J = 7.09 Hz, 3H). **13C NMR** (126 MHz, DMSO-d6) δ 174.2 (C), 79.4 (CH), 75.5 (C), 59.7 (CH2), 50.8 (2 x CH2), 46.3 (2 x CH2), 43.4 (CH2), 27.8 (CH), 14.1 (CH3).

Synthesis of (4-prop-2-yn-1-yl)-piperazin-1-carboxylic acid ethyl ester (10w). Ethyl piperazine-1-carboxylate (1.00 g, 6.32 mmol, 3.0 eq.) was added to a 100 mL round-bottomed flask containing potassium carbonate (726.1 mg, 5.25 mmol, 2.5 eq.) stirring in acetonitrile (20 mL) at 0 °C. The reaction was stirred to a homogeneous suspension at 0 °C and was fitted with septum and flushed with nitrogen. Propargyl bromide (224.4 µL, 2.10 mmol, 80 % in toluene) was added dropwise and the reaction was stirred at 0 °C for 10 minutes. The ice bath was removed, and the reaction was stirred overnight at ambient temperature under nitrogen. The reaction was poured into water (20 mL) and the organic layers were extracted with DCM (4 x 20 mL). The organic layers were collected and combined, washed with water (20 mL) and brine (20 mL). The organic layers were dried over MgSO4, filtered and concentrated, to give a golden oil. The crude product was purified by flash column chromatography on silica using a 0-15 % MeOH/DCM eluent gradient. The appropriate fractions by TLC were combined and concentrated to give the product, (4-prop-2-yn-1-yl)-piperazin-1-carboxylic acid ethyl ester (421.1 mg, 2.14 mmol, 102 %). Carried through wet with water. **1H NMR** (500 MHz, CHLOROFORM-d) δ 4.14 (q, J = 7.09 Hz, 2H), 3.52 (t, J = 5.10 Hz, 4H), 3.32 (d, J = 2.44 Hz, 2H), 2.53 (t, J = 4.85 Hz, 4H), 2.26 (t, J = 2.44 Hz, 1H), 1.26 (t, J = 7.13 Hz, 3H). **13C NMR** (126 MHz, CHLOROFORM-d) δ 155.4 (C), 78.3 (CH), 73.5 (C), 61.3 (CH2), 51.5 (2 x CH2), 47.0 (2 x CH2), 43.5 (CH2), 14.6 (CH3).

6. Synthesis and characterization of compounds B14-25

General Synthesis of Final Compounds B14-25. 1-(cyclopentylmethyl)-3-iodo-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (0.300 mmol) was added to a 5 mL microwave vial equipped with stirrer bar. N-propargylated cyclic amine (1.2 eq.), bis(triphenylphosphine)palladium dichloride (5 mol%), copper (I) iodide (10 mol%) and triethylamine (2.0 eq.) were added to the vial. The reagents were suspended in THF (5 ml) and the vial was sealed with septum cap. The vial was placed into a microwave reactor and heated to and stirred at 70 °C for 2 h under microwave irradiation, yielding a reaction mixture, which was cooled whilst stirring for an hour. The reaction mixture was partitioned between water (10 ml) and EtOAc (20 ml). The organic phase was collected, and the aqueous layer was washed with EtOAc (3 x 25 ml). The organic layers were combined, dried over $MgSO_4$, and concentrated in vacuo to give the crude product. The crude product was purified by preparative-TLC on silica using an 8 % MeOH/DCM eluent system. The appropriate dual-active band by UV and KMnO4 visualization was scraped off the plate and washed with 15 % MeOH/DCM solution. The filtrate was collected and concentrated to give the product.

1-(cyclopentylmethyl)-3-(3-(3-(dimethylamino)piperidin-1-yl)prop-1-yn-1-yl)-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B14). Yield: 10.5 mg, 0.027 mmol, 9 %, as a golden solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.69 (br. s., 1H), 7.56 (br. s., 1H), 4.11 (d, J = 6.23 Hz, 2H), 3.62 (d, J = 6.00 Hz, 2H), 2.97 (d, J = 7.80 Hz, 1H), 2.85 (d, J = 3.78 Hz, 3H), 2.76 (d, J = 10.80 Hz, 1H), 2.46 (quin, J = 7.30 Hz, 1H), 2.29 (br. s., 6H), 2.15 (br. s., 2H), 1.81 (d, J = 10.09 Hz, 1H), 1.73 (td, J = 3.31, 13.16 Hz, 1H), 1.55 - 1.64 (m, 4H), 1.42 - 1.54 (m, 4H), 1.22 - 1.33 (m, 2H), 1.16 (d, J = 12.45 Hz, 1H). **13C NMR** (126 MHz, DMSO-d6) δ 161.6 (C), 154.5 (2 x C), 152.7 (CH), 126.7 (C), 89.3 (CH), 76.2 (2 x C), 60.9 (2 x CH2), 51.8 (CH2), 50.2 (CH), 47.2 (2 x CH2), 41.5 (CH2), 29.6 (4 x CH2), 24.4 (3 x CH3). **LRMS** (ESI +ve) [M+H] 396.10.

1-(3-(1-(cyclopentylmethyl)-6-(methylamino)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)prop-2-yn-1-yl)-N-methylpiperidine-4-carboxamide (B15). Yield: 15.7 mg, 0.038 mmol, 25 %, as a pale-yellow solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.70 (br. s., 1H), 7.66 (q, J = 4.33 Hz, 1H), 7.56 (br. s, 1H), 4.11 (d, J = 6.23 Hz, 2H), 3.59 (s, 2H), 2.88 (t, J = 3.00 Hz, 2H), 2.85 (t, J = 4.10 Hz, 3H), 2.55 (d, J = 4.57 Hz, 3H), 2.46 (quin, J = 7.10 Hz, 1H), 2.21 (dt, J = 2.40, 11.57 Hz, 2H), 2.05 (tt, J = 3.90, 11.80 Hz, 1H), 1.65 - 1.72 (m, 2H), 1.55 - 1.65 (m, 6H), 1.46 - 1.54 (m, 2H), 1.23 - 1.34 (m, 2H**). 13C NMR** (126 MHz, DMSO-d6) δ 174.7 (C), 161.6 (C), 154.5 (2 x C), 152.7 (CH), 126.7 (C), 76.1 (2 x C), 51.4 (4 x CH2), 47.1 (CH), 41.5 (CH2), 29.6 (4 x CH2), 28.5 (2 x CH2), 25.4 (CH3), 24.4 (CH3). **LRMS** (ESI +ve) [M+H] 410.10.

N-(1-(3-(1-(cyclopentylmethyl)-6-(methylamino)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)prop-2-yn-1-yl)piperidin-4-yl)acetamide (B16). Yield: 8.9 mg, 0.022 mmol, 16 %, as a white solid.

1H NMR (500 MHz, DMSO-d6) δ 8.71 (br. s., 1H), 7.76 (d, J = 7.57 Hz, 1H), 7.51 - 7.60 (m, 1H), 4.11 (d, J = 6.31 Hz, 2H), 3.57 (s, 2H), 3.45 - 3.54 (m, 1H), 2.85 (br. s., 3H), 2.83 (br. s., 2H), 2.46 (quin, J = 7.70 Hz, 1H), 2.27 (t, J = 10.68 Hz, 2H), 1.78 (s, 3H), 1.75 (d, J = 10.01 Hz, 2H), 1.54 - 1.63 (m, 4H), 1.47 - 1.54 (m, 2H), 1.41 (dq, J = 4.02, 11.90 Hz, 2H), 1.22 - 1.33 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 168.3 (C), 161.7 (C), 154.5 (2 x C), 152.8 (CH), 126.7 (C), 76.4 (2 x C), 50.9 (2 x CH2), 46.9 (2 x CH2), 45.6 (2 x CH), 31.5 (CH2), 29.6 (4 x CH2), 24.4 (CH3), 22.7 (CH3) [NB. Due to weak sample concentration, expected CH2 at approx. 80.0 not present]. **LRMS** (ESI +ve) [M+H] 410.10.

1-(cyclopentylmethyl)-N-methyl-3-(3-(4-(pyrrolidin-1-yl)piperidin-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B17). Yield: 41.6 mg, 0.099 mmol, 27 %, as a golden solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.70 (br. s., 1H), 7.56 (br. s., 1H), 4.11 (d, J = 5.99 Hz, 2H), 3.58 (s, 2H), 2.85 (d, J = 3.86 Hz, 5H), 2.45 (quin, J = 7.30 Hz, 1H), 2.25 (dt, J = 1.97, 11.35 Hz, 2H), 1.98 (d, J = 12.69 Hz, 1H), 1.86 (d, J = 11.35 Hz, 2H), 1.67 (br. s., 6H), 1.55 - 1.63 (m, 4H), 1.47 - 1.54 (m, 2H), 1.39 - 1.47 (m, 2H), 1.20 - 1.36 (m, 4H). **13C NMR** (126 MHz, DMSO-d6) δ 161.6 (C), 154.5 (2 x C), 152.7 (CH), 126.7 (C), 89.4 (CH2), 76.1 (2 x C), 60.6 (CH2), 50.9 (2 x CH2), 50.4 (CH2), 50.2 (CH2), 46.9 (2 x CH2), 30.9 (CH), 29.6 (4 x CH2), 27.8 (CH), 24.4 (2 x CH2), 22.9 (CH3). **LRMS** (ESI +ve) [M+H] 422.10.

3-(3-([1,4'-bipiperidin]-1'-yl)prop-1-yn-1-yl)-1-(cyclopentylmethyl)-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B18). Yield: 52.3 mg, 0.120 mmol, 42 %, as a golden solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.70 (br. s., 1H), 7.56 (br. s., 1H), 4.11 (d, J = 6.31 Hz, 2H), 3.58 (s, 2H), 2.92 (d, J = 11.35 Hz, 2H), 2.85 (d, J = 3.70 Hz, 3H), 2.53 - 2.61 (m, 2H), 2.42 - 2.48 (m, 1H), 2.21 (t, J = 10.80 Hz, 2H), 1.91 (s, 1H), 1.77 (d, J = 9.62 Hz, 2H), 1.55 - 1.65 (m, 4H), 1.50 (d, J = 9.46 Hz, 10H), 1.34 - 1.43 (m, 2H), 1.21 - 1.33 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 161.6 (C), 160.5 (C), 154.5 (CH), 152.7 (C), 126.7 (C), 89.4 (CH2), 76.1 (2 x C), 61.6 (2 x CH2), 51.4 (2 x CH2), 50.2 (2 x CH), 49.6 (3 x CH2), 46.8 (CH2), 29.6 (4 x CH2), 27.2 (CH2), 25.5 (CH2), 24.4 (CH2). **LRMS** (ESI +ve) [M+H] 436.10.

1-(cyclopentylmethyl)-3-(3-(4-isopropylpiperidin-1-yl)prop-1-yn-1-yl)-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B19). Yield: 50.4 mg, 0.128 mmol, 41 %, as a brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.69 (br. s., 1H), 7.37 - 7.52 (m, 1H), 4.11 (d, J = 6.31 Hz, 2H), 3.56 (s, 2H), 2.89 (d, J = 11.19 Hz, 2H), 2.85 (d, J = 3.86 Hz, 3H), 2.46 (quin, J = 7.30 Hz, 1H), 2.16 (dt, J = 2.21, 11.59 Hz, 2H), 2.05 (dt, J = 2.33, 11.61 Hz, 0H), 1.64 (d, J = 12.85 Hz, 2H), 1.55 - 1.61 (m, 4H), 1.45 - 1.54 (m, 2H), 1.40 (qd, J = 6.68, 13.30 Hz, 1H), 1.26 - 1.33 (m, 2H), 1.21 (dq, J = 3.47, 12.20 Hz, 2H), 0.93 - 1.02 (m, 1H), 0.85 (d, J = 6.78 Hz, 6H). **13C NMR**

(126 MHz, DMSO-d6) δ 161.6 (C), 154.5 (2 x C), 152.7 (CH), 126.7 (C), 89.6 (CH2), 76.0 (2 x C), 52.4 (2 x CH2), 50.2 (CH), 47.1 (CH2), 41.4 (CH), 31.9 (CH), 29.6 (4 x CH2), 28.8 (2 x CH2), 24.4 (CH3), 19.7 (2 x CH3). **LRMS** (ESI +ve) [M+H] 395.10.

1-(cyclopentylmethyl)-N-methyl-3-(3-(4-phenylpiperidin-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B20). Yield: 43.0 mg, 0.100 mmol, 34 %, as a light-brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.73 (br. s., 1H), 7.23 - 7.32 (m, 4H), 7.16 - 7.21 (m, 1H), 4.12 (d, J = 6.31 Hz, 2H), 3.64 (s, 2H), 2.99 (d, J = 11.19 Hz, 2H), 2.86 (d, J = 3.63 Hz, 3H), 2.47 (quin, J = 7.30 Hz, 1H), 2.37 (dt, J = 2.29, 11.59 Hz, 2H), 1.76 - 1.82 (m, 2H), 1.70 (dq, J = 3.63, 12.10 Hz, 2H), 1.55 - 1.64 (m, 4H), 1.47 - 1.55 (m, 2H), 1.42 (dq, J = 4.18, 12.50 Hz, 1H), 1.23 - 1.34 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 161.7 (C), 160.7 (C), 154.5 (2 x C), 152.8 (CH), 146.2 (CH), 128.3 (2 x CH), 126.7 (2 x CH), 126.0 (C), 76.1 (2 x C), 52.4 (CH2), 50.2 (CH2), 47.2 (CH2), 45.4 (CH2), 41.9 (CH2), 41.3 (CH2), 33.5 (CH), 33.0 (2 x CH2), 32.2 (CH), 29.6 (2 x CH2), 24.4 (CH3). **LRMS** (ESI +ve) [M+H] 429.10.

3-(3-(azepan-1-yl)prop-1-yn-1-yl)-1-(cyclopentylmethyl)-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B21). Yield: 56.7 mg, 0.155 mmol, 51 %, as a light-brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.69 (br. s., 1H), 7.55 (br. s., 1H), 4.11 (d, J = 6.15 Hz, 2H), 3.65 (s, 2H), 2.85 (d, J = 4.02 Hz, 3H), 2.67 - 2.72 (m, 4H), 2.46 (quin, J = 6.90 Hz, 1H), 1.59 - 1.67 (m, 8H), 1.54 - 1.58 (m, 4H), 1.47 - 1.54 (m, 2H), 1.21 - 1.33 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 161.6 (C), 154.5 (2 x C), 152.7 (CH), 126.8 (C), 90.6 (CH2), 75.1 (2 x C), 54.9 (2 x CH2), 54.5 (2 x CH2), 50.2 (CH), 48.1 (CH2), 29.6 (2 x CH2), 27.9 (2 x CH2), 26.4 (2 x CH2), 24.4 (CH3). **LRMS** (ESI +ve) [M+H] 367.10.

Ethyl-1-(3-(1-(cyclopentylmethyl)-6-(methylamino)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)prop-2-yn-1-yl)piperidine-4-carboxylate (B22). Yield: 47.7 mg, 0.112 mmol, 40 %, as a light brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.71 (br. S., 1H), 7.56 (br. S, 1H), 4.11 (d, J = 6.23 Hz, 2H), 4.06 (q, J = 7.09 Hz, 2H), 3.59 (s, 2H), 2.85 (d, J = 3.55 Hz, 3H), 2.82 (br. S., 2H), 2.45 (quin, J = 7.20 Hz, 1H), 2.25 – 2.35 (m, 3H), 1.85 (dd, J = 3.19, 13.12 Hz, 2H), 1.55 – 1.66 (m, 6H), 1.44 – 1.54 (m, 2H), 1.23 – 1.34 (m, 2H), 1.17 (t, J = 7.09 Hz, 3H). **13C NMR** (126 MHz, DMSO-d6) δ 174.3 (C), 161.7 (C), 154.5 (2 x C), 152.8 (CH), 126.7 (C), 89.3 (CH2), 76.1 (2 x C), 59.8 (2 x CH2), 50.9 (2 x CH2), 50.2 (CH), 47.0 (CH), 29.6 (4 x CH2), 27.9 (2 x CH2), 24.4 (CH3), 14.1 (CH3). **LRMS** (ESI +ve) [M+H] 425.10.

Ethyl-4-(3-(1-(cyclopentylmethyl)-6-(methylamino)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)prop-2-yn-1-yl)piperazine-1-carboxylate (B23). Yield: 56.4 mg, 0.133 mmol, 37 %, as a

golden solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.70 (br. s., 1H), 7.51 - 7.59 (m, 1H), 4.11 (d, J = 6.46 Hz, 2H), 4.04 (q, J = 7.09 Hz, 2H), 3.64 (s, 2H), 3.41 (br. s., 4H), 3.34 - 3.39 (m, 1H), 2.85 (d, J = 4.10 Hz, 3H), 2.51 - 2.54 (m, 2H), 2.46 (quin, J = 7.30 Hz, 1H), 2.38 - 2.42 (m, 1H), 1.54 - 1.65 (m, 4H), 1.44 - 1.54 (m, 2H), 1.23 - 1.33 (m, 2H), 1.18 (t, J = 7.09 Hz, 3H). **13C NMR** (126 MHz, DMSO-d6) δ 161.6 (C), 154.6 (2 x C), 154.5 (C), 152.7 (CH), 126.5 (C), 88.8 (CH2), 76.4 (2 x C), 60.7 (2 x CH2), 51.0 (2 x CH2), 50.9 (CH), 46.8 (CH2), 43.2 (CH2), 29.6 (4 x CH2), 24.4 (CH3), 14.5 (CH3). **LRMS** (ESI +ve) [M+H] 426.10.

1-(cyclopentylmethyl)-3-(3-(3,5-dimethylpiperidin-1-yl)-prop-2-yn-1-yl)-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B24). Yield: 35.6 mg, 0.094 mmol, 30 %, as a beige solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.68 (br. s., 1H), 7.52 - 7.58 (m, 1H), 4.11 (d, J = 6.07 Hz, 2H), 3.59 (s, 2H), 2.85 (d, J = 3.86 Hz, 3H), 2.80 (td, J = 1.45, 10.11 Hz, 2H), 2.46 (quin, J = 7.20 Hz, 1H), 1.73 (t, J = 10.70 Hz, 2H), 1.62 - 1.69 (m, 4H), 1.55 - 1.62 (m, 4H), 1.45 - 1.54 (m, 2H), 1.22 - 1.33 (m, 2H), 0.85 (d, J = 6.38 Hz, 6H). **13C NMR** (126 MHz, DMSO-d6) δ 161.6 (C), 154.5 (2 x C), 152.7 (CH), 126.7 (C), 89.5 (CH2), 76.0 (2 x C), 59.6 (2 x CH2), 50.2 (2 x CH), 47.0 (CH2), 41.4 (CH2), 30.6 (2 x CH2), 29.6 (2 x CH2), 27.8 (CH2), 24.4 (CH3), 19.4 (2 x CH3). **LRMS** (ESI +ve) [M+H] 381.00.

1-(cyclopentylmethyl)-N-methyl-3-(3-(3-methylpiperidin-1-yl)prop-2-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B25). Yield: 64.7 mg, 0.177 mmol, 45 %, as a beige solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.69 (br. S., 1H), 7.53 – 7.59 (m, 1H), 4.11 (d, J = 6.23 Hz, 2H), 3.57 (s, 2H), 2.85 (d, J = 3.86 Hz, 3H), 2.75 – 2.82 (m, 2H), 2.46 (quin, J = 7.20 Hz, 1H), 2.13 (dt, J = 2.36, 11.31 Hz, 1H), 1.84 (t, J = 10.48 Hz, 1H), 1.54 – 1.68 (m, 8H), 1.42 – 1.54 (m, 3H), 1.21 – 1.34 (m, 2H), 0.86 (d, J = 6.54 Hz, 3H). **13C NMR** (126 MHz, DMSO-d6) δ 161.6 (C), 154.5 (2 x C), 152.7 (CH), 126.7 (C), 89.5 (CH2), 76.0 (2 x C), 60.0 (CH), 52.0 (2 x CH2), 50.2 (CH), 47.3 (2 x CH2), 32.1 (CH2), 30.6 (CH2), 29.6 (2 x CH2), 24.9 (CH2), 24.4 (CH3), 19.5 (CH3). **LRMS** (ESI +ve) [M+H] 367.10.

7. Synthesis and characterization of compounds B26-32

General Synthesis of 1H-pyrazolo[3,4-d]pyrimidin-6-amine (20) cores. 6-chloro-3-iodo-1H-pyrazolo[3,4-d]pyrimidine (1.00 mmol) was added to a 20 mL microwave vial equipped with stirrer bar. The solid was suspended in THF (1.5 mL) and dissolved through sonication. The mixture was stirred, and amine solution (2 M in THF, 3.0 eq.) was added. The vial was sealed with septum cap and heated to 150 °C while stirring in a microwave reactor for 60 minutes under microwave irradiation. The reaction vial was removed from the microwave and allowed to cool to ambient temperature, resulting the formation of a precipitate, which was collected by vacuum filtration. The solid was washed with water (3 x 4 mL) and dried to yield the product.

N-ethyl-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-6-amine (11a). Yield: 182.8 mg, 0.632 mmol, 58 %, as a white solid. **1H NMR** (500 MHz, DMSO-d6) δ 13.35 (br. s., 1H), 8.44 (s, 1H), 7.50 (d, J = 12.37 Hz, 1H), 3.28 (s, 2H), 1.14 (t, J = 7.17 Hz, 3H). **13C NMR** (126 MHz, DMSO-d6) δ 161.5 (C), 156.9 (CH), 153.4 (2 x C), 92.9 (C), 35.6 (CH2), 14.3 (CH3). **LRMS** (ESI +ve) [M+H] 289.90.

3-iodo-N-propyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (11b). Yield: 212.6 mg, 0.701 mmol, 62 %, as a cream solid. **1H NMR** (500 MHz, DMSO-d6) δ 13.33 (br. s., 1H), 8.45 (br. s., 1H), 7.62 (br. s., 1H), 3.31 (s, 2H), 1.56 (sxt, J = 7.27 Hz, 2H), 0.89 (t, J = 7.41 Hz, 3H). **13C NMR** (126 MHz, DMSO-d6) δ 161.7 (C), 156.9 (CH), 153.4 (2 x C), 92.9 (C), 42.7 (CH2), 21.7 (CH2), 11.4 (CH3). **LRMS** (ESI +ve) [M+H] 303.90.

3-iodo-N-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (11c). Yield: 175.4 mg, 0.579 mmol, 53 %, as a beige solid. **1H NMR** (500 MHz, DMSO-d6) δ 13.30 (br. s., 1H), 8.46 (br. s., 1H), 7.45 (br. s., 1H), 4.04 (br. s., 1H), 1.17 (d, J = 6.54 Hz, 6H). **13C NMR** (126 MHz, DMSO-d6) δ 160.9 (C), 156.9 (CH), 153.5 (2 x C), 92.9 (C), 42.2 (CH), 22.1 (2 x CH3). **LRMS** (ESI +ve) [M+H] 303.90.

3-iodo-N,N-dimethyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (11d). Yield: 153.4 mg, 0.531 mmol, 46 %, as a grey solid. **1H NMR** (500 MHz, DMSO-d6) δ 13.38 (br. s., 1H), 8.54 - 8.56 (m, 1H), 3.18 (br. s., 6H). **13C NMR** (126 MHz, DMSO-d6) δ 161.2 (C), 156.9 (CH), 153.0 (C), 110.9 (C), 93.0 (C), 37.2 (2 x CH3). **LRMS** (ESI +ve) [M+H] 289.90.

3-iodo-N-(pentan-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (11e). Yield: 234.9 mg, 0.709 mmol, 64 %, as a light brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 13.31 (br. s., 1H), 8.44

(br. s., 1H), 7.41 (d, J = 6.38 Hz, 1H), 3.77 (br. s., 1H), 1.43 - 1.60 (m, 4H), 0.86 (t, J = 7.37 Hz, 6H). **13C NMR** (126 MHz, DMSO-d6) δ 162.0 (C), 156.8 (CH), 153.4 (2 x C), 92.9 (C), 53.1 (CH), 29.5 (2 x CH2), 26.2 (2 x CH3). **LRMS** (ESI +ve) [M+H] 331.90.

N-benzyl-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-6-amine (11f). Yield: 273.3 mg, 0.778 mmol, 62 %, as a white solid. **1H NMR** (500 MHz, DMSO-d6) δ 13.34 (br. s., 1H), 8.50 (s, 1H), 8.14 (br. s., 1H), 7.27 - 7.33 (m, 4H), 7.18 - 7.23 (m, 1H), 4.54 (br. s., 2H). **13C NMR** (126 MHz, DMSO-d6) δ 161.7 (C), 156.7 (CH), 153.7 (2 x C), 128.2 (2 x C), 126.9 (2 x C), 126.5 (C), 93.0 (C), 44.1 (CH2). **LRMS** (ESI +ve) [M+H] 351.90.

N-(tert-butyl)-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-6-amine (11g). Yield: 266.8 mg, 0.841 mmol, 73 %, as a golden solid. **1H NMR** (500 MHz, DMSO-d6) δ 13.35 (br. S., 1H), 8.46 (s, 1H), 7.15 (br. S., 1H), 1.42 (s, 9H). **13C NMR** (126 MHz, DMSO-d6) δ 161.1 (C), 156.4 (CH), 153.1 (2 x C), 92.8 (C), 50.3 (C), 29.5 (CH3), 28.4 (2 x CH3). **LRMS** (ESI +ve) [M+H] 317.90.

General Synthesis of 1-(cyclopentylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine cores. 3-iodo-1H-pyrazolo[3,4-d]pyrimidin-6-amine core (0.500 -0.800 mmol) was added to a 20 mL microwave vial equipped with a stirrer bar. The solid was suspended in DMF (5 mL) and stirred to a homogeneous solution. Sodium hydride (1.5 eq., 60 % dispersion in mineral oil) was added portion wise, and the reaction was stirred for an hour to allow gas evolution to subside. Iodo(cyclopentylmethyl) (1.5 eq.) was added, and the vial was sealed with septum cap and heated to 150 °C while stirring for 1 hour 20 minutes under microwave irradiation in a microwave reactor, yielding a solution, which was cooled whilst stirring overnight. The reaction was partitioned between water (20 mL) and EtOAc (40 mL). The organic layer was collected, and the aqueous layer was washed with further EtOAc (3 x 20 mL). The organic fractions were combined, washed with brine (20 mL), dried over MgSO4, and concentrated to give the crude product. The crude was loaded onto a SNAP HP-Sil 10 g column and purified by normal phase chromatography using a 0-10 % MeOH/DCM eluent gradient over 12 CV. The appropriate fractions were concentrated to give the product.

1-(cyclopentylmethyl)-N-ethyl-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-6-amine (12a). Yield: 95.7 mg, 0.257 mmol, 50 %, as a pale pink solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.44 (br. s., 1H), 7.66 (br. s., 1H), 4.09 (d, J = 7.01 Hz, 2H), 3.34 (br. s., 2H), 2.43 (quin, J = 7.20 Hz, 1H), 1.54 - 1.64 (m, 4H), 1.45 - 1.54 (m, 2H), 1.27 (d, J = 6.31 Hz, 2H), 1.15 (t, J = 7.09 Hz, 3H). **13C NMR** (126 MHz, DMSO-d6) δ 161.3 (C), 155.2 (2 x C), 153.7 (CH), 91.8 (C), 50.2 (CH2), 35.6 (CH), 29.5 (4 x CH2), 24.4 (CH2), 14.1 (CH3). **LRMS** (ESI +ve) [M+H] 371.80.

1-(cyclopentylmethyl)-3-iodo-N-propyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (12b). Yield: 81.1 mg, 0.211 mmol, 42 %, as an off-white solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.43 (br. s., 1H), 7.69 (br. s., 1H), 4.08 (d, J = 7.17 Hz, 2H), 3.28 (br. s., 2H), 2.43 (quin, J = 7.20 Hz, 1H), 1.54 - 1.63 (m, 6H), 1.44 - 1.54 (m, 2H), 1.28 (d, J = 5.99 Hz, 2H), 0.89 (t, J = 7.37 Hz, 3H). **13C NMR** (126 MHz, DMSO-d6) δ 161.6 (C), 155.2 (CH), 153.7 (2 x C), 91.8 (C), 50.2 (CH2), 42.6 (CH2), 29.5 (4 x CH2), 24.4 (CH2), 21.7 (CH), 11.5 (CH3). **LRMS** (ESI +ve) [M+H] 386.00.

1-(cyclopentylmethyl)-3-iodo-N-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (12c). Yield: 100.0 mg, 0.260 mmol, 52 %, as a white solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.44 (br. S., 1H), 7.53 (br. S., 1H), 4.08 (d, J = 7.25 Hz, 3H), 2.39 – 2.47 (m, 1H), 1.54 – 1.64 (m, 4H), 1.43 – 1.53 (m, 2H), 1.21 – 1.33 (m, 2H), 1.18 (d, J = 6.54 Hz, 6H). **13C NMR** (126 MHz, DMSO-d6) δ 160.8 (C), 155.2 (CH), 153.7 (2 x C), 91.8 (C), 50.1 (CH2), 42.3 (CH), 29.5 (4 x CH2), 24.4 (2 x CH3), 21.9 (CH). **LRMS** (ESI +ve) [M+H] 386.00.

1-(cyclopentylmethyl)-3-iodo-N,N-dimethyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (12d). Yield: 108.1 mg, 0.291 mmol, 61 %, as a cream solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.53 (s, 1H), 4.12 (d, J = 7.49 Hz, 2H), 3.21 (s, 6H), 2.45 (td, J = 7.18, 14.40 Hz, 1H), 1.55 - 1.66 (m, 4H), 1.41 - 1.55 (m, 2H), 1.23 - 1.35 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 161.0 (C), 155.2 (CH), 153.3 (C), 111.3 (C), 91.8 (C), 50.2 (CH2), 37.0 (CH), 29.6 (4 x CH2), 24.4 (2 x CH3). **LRMS** (ESI +ve) [M+H] 371.90 .

1-(cyclopentylmethyl)-3-iodo-N-(pentan-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (12e). Yield: 72.0 mg, 0.174 mmol, 37 %, as a clear pale oil. **1H NMR** (500 MHz, DMSO-d6) δ 8.41 (br. s., 1H), 7.46 (d, J = 7.57 Hz, 1H), 4.07 (d, J = 7.33 Hz, 2H), 2.43 (spt, J = 7.00 Hz, 1H), 1.52 - 1.64 (m, 6H), 1.41 - 1.53 (m, 5H), 1.23 - 1.33 (m, 2H), 0.85 (t, J = 7.29 Hz, 6H). **13C NMR** (126 MHz, DMSO-d6) δ 161.8 (C), 155.1 (CH), 153.7 (2 x C), 91.7 (C), 53.4 (CH2), 50.1 (CH), 29.5 (4 x CH2), 26.4 (CH), 24.4 (2 x CH2), 10.5 (2 x CH3). **LRMS** (ESI +ve) [M+H] 414.2.

N-benzyl-1-(cyclopentylmethyl)-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-6-amine (12f). Yield: 106.6 mg, 0.246 mmol, 43 %, as a pale pink fluffy solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.47 (br. s., 1H), 8.28 (br. s., 1H), 7.34 (br. s., 2H), 7.28 (t, J = 7.53 Hz, 2H), 7.17 - 7.22 (m, 1H), 4.51 (br. s., 2H), 4.06 (d, J = 7.41 Hz, 2H), 2.29 - 2.40 (m, 1H), 1.37 - 1.61 (m, 6H), 1.10 - 1.28 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 161.4 (C), 153.9 (2 x C, CH), 128.0 (2 x CH), 127.4

(C), 126.5 (3 x CH), 91.8 (C), 50.4 (CH2), 44.3 (CH2), 33.9 (CH), 29.5 (2 x CH2), 24.5 (2 x CH2). **LRMS** (ESI +ve) [M+H] 434.00.

N-(tert-butyl)-1-(cyclopentylmethyl)-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-6-amine (12g). Yield: 156.6 mg, 0.392 mmol, 68 %, as a golden solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.44 (s, 1H), 7.24 (br. s., 1H), 4.10 (d, J = 7.33 Hz, 2H), 2.44 (quin, J = 7.20 Hz, 1H), 1.54 - 1.62 (m, 4H), 1.45 - 1.53 (m, 2H), 1.42 (s, 9H), 1.21 - 1.33 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 160.9 (C), 154.7 (CH), 153.4 (C), 111.3 (C), 91.7 (C), 50.5 (CH2), 50.3 (C), 29.5 (4 x CH2), 28.3 (CH), 24.5 (3 x CH3). **LRMS** (ESI +ve) [M+H] 399.90.

General Synthesis of Final Compounds B26-32. 1-(cyclopentylmethyl)-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-6-amine core (0.150 - 0.300 mmol) was added to a 5 mL microwave vial equipped with a stirrer bar. To the vial were added N,N-dimethyl-1-(prop-2-yn-1-yl)piperidin-3-amine (1.2 eq.), bis(triphenylphosphine)palladium (II) dichloride (6 mol%) and copper (I) iodide (15 mol%). The reagents were suspended in THF (4 mL) and stirred to a homogeneous solution. Triethylamine (2.0 eq.) was added, and the vial was sealed with septum cap and placed into a microwave reactor. The reaction was heated to and stirred at 70 °C for 2 hours under microwave irradiation, and the reaction was cooled while stirring at room temperature overnight. The reaction was portioned between water (40 mL) and EtOAc (50 mL). The organic layer was collected, and the aqueous layer was extracted with further EtOAc (4 x 50 mL). The organic extracts were combined and washed with water (100 mL) and brine (100 mL). The resultant phase was dried over $MgSO_4$ and concentrated in vacuo to give the crude product. The crude product was purified by preparative-TLC on silica using a 10 % MeOH/DCM eluent system. The appropriate dual active band by UV and KMnO4 visualization was scraped from the plate and the silica was washed with 20 % MeOH/DCM solution. The filtrate was collected and concentrated in vacuo to give the product.

1-(cyclopentylmethyl)-3-(3-(3-(dimethylamino)piperidin-1-yl)prop-1-yn-1-yl)-N-ethyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B26). Yield: 28.6 mg, 0.070 mmol, 26 %, as a golden solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.69 (br. s., 1H), 7.61 (br. s, 1H), 4.09 (d, J = 7.09 Hz, 2H), 3.61 (dd, J = 17.10, 22.54 Hz, 2H), 2.97 (td, J = 1.64, 10.36 Hz, 1H), 2.77 (d, J = 10.80 Hz, 1H), 2.45 (quin, J = 7.20 Hz, 1H), 2.31 (br. s., 1H), 2.22 (s, 6H), 2.20 (d, J = 5.52 Hz, 2H), 2.04 - 2.16 (m, 2H), 1.76 - 1.83 (m, 1H), 1.71 (qd, J = 3.23, 9.86 Hz, 1H), 1.54 - 1.64 (m, 4H), 1.47 - 1.54 (m, 2H), 1.40 - 1.47 (m, 1H), 1.22 - 1.34 (m, 2H), 1.13 - 1.19 (m, 3H), 1.06 - 1.13 (m, 1H). **13C NMR** (126 MHz, DMSO-d6) δ 161.0 (C), 154.5 (CH), 152.7 (C), 126.7 (C), 107.8 (C), 89.4 (C), 76.1 (C), 60.9 (2 x CH2), 54.7 (CH2), 51.9 (CH2), 50.1 (CH), 47.2 (CH2), 41.7 (2 x CH2), 35.5 (CH), 29.5 (2 x CH2), 26.1 (2 x CH2), 24.4 (2 x CH3), 23.9 (CH3). **LRMS** (ESI +ve) [M+H] 410.40.

1-(cyclopentylmethyl)-3-(3-(3-(dimethylamino)piperidin-1-yl)prop-1-yn-1-yl)-N-propyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B27). Yield: 33.2 mg, 0.078 mmol, 38 %, as a pale golden solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.69 (br. s., 1H), 7.66 (br. s., 1H), 4.09 (d, J = 7.01 Hz, 2H), 3.65 (dd, J = 17.02, 23.57 Hz, 2H), 2.98 (d, J = 9.77 Hz, 1H), 2.74 (d, J = 10.80 Hz, 1H), 2.28 - 2.48 (m, 9H), 2.17 - 2.28 (m, 2H), 1.84 (d, J = 12.06 Hz, 1H), 1.74 (td, J = 3.54, 13.10 Hz, 1H), 1.54 - 1.66 (m, 6H), 1.41 - 1.53 (m, 4H), 1.22 - 1.34 (m, 3H), 0.90 (t, J = 7.41 Hz, 3H). **13C NMR** (126 MHz, DMSO-d6) δ 161.2 (C), 154.5 (CH), 152.7 (C), 126.6 (C), 105.9 (C), 79.2 (C), 76.4 (C), 61.1 (2 x CH2), 51.7 (CH2), 50.1 (CH), 47.1 (2 x CH2), 46.4 (CH2), 42.6 (CH2), 41.2 (CH2), 29.5 (4 x CH2), 24.4 (2 x CH3), 23.3 (CH), 11.5 (CH3). **LRMS** (ESI +ve) [M+H] 424.50.

1-(cyclopentylmethyl)-3-(3-(3-(dimethylamino)piperidin-1-yl)prop-1-yn-1-yl)-N-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B28). Yield: 16.6 mg, 0.039 mmol, 28 %, as a golden-brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.72 (br. s., 1H), 7.51 (br. s., 1H), 4.10 (d, J = 7.17 Hz, 3H), 3.58 - 3.70 (m, 2H), 3.00 (d, J = 9.93 Hz, 1H), 2.76 (d, J = 10.80 Hz, 1H), 2.28 - 2.49 (m, 8H), 2.21 (d, J = 9.54 Hz, 2H), 1.86 (d, J = 9.54 Hz, 1H), 1.75 (td, J = 3.41, 13.20 Hz, 1H), 1.55 - 1.67 (m, 4H), 1.43 - 1.54 (m, 3H), 1.22 - 1.34 (m, 3H), 1.19 (d, J = 6.46 Hz, 6H). **13C NMR** (126 MHz, DMSO-d6) δ 160.4 (C), 154.5 (CH), 152.8 (C), 126.6 (C), 107.5 (C), 89.2 (C), 76.3 (C), 61.0 (2 x CH2), 51.7 (CH2), 50.0 (CH), 47.1 (2 x CH2), 41.2 (2 x CH2), 29.5 (4 x CH2), 24.4 (2 x CH3), 23.4 (CH), 21.8 (2 x CH3). **LRMS** (ESI +ve) [M+H] 424.50.

1-(cyclopentylmethyl)-3-(3-(3-(dimethylamino)piperidin-1-yl)prop-1-yn-1-yl)-N,N-dimethyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B29). Yield: 46.3 mg, 0.113 mmol, 35 %, as a brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.78 (s, 1H), 4.12 (d, J = 7.49 Hz, 2H), 3.62 (dd, J = 17.10, 23.64 Hz, 2H), 3.19 (s, 6H), 2.95 - 3.00 (m, 1H), 2.76 (d, J = 10.80 Hz, 1H), 2.45 (quin, J = 7.20 Hz, 1H), 2.34 (br. s., 1H), 2.24 (s, 6H), 2.06 - 2.17 (m, 2H), 1.80 (d, J = 9.46 Hz, 1H), 1.72 (qd, J = 3.26, 9.84 Hz, 1H), 1.54 - 1.65 (m, 4H), 1.46 - 1.53 (m, 2H), 1.40 - 1.46 (m, 1H), 1.24 - 1.33 (m, 2H), 1.13 (dq, J = 3.59, 11.94 Hz, 1H). **13C NMR** (126 MHz, DMSO-d6) δ 160.6 (C), 154.5 (CH), 152.3 (C), 126.7 (C), 107.5 (C), 89.5 (C), 76.2 (C), 60.9 (2 x CH2), 54.6 (CH), 51.9 (CH2), 50.2 (CH2), 47.2 (CH2), 41.6 (2 x CH2), 37.0 (CH2), 29.6 (2 x CH2), 24.5 (2 x CH3), 24.4 (2 x CH3), 23.9 (CH). **LRMS** (ESI +ve) [M+H] 410.40.

1-(cyclopentylmethyl)-3-(3-(3-(dimethylamino)piperidin-1-yl)prop-1-yn-1-yl)-N-(pentan-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B30). Yield: 43.8 mg, 0.096 mmol, 59 %, as a golden-brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.67 (br. s., 1H), 7.43 (d, J = 8.12 Hz, 1H), 4.08 (d, J = 7.33 Hz, 2H), 3.80 (br. s., 1H), 3.62 (dd, J = 17.18, 19.78 Hz, 2H), 2.97 (d, J = 8.91 Hz, 1H), 2.76 (d, J = 10.80 Hz, 1H), 2.44 (td, J = 7.09, 14.19 Hz, 2H), 2.28 (br. s., 6H),

2.09 - 2.19 (m, 2H), 1.81 (d, J = 10.01 Hz, 1H), 1.72 (td, J = 3.24, 13.14 Hz, 1H), 1.53 - 1.64 (m, 6H), 1.41 - 1.53 (m, 5H), 1.22 - 1.33 (m, 2H), 1.16 (d, J = 9.85 Hz, 1H), 0.86 (t, J = 7.21 Hz, 6H). **13C NMR** (126 MHz, DMSO-d6) δ 160.6 (C), 154.5 (CH), 152.6 (C), 126.6 (C), 107.5 (C), 89.2 (C), 76.2 (C), 60.9 (2 x CH2), 54.4 (CH), 53.4 (CH), 51.8 (CH2), 47.2 (2 x CH2), 41.5 (2 x CH2), 29.5 (2 x CH2), 26.4 (CH2), 25.9 (2 x CH2), 24.4 (2 x CH3), 23.7 (CH), 10.5 (2 x CH3). **LRMS (ESI +ve)** [M+H] 452.50. **HRMS (ESI +ve)** [M+H] 452.3491 ([C26H42N7]+, Calc. Exact Mass: 451.3420).

N-benzyl-1-(cyclopentylmethyl)-3-(3-(3-(dimethylamino)piperidin-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B31). Yield: 35.4 mg, 0.075 mmol, 58 %, as a pale brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.72 (br. s., 1H), 8.24 (br. s., 1H), 7.35 (br. s., 2H), 7.18 - 7.31 (m, 3H), 4.51 (br. s., 2H), 4.07 (d, J = 7.41 Hz, 2H), 3.61 (dd, J = 17.18, 20.10 Hz, 2H), 2.96 (d, J = 8.91 Hz, 1H), 2.75 (d, J = 10.88 Hz, 1H), 2.36 (dd, J = 1.85, 3.67 Hz, 2H), 2.26 (br. s., 6H), 2.08 - 2.17 (m, 2H), 1.80 (d, J = 9.69 Hz, 1H), 1.72 (qd, J = 3.30, 9.79 Hz, 1H), 1.54 (br. s., 2H), 1.40 - 1.51 (m, 4H), 1.08 - 1.30 (m, 4H). **13C NMR** (126 MHz, DMSO-d6) δ 161.0 (C), 154.3 (C), 152.9 (CH), 135.9 (C), 128.0 (2 x CH), 127.4 (2 x C), 126.7 (CH), 126.5 (2 x CH), 89.4 (C), 76.1 (C), 60.9 (2 x CH2), 51.8 (CH2), 50.3 (CH), 47.2 (2 x CH2), 44.3 (CH2), 41.6 (2 x CH2), 29.5 (2 x CH2), 25.9 (CH2), 24.5 (2 x CH3), 23.7 (CH). **LRMS** (ESI +ve) [M+H] 472.50.

N-(tert-butyl)-1-(cyclopentylmethyl)-3-(3-(3-(dimethylamino)piperidin-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B32). Yield: 27.1 mg, 0.062 mmol, 54 %, as light brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.70 (s, 1H), 7.22 (br. s., 1H), 4.10 (d, J = 7.41 Hz, 2H), 3.62 (dd, J = 17.02, 22.62 Hz, 2H), 2.97 (d, J = 10.09 Hz, 1H), 2.76 (d, J = 10.72 Hz, 1H), 2.46 (quin, J = 6.80 Hz, 1H), 2.41 (br. s., 1H), 2.28 (br. s., 6H), 2.10 - 2.18 (m, 2H), 1.81 (d, J = 9.62 Hz, 1H), 1.72 (quind, J = 3.30, 13.20 Hz, 1H), 1.54 - 1.64 (m, 4H), 1.44 - 1.54 (m, 2H), 1.39 - 1.44 (m, 9H), 1.22 - 1.34 (m, 3H), 1.16 (d, J = 11.03 Hz, 1H). **13C NMR** (126 MHz, DMSO-d6) δ 160.5 (C), 154.0 (CH), 152.4 (C), 126.6 (2 x C), 89.3 (C), 76.2 (C), 60.9 (2 x CH2), 54.4 (C), 51.8 (CH2), 50.3 (CH2), 47.2 (CH2), 41.5 (CH2), 29.5 (4 x CH2), 28.3 (2 x CH3), 25.8 (CH), 24.5 (3 x CH3), 23.7 (CH). **LRMS** (ESI +ve) [M+H] 438.50.

8. Synthesis and characterization of compounds B33-39

General Synthesis of 1-substituted-1H-pyrazolo[3,4-d]pyrimidin-6-amine cores. 3-iodo-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (0.800 mmol) was added to a 20 mL microwave vial equipped with a stirrer bar. The solid was suspended in DMF (5 mL) and stirred to a homogeneous solution. Sodium hydride (1.5 eq., 60 % dispersion in mineral oil) was added portion wise, and the reaction was stirred for an hour to allow gas evolution to subside. Alkyl bromide (1.5 eq.) was added, and the vial was sealed

with septum cap and heated to 150 °C while stirring for 1 hour 20 minutes under microwave irradiation in a microwave reactor, yielding a solution, which was cooled whilst stirring overnight. The reaction was partitioned between water (20 mL) and EtOAc (40 mL). The organic layer was collected, and the aqueous layer was washed with further EtOAc (3 x 20 mL). The organic fractions were combined, washed with brine (20 mL), dried over $MgSO_4$, and concentrated to give the crude product. The crude was loaded onto a SNAP HP-Sil 10 g column and purified by normal phase chromatography using a 0-10 % MeOH/DCM eluent gradient over 12 CV. The appropriate fractions were concentrated to give the product.

1-cyclopentyl-3-iodo-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (13a). Yield: 254.8 mg, 0.742 mmol, 77 %, as a pale-yellow solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.41 (br. s., 1H), 7.59 (br. s., 1H), 5.05 (br. s., 1H), 2.86 (d, J = 4.18 Hz, 3H), 2.00 - 2.10 (m, 2H), 1.90 - 1.99 (m, 2H), 1.80 - 1.89 (m, 2H), 1.59 - 1.70 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 161.8 (C), 154.8 (2 x C), 153.7 (CH), 91.7 (C), 56.8 (2 x CH2), 31.6 (2 x CH2), 27.8 (CH), 24.2 (CH3). **LRMS** (ESI +ve) [M+H] 343.9.

1-(cyclohexylmethyl)-3-iodo-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (13b). Yield: 218.1 mg, 0.588 mmol, 70 %, as a white solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.43 (br. s., 1H), 7.60 (br. s., 1H), 4.03 (br. s., 2H), 2.85 (d, J = 3.07 Hz, 3H), 1.89 (br. s., 1H), 1.65 (dd, J = 2.56, 9.42 Hz, 2H), 1.59 (br. s., 1H), 1.51 (d, J = 11.82 Hz, 2H), 1.07 - 1.23 (m, 3H), 0.97 (q, J = 10.60 Hz, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 162.0 (C), 155.5 (2 x C), 153.7 (CH), 91.9 (C), 51.6 (CH2), 37.5 (CH), 30.0 (3 x CH2), 25.8 (2 x CH2), 25.0 (CH3). **LRMS** (ESI +ve) [M+H] 372.00.

1-cyclohexyl-3-iodo-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (13c). Yield: 66.0 mg, 0.185 mmol, 24 %, as an off-white solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.45 (br. s., 1H), 7.54 (br. s., 1H), 4.48 (br. s., 1H), 2.86 (d, J = 4.57 Hz, 3H), 1.79 - 1.93 (m, 6H), 1.68 (d, J = 13.00 Hz, 1H), 1.41 (br. s., 2H), 1.18 - 1.29 (m, 1H). **13C NMR** (126 MHz, DMSO-d6) δ 162.2 (C), 156.9 (2 x C), 154.4 (CH), 93.0 (C), 55.2 (CH), 31.7 (3 x CH2), 28.0 (2 x CH2), 25.0 (CH3). **LRMS** (ESI +ve) [M+H] 357.80.

3-iodo-N-methyl-1-(tetrahydro-2H-pyran-4-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (13d). Yield: 176.4 mg, 0.491 mmol, 58 %, as a cream solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.45 (br. s., 1H), 7.63 (br. s., 1H), 4.64 - 4.78 (m, 1H), 3.97 (dd, J = 3.94, 11.27 Hz, 2H), 3.45 - 3.55 (m, 2H), 2.87 (d, J = 4.49 Hz, 3H), 2.06 - 2.18 (m, 2H), 1.84 (d, J = 11.35 Hz, 2H). **13C**

NMR (126 MHz, DMSO-d6) δ 161.8 (C), 156.9 (CH), 154.6 (C), 153.4 (C), 93.0 (C), 66.1 (4 x CH2), 52.7 (CH), 31.7 (CH3). **LRMS** (ESI +ve) [M+H] 359.80.

3-iodo-N-methyl-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (13e). Yield: 221.7 mg, 0.617 mmol, 81 %, as a cream solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.44 (br. s., 1H), 7.62 (br. s., 1H), 4.27 (d, J = 5.99 Hz, 2H), 4.09 - 4.16 (m, 1H), 3.76 (q, J = 6.90 Hz, 1H), 3.61 (q, J = 7.38 Hz, 1H), 2.85 (d, J = 3.31 Hz, 3H), 1.87 - 1.96 (m, 1H), 1.75 - 1.87 (m, 2H), 1.69 (br. s., 1H). **13C NMR** (126 MHz, DMSO-d6) δ 162.1 (C), 155.5 (2 x C), 153.7 (CH), 92.2 (C), 76.3 (2 x CH2), 67.1 (CH2), 49.6 (CH), 28.6 (CH2), 24.8 (CH3). **LRMS** (ESI +ve) [M+H] 359.80.

1-((1,3-dioxolan-2-yl)methyl)-3-iodo-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (13f). Yield: 184.9 mg, 0.512 mmol, 82 %, as a white solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.40 – 8.49 (m, 1H), 7.66 (br. S., 1H), 5.29 (br. S., 1H), 4.29 (d, J = 2.36 Hz, 2H), 3.87 – 3.98 (m, 2H), 3.78 – 3.87 (m, 2H), 2.86 (d, J = 3.78 Hz, 3H). **13C NMR** (126 MHz, DMSO-d6) δ 162.1 (C), 155.7 (2 x C), 153.7 (CH), 100.7 (C), 93.3 (CH), 64.3 (2 x CH2), 48.2 (CH2), 27.8 (CH3). **LRMS** (ESI +ve) [M+H] 361.80.

1-benzyl-3-iodo-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (13g). Yield: 132.0 mg, 0.362 mmol, 38 %, as a white solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.46 (br. s., 1H), 7.69 (br. s., 1H), 7.31 - 7.35 (m, 2H), 7.20 - 7.30 (m, 3H), 5.40 (br. s., 2H), 2.88 (d, J = 4.49 Hz, 3H). **13C NMR** (126 MHz, DMSO-d6) δ 162.2 (C), 155.3 (2 x C), 153.8 (CH), 137.0 (CH), 128.6 (2 x CH), 127.8 (2 x CH), 127.6 (CH), 99.3 (C), 49.3 (CH2), 27.9 (CH3). **LRMS** (ESI +ve) [M+H] 365.90.

General Synthesis of Final Compounds B33-39. 3-iodo-1H-pyrazolo[3,4-d]pyrimidin-6-amine core (0.150 - 0.300 mmol) was added to a 5 mL microwave vial equipped with a stirrer bar. To the vial were added N,N-dimethyl-1-(prop-2-yn-1-yl)piperidin-3-amine (1.2 eq.), bis(triphenylphosphine)palladium (II) dichloride (6 mol%) and copper (I) iodide (15 mol%). The reagents were suspended in THF (4 mL) and stirred to a homogeneous solution. Triethylamine (2.0 eq.) was added, and the vial was sealed with septum cap and placed into a microwave reactor. The reaction was heated to and stirred at 70 °C for 2 hours under microwave irradiation, and the reaction was cooled while stirring at room temperature overnight. The reaction was portioned between water (40 mL) and EtOAc (50 mL). The organic layer was collected, and the aqueous layer was extracted with further EtOAc (4 x 50 mL). The organic extracts were combined and washed with water (100 mL) and brine (100 mL). The resultant phase was dried over $MgSO_4$ and concentrated in vacuo to give the crude product. The crude product was purified by preparative-TLC on silica using a 10 % MeOH/DCM eluent system. The appropriate dual active band

by UV and KMnO4 visualization was scraped from the plate and the silica was washed with 20 % MeOH/DCM solution. The filtrate was collected and concentrated in vacuo to give the product.

1-cyclopentyl-3-(3-(3-(dimethylamino)piperidin-1-yl)prop-1-yn-1-yl)-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B33). Yield: 42.1 mg, 0.110 mmol, 44 %, as a pale golden solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.67 (br. s., 1H), 7.55 (br. s., 1H), 5.08 (br. s., 1H), 3.60 (dd, J = 17.02, 20.10 Hz, 2H), 2.97 (td, J = 1.59, 10.38 Hz, 1H), 2.86 (d, J = 4.41 Hz, 3H), 2.77 (d, J = 10.80 Hz, 1H), 2.30 (br. s., 1H), 2.21 (s, 6H), 2.01 - 2.14 (m, 4H), 1.95 (dt, J = 6.03, 12.75 Hz, 2H), 1.86 (dq, J = 5.16, 8.20 Hz, 2H), 1.76 - 1.82 (m, 1H), 1.71 (td, J = 3.10, 12.90 Hz, 1H), 1.61 - 1.68 (m, 2H), 1.45 (tq, J = 3.95, 12.50 Hz, 1H), 1.11 (dq, J = 3.86, 12.01 Hz, 1H). **13C NMR** (126 MHz, DMSO-d6) δ 161.5 (C), 154.0 (CH), 152.6 (2 x C), 126.6 (C), 89.4 (C), 76.2 (C), 75.6 (CH), 60.9 (CH2), 56.5 (CH2), 54.8 (CH), 52.0 (CH2), 47.3 (CH2), 41.7 (2 x CH2), 31.6 (2 x CH3), 26.1 (CH2), 24.3 (CH3), 23.9 (CH2). **LRMS** (ESI +ve) [M+H] 382.50.

1-(cyclohexylmethyl)-3-(3-(3-(dimethylamino)piperidin-1-yl)prop-1-yn-1-yl)-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B34). Yield: 35.5 mg, 0.087 mmol, 38 %, as a golden solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.68 (br. s., 1H), 7.56 (br. s., 1H), 4.04 (d, J = 5.20 Hz, 2H), 3.61 (dd, J = 17.18, 19.86 Hz, 2H), 2.97 (td, J = 1.66, 10.40 Hz, 1H), 2.85 (d, J = 4.02 Hz, 3H), 2.77 (d, J = 10.80 Hz, 1H), 2.29 (br. s., 1H), 2.21 (s, 6H), 2.11 (dt, J = 1.89, 10.60 Hz, 1H), 2.07 (t, J = 10.00 Hz, 1H), 1.91 (br. s., 1H), 1.80 (d, J = 10.64 Hz, 1H), 1.71 (quind, J = 2.90, 13.16 Hz, 1H), 1.65 (dd, J = 3.03, 9.42 Hz, 2H), 1.59 (br. s., 1H), 1.50 (d, J = 11.98 Hz, 2H), 1.44 (tt, J = 3.90, 12.60 Hz, 1H), 1.05 - 1.22 (m, 4H), 0.98 (d, J = 11.82 Hz, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 161.7 (C), 154.7 (CH), 152.6 (C), 126.7 (2 x C), 89.5 (C), 76.1 (C), 60.9 (CH2), 54.8 (CH), 51.9 (CH2), 51.5 (CH2), 47.3 (CH2), 41.7 (3 x CH2), 37.4 (CH2), 30.0 (3 x CH2), 25.8 (2 x CH3), 25.0 (CH3), 23.9 (CH). **LRMS** (ESI +ve) [M+H] 410.04.

1-cyclohexyl-3-(3-(3-(dimethylamino)piperidin-1-yl)prop-1-yn-1-yl)-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B35). Yield: 17.0 mg, 0.043 mmol, 26 %, as a golden solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.68 (br. s., 1H), 7.55 (br. s, 1H), 4.50 (br. s., 1H), 3.62 (dd, J = 17.10, 23.57 Hz, 2H), 2.97 (d, J = 9.46 Hz, 1H), 2.86 (d, J = 4.57 Hz, 3H), 2.76 (d, J = 10.88 Hz, 1H), 2.28 (d, J = 12.22 Hz, 6H), 2.15 (t, J = 9.58 Hz, 2H), 1.97 - 2.06 (m, 1H), 1.86 (d, J = 4.10 Hz, 4H), 1.78 - 1.84 (m, 2H), 1.65 - 1.75 (m, 2H), 1.35 - 1.52 (m, 4H), 1.21 - 1.30 (m, 1H), 1.07 - 1.20 (m, 1H). **13C NMR** (126 MHz, DMSO-d6) δ 172.0 (C), 161.4 (C), 153.6 (CH), 152.7 (C), 126.4 (C), 89.2 (C), 79.4 (C), 76.3 (CH), 60.9 (CH2), 55.0 (CH), 51.8 (CH2), 51.6 (CH2), 47.2 (CH2), 46.4 (CH2), 41.5 (CH2), 31.6 (2 x CH2), 25.0 (CH2), 24.9 (2 x CH3), 23.7 (CH2), 21.0 (CH3). **LRMS** (ESI +ve) [M+H] 396.40.

3-(3-(3-(dimethylamino)piperidin-1-yl)prop-1-yn-1-yl)-N-methyl-1-(tetrahydro-2H-pyran-4-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B36). Yield: 40.0 mg, 0.100 mmol, 39 %, as a dark golden solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.69 (br. s., 1H), 7.58 (br. s, 1H), 4.76 (br. s., 1H), 3.98 (dd, J = 3.98, 11.07 Hz, 2H), 3.60 (dd, J = 17.18, 22.62 Hz, 2H), 3.51 (t, J = 11.43 Hz, 2H), 2.97 (td, J = 1.68, 10.34 Hz, 1H), 2.87 (d, J = 4.65 Hz, 3H), 2.77 (d, J = 10.80 Hz, 1H), 2.26 (tt, J = 3.60, 10.60 Hz, 1H), 2.16 - 2.20 (m, 6H), 2.09 - 2.15 (m, 2H), 2.02 - 2.08 (m, 1H), 1.89 - 2.00 (m, 1H), 1.85 (d, J = 11.66 Hz, 2H), 1.79 (d, J = 12.69 Hz, 1H), 1.63 - 1.75 (m, 1H), 1.45 (tq, J = 3.98, 12.69 Hz, 1H), 1.08 (dquin, J = 4.02, 12.30 Hz, 1H). **13C NMR** (126 MHz, DMSO-d6) δ 161.5 (C), 153.8 (2 x C), 152.7 (CH), 126.7 (C), 89.6 (C), 79.5 (C), 76.1 (CH), 66.1 (CH2), 60.8 (CH2), 54.9 (CH2), 52.5 (CH2), 47.3 (CH2), 46.5 (CH), 41.8 (2 x CH2), 31.5 (2 x CH2), 26.2 (2 x CH3), 24.0 (CH3). **LRMS** (ESI +ve) [M+H] 398.40.

3-(3-(3-(dimethylamino)piperidin-1-yl)prop-1-yn-1-yl)-N-methyl-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B37). Yield: 40.6 mg, 0.102 mmol, 43 %, as a golden solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.69 (br. s., 1H), 7.58 (br. s., 1H), 4.28 (d, J = 5.99 Hz, 2H), 4.12 (q, J = 7.96 Hz, 1H), 3.76 (q, J = 7.10 Hz, 1H), 3.57 - 3.66 (m, 3H), 2.97 (dd, J = 1.54, 8.87 Hz, 1H), 2.86 (d, J = 3.78 Hz, 3H), 2.76 (d, J = 10.88 Hz, 1H), 2.34 (br. s., 1H), 2.18 - 2.28 (m, 6H), 2.11 (dq, J = 2.13, 11.80 Hz, 2H), 1.88 - 1.95 (m, 1H), 1.76 - 1.87 (m, 3H), 1.64 - 1.75 (m, 2H), 1.46 (tq, J = 3.88, 12.41 Hz, 1H), 1.13 (dq, J = 2.17, 11.30 Hz, 1H). **13C NMR** (126 MHz, DMSO-d6) δ 161.7 (C), 154.8 (2 x C), 152.6 (CH), 126.9 (C), 89.5 (C), 76.2 (C), 67.1 (CH2), 60.9 (CH2), 54.6 (CH), 51.9 (CH2), 49.5 (CH), 47.2 (CH2), 41.6 (2 x CH2), 28.6 (2 x CH3), 27.8 (CH2), 26.0 (CH2), 24.8 (CH3), 23.9 (CH2). **LRMS** (ESI +ve) [M+H] 398.40.

1-((1,3-dioxolan-2-yl)methyl)-3-(3-(3-(dimethylamino)piperidin-1-yl)prop-1-yn-1-yl)-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B38). Yield: 37.1 mg, 0.093 mmol, 36 %, as a cream solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.70 (br. s., 1H), 7.62 (br. s., 1H), 5.30 (br. s., 1H), 4.30 (d, J = 3.94 Hz, 2H), 3.94 (br. s., 2H), 3.78 - 3.86 (m, 2H), 3.62 (dd, J = 17.00, 20.41 Hz, 2H), 2.97 (td, J = 1.60, 10.36 Hz, 1H), 2.86 (d, J = 3.31 Hz, 3H), 2.76 (d, J = 10.80 Hz, 1H), 2.33 (br. s., 1H), 2.23 (br. s., 6H), 2.11 (q, J = 11.64 Hz, 2H), 1.80 (d, J = 9.77 Hz, 1H), 1.72 (qd, J = 3.24, 9.83 Hz, 1H), 1.46 (tq, J = 3.86, 12.43 Hz, 1H), 1.13 (q, J = 11.00 Hz, 1H). **13C NMR** (126 MHz, DMSO-d6) δ 172.7 (C), 161.7 (C), 155.0 (CH), 152.7 (C), 127.3 (C), 100.6 (CH), 89.6 (C), 76.0 (C), 64.3 (CH2), 60.9 (CH2), 54.6 (C), 51.9 (CH2), 48.1 (CH2), 47.2 (CH2), 41.7 (2 x CH2), 27.8 (CH2), 26.0 (2 x CH3), 23.9 (CH3). **LRMS** (ESI +ve) [M+H] 400.30.

1-benzyl-3-(3-(3-(dimethylamino)piperidin-1-yl)prop-1-yn-1-yl)-N-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B39). Yield: 26.8 mg, 0.066 mmol, 30 %, as a white solid.

1H NMR (500 MHz, DMSO-d6) δ 8.71 (br. s., 1H), 7.65 (br. s., 1H), 7.17 - 7.36 (m, 5H), 5.40 (br. s., 2H), 3.61 (dd, J = 17.42, 21.04 Hz, 2H), 2.95 (d, J = 10.17 Hz, 1H), 2.88 (d, J = 4.57 Hz, 3H), 2.74 (d, J = 10.96 Hz, 1H), 2.31 - 2.41 (m, J = 1.90, 1.90, 3.70 Hz, 1H), 2.25 (br. s., 6H), 2.06 - 2.16 (m, 2H), 1.80 (d, J = 10.09 Hz, 1H), 1.71 (qd, J = 3.31, 9.86 Hz, 1H), 1.45 (tq, J = 3.94, 12.32 Hz, 1H), 1.15 (br. s., 1H). **13C NMR** (126 MHz, DMSO-d6) δ 161.8 (C), 154.5 (CH), 152.8 (C), 136.8 (C), 128.6 (4 x CH), 127.8 (C), 127.6 (CH), 100.5 (C), 89.7 (C), 76.0 (C), 60.9 (CH2), 54.5 (CH), 51.8 (CH2), 49.3 (CH2), 47.2 (CH2), 41.6 (2 x CH2), 28.0 (2 x CH3), 25.9 (CH3). **LRMS** (ESI +ve) [M+H] 404.40.

9. Synthesis of Combinatorial Library of the B Core (B40-B63)

General Synthesis of 1H-pyrazolo[3,4-d]pyrimidin-6-amine cores. Synthesized by the methods described above in Section 7.

General Synthesis of 1-(substituted)-1H-pyrazolo[3,4-d]pyrimidin-6-amine cores. 3-iodo-1H-pyrazolo[3,4-d]pyrimidin-6-amine core (1.00 mmol) was added to a 20 mL microwave vial equipped with a stirrer bar. The solid was suspended in DMF (10 mL) and stirred to a homogeneous solution. Sodium hydride (1.5 eq., 60 % dispersion in mineral oil) was added, and the reaction was stirred for 30 minutes for gas evolution to subside. Bromo cycloalkane (1.5 eq.) was added, and the vial was sealed with a septum cap and placed into a microwave reactor. The reaction was heated to and stirred at 150 °C for 90 minutes under microwave irradiation, and the reaction was cooled whilst stirring overnight. The reaction was partitioned between water (50 mL) and EtOAc (50 mL). The organic layer was collected, and the aqueous layer was washed with further EtOAc (4 x 50 mL). The organic fractions were collected, combined, washed with water (100 mL) and brine (100 mL). The resultant organic layer was dried over $MgSO_4$ and concentrated in vacuo to give the crude product. The crude was purified by flash column chromatography on silica using a 0-8 % MeOH/DCM eluent system. The appropriate fractions by TLC were combined and concentrated to give the product.

1-cyclopentyl-3-iodo-N-propyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (14a). Yield: 292.7 mg, 0.788 mmol, 81 %, as a white solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.41 (br. s., 1H), 7.67 (br. s., 1H), 5.01 (br. s., 1H), 3.28 (d, J = 4.10 Hz, 2H), 2.04 (br. s., 2H), 1.90 - 2.00 (m, 2H), 1.79 - 1.90 (m, 2H), 1.62 - 1.71 (m, 2H), 1.51 - 1.61 (m, 2H), 0.87 - 0.93 (m, 3H). **13C NMR** (126 MHz, DMSO-d6) δ 161.4 (C), 157.2 (CH), 153.7 (2 x C), 91.6 (C), 42.6 (CH2), 32.7 (CH), 31.5 (4 x CH2), 24.2 (CH2), 11.5 (CH3). **LRMS** (ESI +ve) [M+H] 371.90.

1-(cyclohexylmethyl)-3-iodo-N-propyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (14b). Yield: 219.5 mg, 0.550 mmol, 58 %, as an off-white solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.43 (br. s., 1H), 7.68 (br. s., 1H), 4.02 (br. s., 2H), 3.28 (br. s., 2H), 1.90 (d, J = 11.03 Hz, 1H), 1.65 (dd,

J = 3.19, 9.81 Hz, 2H), 1.57 (d, J = 6.78 Hz, 3H), 1.51 (d, J = 12.06 Hz, 2H), 1.10 - 1.23 (m, 3H), 0.96 (d, J = 11.51 Hz, 2H), 0.90 (t, J = 7.33 Hz, 3H). **13C NMR** (126 MHz, DMSO-d6) δ 161.7 (C), 153.9 (C), 148.4 (CH), 111.9 (C), 91.3 (C), 51.6 (CH2), 37.6 (CH), 30.0 (3 x CH2), 25.8 (CH2), 25.0 (3 x CH2), 10.8 (CH3). **LRMS** (ESI +ve) [M+H] 400.00.

1-cyclohexyl-3-iodo-N-propyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (14c). Yield: 18.9 mg, 0.049 mmol, 5 %, as a cream solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.42 (br. s., 1H), 7.65 (br. s., 1H), 4.44 (br. s., 1H), 3.26 - 3.30 (m, 2H), 1.79 - 1.95 (m, 6H), 1.68 (d, J = 12.69 Hz, 1H), 1.57 (br. s., 2H), 1.40 (br. s., 2H), 1.17 - 1.28 (m, 1H), 0.86 - 0.94 (m, 3H). **13C NMR** (126 MHz, DMSO-d6) δ 161.3 (C), 154.3 (CH), 153.8 (C), 153.7 (C), 99.2 (C), 55.6 (CH), 42.7 (CH2), 34.6 (CH2), 31.6 (CH2), 25.0 (CH2), 24.9 (2 x CH2), 21.7 (CH2), 11.5 (CH3). **LRMS** (ESI +ve) [M+H] 386.00.

1-cyclopentyl-3-iodo-N-(pentan-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (14d). Yield: 205.1 mg, 0.514 mmol, 53 %, as a pale-cream solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.40 (br. s., 1H), 7.46 (d, J = 7.41 Hz, 1H), 4.98 (quin, J = 7.41 Hz, 1H), 3.82 (br. s., 1H), 1.91 - 2.09 (m, 4H), 1.81 - 1.90 (m, 2H), 1.62 - 1.71 (m, 2H), 1.42 - 1.61 (m, 4H), 0.86 (t, J = 7.29 Hz, 6H). **13C NMR** (126 MHz, DMSO-d6) δ 161.5 (2 x C), 153.7 (CH), 112.0 (C), 91.4 (C), 57.4 (CH), 53.3 (CH), 31.4 (2 x CH2), 26.2 (2 x CH2), 24.2 (2 x CH2), 10.4 (2 x CH3). **LRMS** (ESI +ve) [M+H] 399.90.

1-(cyclohexylmethyl)-3-iodo-N-(pentan-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (14e). Yield: 255.1 mg, 0.597 mmol, 60 %, as a cream solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.41 (br. s., 1H), 7.46 (d, J = 7.49 Hz, 1H), 4.01 (d, J = 6.31 Hz, 2H), 3.80 (br. s., 1H), 1.88 (ttd, J = 3.48, 7.26, 14.47 Hz, 1H), 1.64 (d, J = 6.54 Hz, 2H), 1.44 - 1.60 (m, 7H), 1.07 - 1.22 (m, 3H), 0.91 - 1.02 (m, 2H), 0.85 (t, J = 7.21 Hz, 6H). **13C NMR** (126 MHz, DMSO-d6) δ 161.7 (C), 161.5 (C), 153.7 (CH), 111.9 (C), 91.3 (C), 53.4 (CH2), 51.7 (CH), 37.6 (CH), 30.1 (2 x CH2), 26.4 (CH2), 25.8 (2 x CH2), 25.0 (2 x CH2), 10.5 (2 x CH3). **LRMS** (ESI +ve) [M+H] 428.00.

1-cyclohexyl-3-iodo-N-(pentan-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (14f). Yield: 27.0 mg, 0.065 mmol, 7 %, as a white solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.37 - 8.49 (m, 1H), 7.45 (d, J = 7.96 Hz, 1H), 4.37 - 4.47 (m, 1H), 3.78 (br. s., 1H), 1.78 - 1.95 (m, 6H), 1.68 (d, J = 13.16 Hz, 1H), 1.53 (d, J = 7.17 Hz, 4H), 1.39 (br. s., 2H), 1.15 - 1.28 (m, 1H), 0.86 (d, J = 6.23 Hz, 6H). **13C NMR** (126 MHz, DMSO-d6) δ 161.7 (C), 161.5 (C), 153.7 (CH), 111.9 (C), 91.3 (C), 55.9 (CH), 53.4 (CH), 31.7 (CH2), 31.4 (2 x CH2), 26.3 (CH2), 25.0 (2 x CH2), 24.9 (2 x CH2), 10.5 (2 x CH3). **LRMS** (ESI +ve) [M+H] 414.00.

N-benzyl-1-cyclopentyl-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-6-amine (14g). Yield: 180.0 mg, 0.429 mmol, 50 %, as a cream solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.45 (br. s., 1H), 8.23 (br. s., 1H), 7.35 (br. s., 2H), 7.29 (t, J = 7.53 Hz, 2H), 7.17 - 7.24 (m, 1H), 4.99 (quin, J = 7.41 Hz, 1H), 4.54 (br. s., 2H), 2.00 (br. s., 2H), 1.88 - 1.96 (m, 2H), 1.82 (br. s., 2H), 1.57 - 1.70 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 153.9 (2 x C), 148.4 (CH), 128.1 (3 x CH), 127.4 (2 x CH), 126.6 (CH), 113.0 (C), 101.9 (C), 44.3 (CH2), 31.4 (CH), 30.8 (2 x CH2), 24.1 (2 x CH2). **LRMS** (ESI +ve) [M+H] 419.90.

N-benzyl-1-(cyclohexylmethyl)-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-6-amine (14h). Yield: 175.1 mg, 0.391 mmol, 44 %, as a white solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.47 (br. s., 1H), 8.28 (br. s., 1H), 7.35 (br. s., 1H), 7.26 - 7.31 (m, 3H), 7.17 - 7.23 (m, 1H), 4.52 (br. s, 2H), 3.99 (d, J = 7.09 Hz, 2H), 1.78 (br. s., 1H), 1.53 - 1.67 (m, 2H), 1.35 - 1.51 (m, 2H), 1.09 (br. s., 4H), 0.80 - 0.99 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 153.9 (C), 152.9 (CH), 143.2 (C), 128.2 (2 x CH), 128.1 (C), 127.5 (2 x CH), 126.9 (CH), 126.5 (C), 99.2 (C), 51.8 (CH2), 44.4 (CH2), 37.5 (CH), 33.9 (CH2), 30.0 (CH2), 25.7 (CH2), 25.0 (2 x CH2). **LRMS** (ESI +ve) [M+H] 447.90.

N-benzyl-1-cyclohexyl-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-6-amine (14i). Yield: 145.5 mg, 0.336 mmol, 36 %, as a cream solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.42 - 8.49 (m, 1H), 8.24 (br. s., 1H), 7.37 (br. s., 1H), 7.26 - 7.32 (m, 3H), 7.17 - 7.23 (m, 1H), 4.53 (d, J = 6.23 Hz, 2H), 4.35 - 4.46 (m, 1H), 1.75 - 1.88 (m, 6H), 1.67 (d, J = 12.93 Hz, 1H), 1.34 - 1.46 (m, 2H), 1.16 - 1.28 (m, 1H). **13C NMR** (126 MHz, DMSO-d6) δ 161.7 (C), 153.9 (CH), 128.2 (C), 128.1 (C), 127.7 (2 x CH), 126.9 (2 x CH), 126.6 (CH), 126.5 (C), 93.0 (C), 44.5 (CH2), 32.3 (CH), 31.3 (CH2), 25.0 (2 x CH2), 24.8 (2 x CH2). **LRMS** (ESI +ve) [M+H] 433.90.

N-(tert-butyl)-1-cyclopentyl-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-6-amine (14j). Yield: 253.3 mg, 0.606 mmol, 62 %, as an off-white solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.43 (s, 1H), 7.24 (br. s., 1H), 4.99 (quin, J = 7.53 Hz, 1H), 1.96 - 2.12 (m, 4H), 1.79 - 1.90 (m, 2H), 1.59 - 1.72 (m, 2H), 1.42 (s, 9H). **13C NMR** (126 MHz, DMSO-d6) δ 160.5 (C), 154.2 (CH), 153.4 (2 x C), 91.3 (C), 57.6 (C), 50.4 (CH), 31.3 (2 x CH2), 28.3 (2 x CH2), 24.1 (3 x CH3). **LRMS** (ESI +ve) [M+H] 386.00.

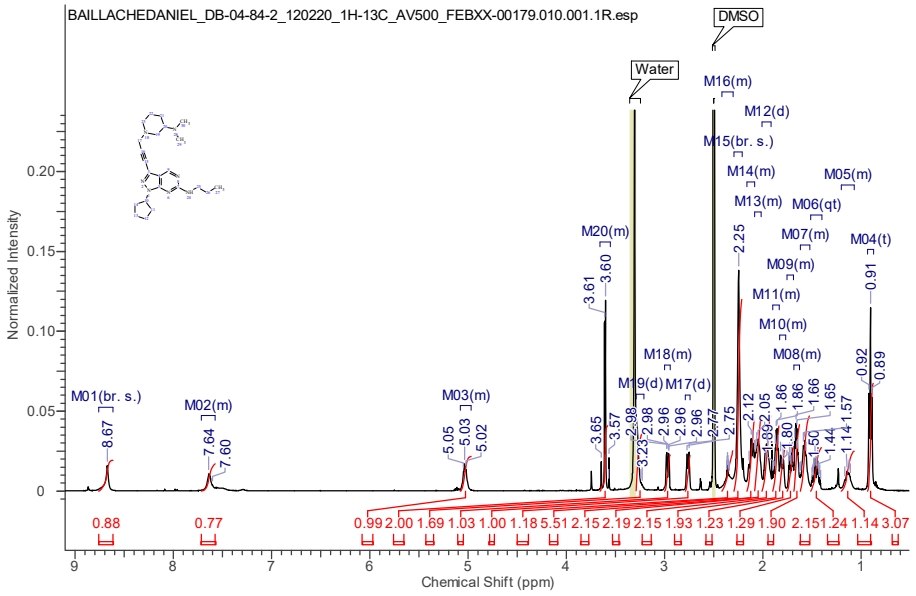
N-(tert-butyl)-1-(cyclohexylmethyl)-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-6-amine (14k). Yield: 319.3 mg, 0.773 mmol, 76 %, as a white solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.44 (s, 1H), 7.25 (br. s., 1H), 4.03 (d, J = 6.94 Hz, 2H), 1.84 - 1.94 (m, 1H), 1.61 - 1.69 (m, 2H), 1.59

(d, J = 8.59 Hz, 1H), 1.53 (d, J = 11.51 Hz, 2H), 1.42 (s, 9H), 1.09 - 1.21 (m, 3H), 0.91 - 1.02 (m, 2H). **13C NMR** (126 MHz, DMSO-d6) δ 160.8 (C), 154.9 (C), 153.4 (CH), 153.2 (C), 91.7 (C), 51.9 (CH2), 50.5 (C), 37.7 (CH), 30.2 (2 x CH2), 28.3 (CH2), 25.8 (2 x CH2), 25.1 (3 x CH3). **LRMS** (ESI +ve) [M+H] 413.90.

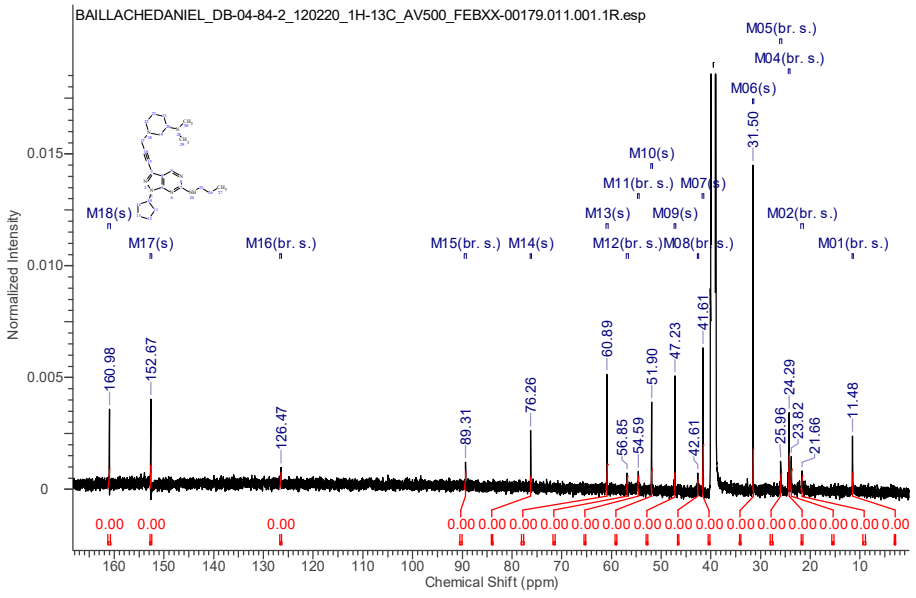
N-(tert-butyl)-1-cyclohexyl-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-6-amine (14l). Yield: 20.4 mg, 0.051 mmol, 5 %, as a white solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.43 (s, 1H), 7.23 (br. s., 1H), 4.42 (tt, J = 5.17, 10.47 Hz, 1H), 1.87 - 2.00 (m, 4H), 1.84 (d, J = 13.32 Hz, 2H), 1.68 (d, J = 12.93 Hz, 1H), 1.43 (s, 9H), 1.33 - 1.41 (m, 2H), 1.19 - 1.28 (m, 1H). **13C NMR** (126 MHz, DMSO-d6) δ 160.5 (C), 154.9 (C), 153.8 (CH), 153.4 (C), 91.8 (C), 50.4 (CH), 31.5 (2 x CH2), 28.3 (C), 25.1 (3 x CH3), 24.9 (3 x CH2). **LRMS** (ESI +ve) [M+H] 400.00.

General Synthesis of Library B Combination Molecules B40-63. 3-iodo-1H-pyrazolo[3,4-d]pyrimidin-6-amine core (0.150 - 0.300 mmol) was added to a 5 mL microwave vial equipped with a stirrer bar. To the vial were added, N-propargylated cyclic amine (1.2 eq.), bis(triphenylphosphine) palladium (II) dichloride (6 mol%) and copper (I) iodide (15 mol%). The reagents were suspended in THF (4 mL) and stirred to a homogeneous solution. Triethylamine (2.0 eq.) was added, and the vial was sealed with septum cap and placed into a microwave reactor. The reaction was heated to and stirred at 70 °C for 2 hours under microwave irradiation, and the reaction was cooled while stirring at room temperature overnight. The reaction was portioned between water (40 mL) and EtOAc (50 mL). The organic layer was collected, and the aqueous layer was extracted with further EtOAc (4 x 50 mL). The organic extracts were combined and washed with water (100 mL) and brine (100 mL). The resultant phase was dried over $MgSO_4$ and concentrated in vacuo to give the crude product. The crude product was purified by preparative-TLC on silica using a 10 % MeOH/DCM eluent system. The appropriate dual active band by UV and $KMnO_4$ visualization was scraped from the plate and the silica was washed with 20 % MeOH/DCM solution. The filtrate was collected and concentrated in vacuo to give the product.

1-cyclopentyl-3-(3-(3-(dimethylamino)piperidine-1-yl)prop-1-yn-1-yl)-N-propyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B40). Yield: 37.7 mg, 0.092 mmol, 47 %, as a golden solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.67 (br. s., 1H), 7.64 (br. s., 1H), 5.03 (t, J = 6.40 Hz, 1H), 3.61 (d, J = 5.44 Hz, 2H), 3.22 - 3.29 (m, 2H), 2.94 - 3.00 (m, 1H), 2.76 (d, J = 10.88 Hz, 1H), 2.30 - 2.43 (m, 1H), 2.25 (br. s., 6H), 2.08 - 2.17 (m, 2H), 2.01 - 2.08 (m, 2H), 1.96 (d, J = 5.28 Hz, 2H), 1.83 - 1.90 (m, 2H), 1.77 - 1.83 (m, 1H), 1.69 - 1.75 (m, 1H), 1.63 - 1.69 (m, 2H), 1.53 - 1.62 (m, 2H), 1.46 (tq, J = 3.93, 12.38 Hz, 1H), 1.07 - 1.20 (m, 1H), 0.91 (t, J = 7.37 Hz, 3H).

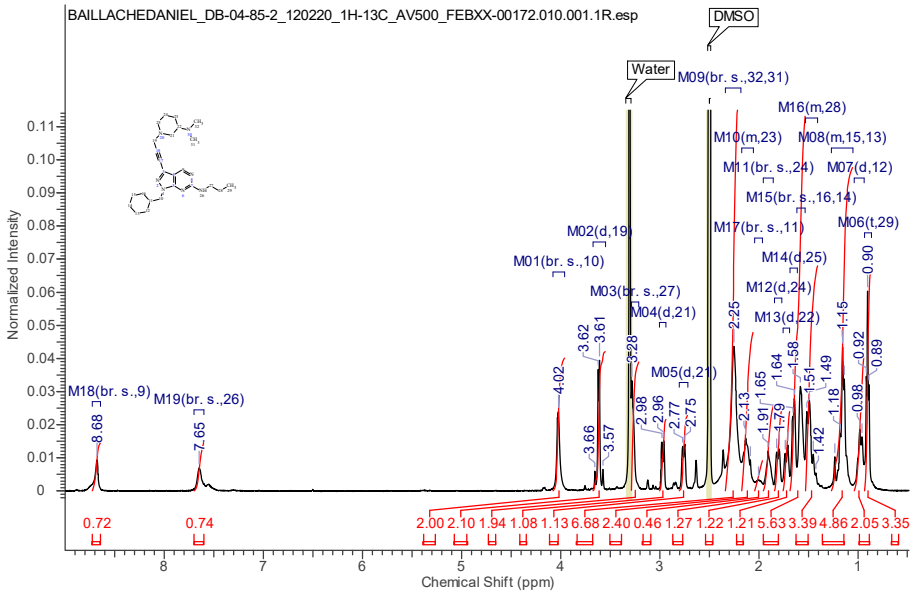


13C NMR (126 MHz, DMSO-d6) δ 161.0 (2 x C), 152.7 (2 x C), 126.5 (CH), 89.3 (C), 76.3 (C), 60.9 (CH2), 56.9 (CH), 54.6 (CH), 51.9 (CH2), 47.2 (CH2), 42.6 (CH2), 41.6 (CH2), 31.5 (3 x CH2), 26.0 (CH2), 24.3 (CH2), 23.8 (2 x CH3), 21.7 (CH2), 11.5 (CH3).

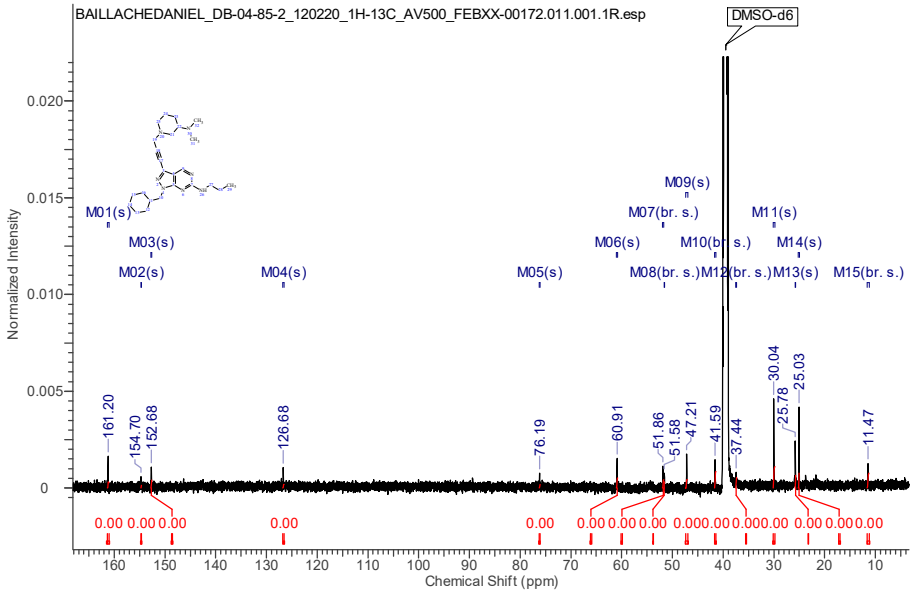


LRMS (ESI +ve) [M+H] 410.40.

1-(cyclohexylmethyl)-3-(3-(3-(dimethylamino)piperidine-1-yl)prop-1-yn-1-yl)-N-propyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B41). Yield: 18.8 mg, 0.043 mmol, 24 %, as a golden brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.68 (br. s., 1H), 7.65 (br. s., 1H), 4.02 (br. s., 2H), 3.61 (d, J = 5.60 Hz, 2H), 3.28 (br. s., 2H), 2.97 (d, J = 9.77 Hz, 1H), 2.76 (d, J = 10.72 Hz, 1H), 2.25 (br. s., 6H), 2.06 - 2.17 (m, 2H), 2.00 (br. s., 1H), 1.91 (br. s., 1H), 1.81 (d, J = 12.45 Hz, 1H), 1.72 (d, J = 13.16 Hz, 1H), 1.65 (d, J = 7.17 Hz, 2H), 1.58 (br. s., 4H), 1.41 - 1.53 (m, 2H), 1.05 - 1.27 (m, 4H), 0.97 (d, J = 11.43 Hz, 2H), 0.90 (t, J = 7.09 Hz, 3H).

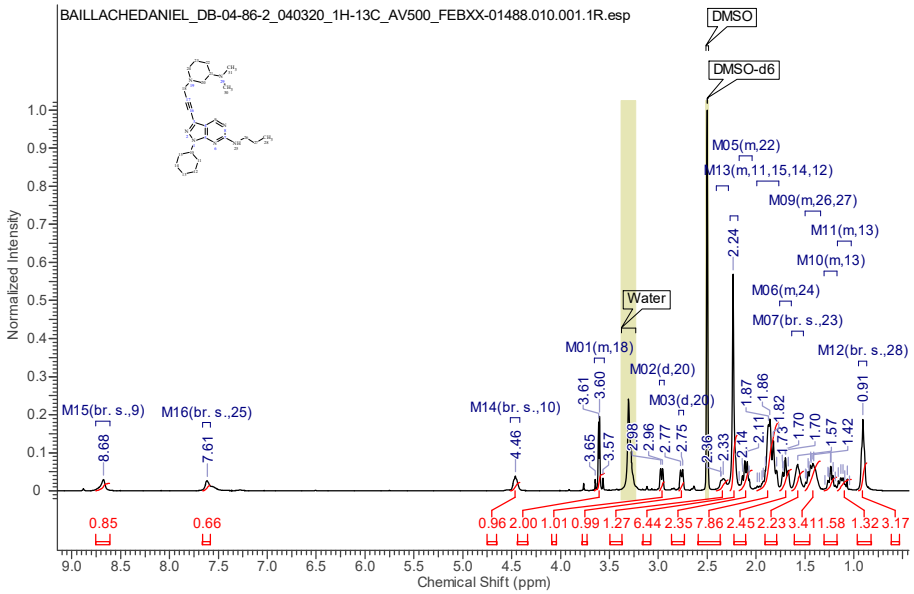


13C NMR (126 MHz, DMSO-d6) δ 161.2 (C), 154.7 (CH), 152.7 (C), 146.2 (C) 126.7 (C), 89.4 (C), 76.2 (C), 60.9 (2 x CH2), 51.9 (CH2), 51.6 (CH2), 47.2 (2 x CH2), 41.6 (CH2), 37.4 (CH), 30.0 (4 x CH2), 25.8 (2 x CH2), 25.0 (2 x CH3), 23.8 (CH), 11.5 (CH3).

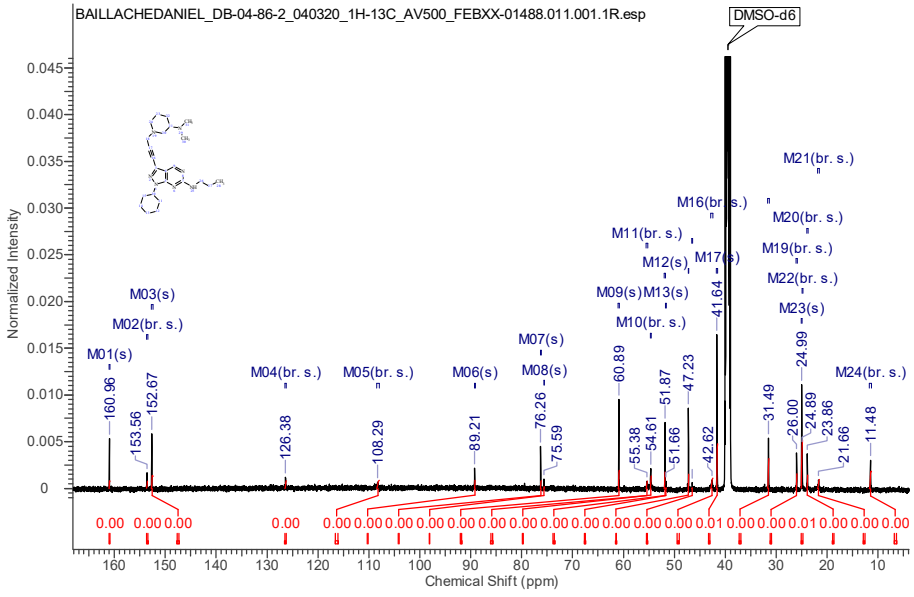


LRMS (ESI +ve) [M+H] 438.50.

1-cyclohexyl-3-(3-(3-(dimethylamino)piperidine-1-yl)prop-1-yn-1-yl)-N-propyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B42). Yield: 37.7 mg, 0.089 mmol, 42 %, as a golden solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.68 (br. s., 1H), 7.61 (br. s., 1H), 4.46 (br. s., 1H), 3.55 - 3.66 (m, 2H), 2.97 (d, J = 10.25 Hz, 1H), 2.76 (d, J = 10.72 Hz, 1H), 2.28 - 2.41 (m, 1H), 2.24 (br. s., 6H), 2.04 - 2.17 (m, 2H), 1.77 - 1.99 (m, 8H), 1.64 - 1.76 (m, 2H), 1.57 (br. s., 2H), 1.34 - 1.50 (m, 4H), 1.17 - 1.30 (m, 1H), 1.03 - 1.17 (m, 1H), 0.91 (br. s., 3H).

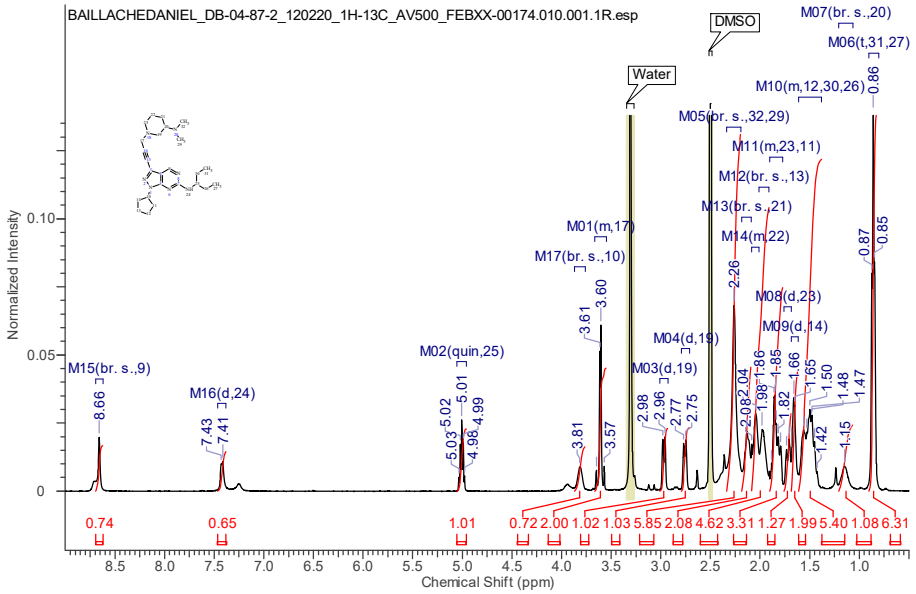


13C NMR (126 MHz, DMSO-d6) δ 161.0 (C), 153.6 (CH), 152.7 (C), 126.4 (C), 108.3 (C), 89.2 (C), 76.3 (C), 75.6 (CH), 60.9 (2 x CH2), 54.6 (CH), 51.9 (CH2), 47.2 (CH2), 46.5 (CH2), 42.6 (CH2), 41.6 (2 x CH2), 31.5 (CH2), 26.0 (CH2), 25.0 (CH2), 24.9 (2 x CH3), 23.9 (CH2), 11.5 (CH3).

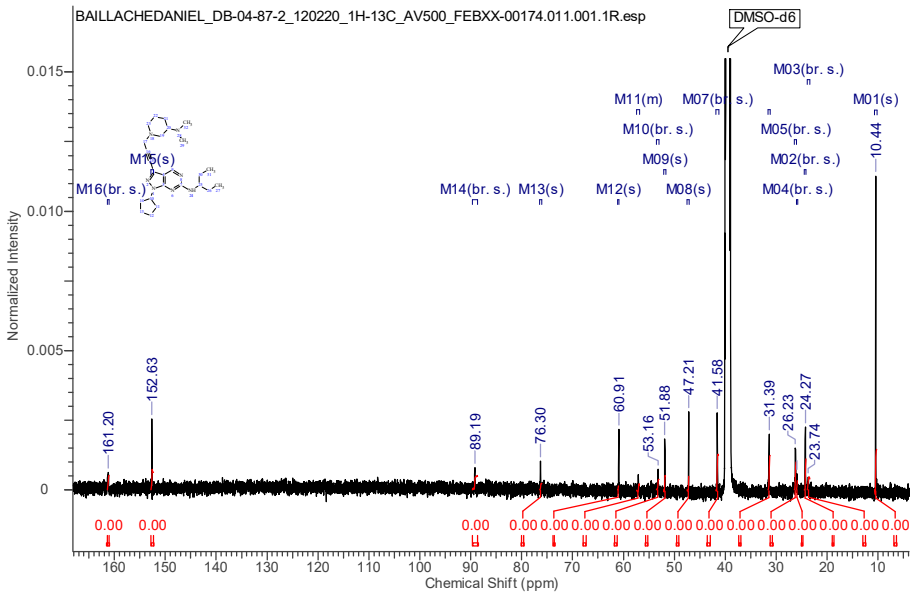


LRMS (ESI +ve) [M+H] 424.50.

1-cyclopentyl-3-(3-(3-(dimethylamino)piperidine-1-yl)prop-1-yn-1-yl)-N-(pentan-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B43). Yield: 40.8 mg, 0.093 mmol, 46 %, as a golden brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.66 (br. s., 1H), 7.42 (d, J = 7.01 Hz, 1H), 5.01 (quin, J = 7.27 Hz, 1H), 3.81 (br. s., 1H), 3.55 - 3.67 (m, 2H), 2.97 (d, J = 9.77 Hz, 1H), 2.76 (d, J = 10.64 Hz, 1H), 2.26 (br. s., 6H), 2.13 (br. s., 2H), 2.01 - 2.09 (m, 2H), 1.98 (br. s., 2H), 1.77 - 1.90 (m, 3H), 1.72 (d, J = 13.16 Hz, 1H), 1.66 (d, J = 3.94 Hz, 2H), 1.38 - 1.61 (m, 6H), 1.15 (br. s., 1H), 0.86 (t, J = 6.82 Hz, 6H).



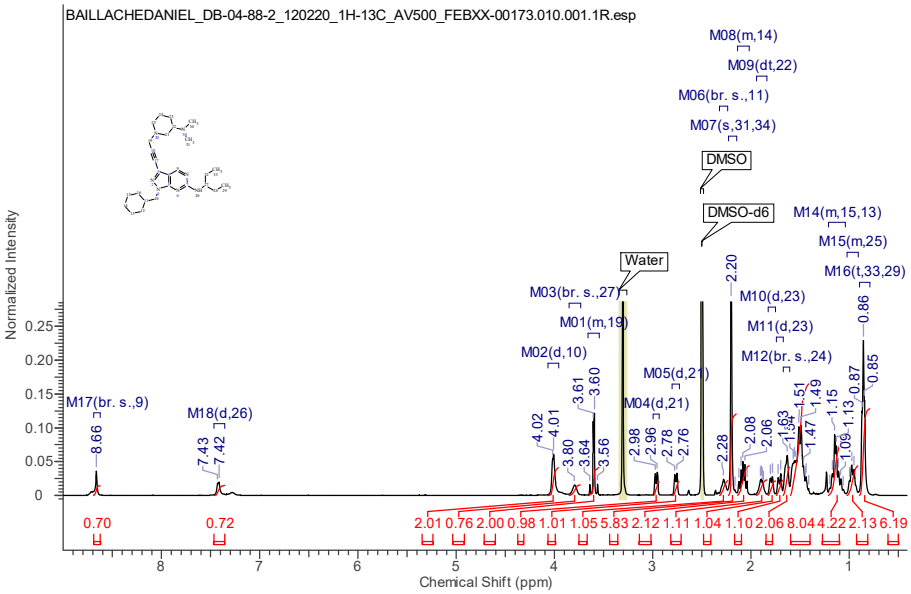
13C NMR (126 MHz, DMSO-d6) δ 161.2 (CH), 152.6 (3 x C), 108.3 (C), 89.2 (C), 76.3 (C), 60.9 (CH2), 57.1 (CH), 53.2 (2 x CH), 51.9 (CH2), 47.2 (2 x CH2), 41.6 (2 x CH2), 31.4 (CH2), 26.2 (CH2), 25.9 (CH2), 24.3 (2 x CH3), 23.7 (2 x CH2), 10.4 (2 x CH3).



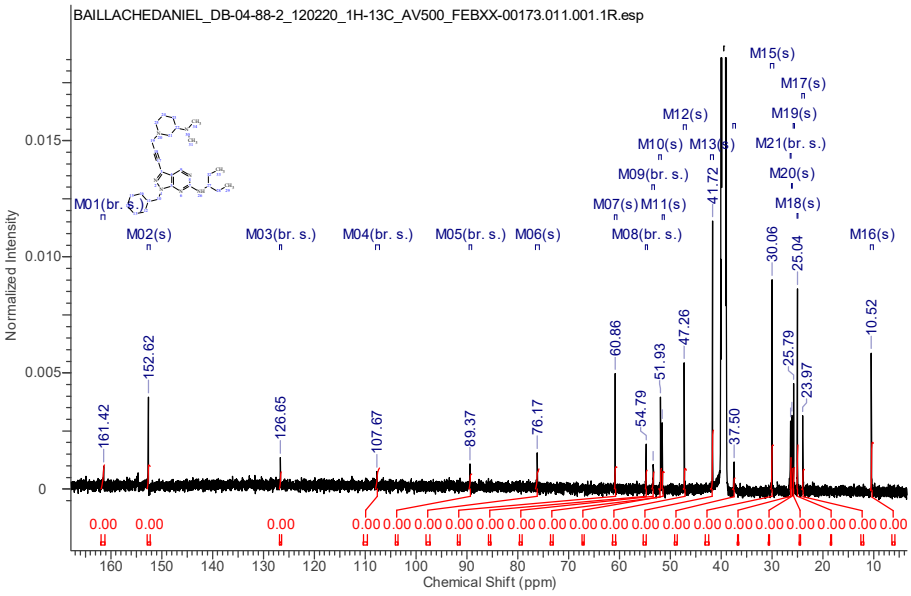
LRMS (ESI +ve) [M+H] 438.50. **HRMS** (ESI +ve) [M+H] 438.3352 ([C25H40N7]+, Calc. Exact Mass: 437.3270).

1-(cyclohexylmethyl)-3-(3-(3-(dimethylamino)piperidine-1-yl)prop-1-yn-1-yl)-N-(pentan-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B44). Yield: 25.6 mg, 0.055 mmol, 32 %, as a light brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.66 (br. s., 1H), 7.42 (d, J = 7.09 Hz, 1H), 4.02 (d, J = 5.12 Hz, 2H), 3.80 (br. s., 1H), 3.55 - 3.66 (m, 2H), 2.97 (d, J = 9.69 Hz, 1H), 2.77 (d, J = 10.64 Hz, 1H), 2.28 (br. s., 1H), 2.20 (s, 6H), 2.02 - 2.14 (m, 2H), 1.89 (td, J = 3.66, 7.01

Hz, 1H), 1.79 (d, J = 11.66 Hz, 1H), 1.71 (d, J = 13.08 Hz, 1H), 1.63 (br. s., 2H), 1.40 - 1.60 (m, 8H), 1.04 - 1.21 (m, 4H), 0.91 - 1.02 (m, 2H), 0.86 (t, J = 6.50 Hz, 6H).



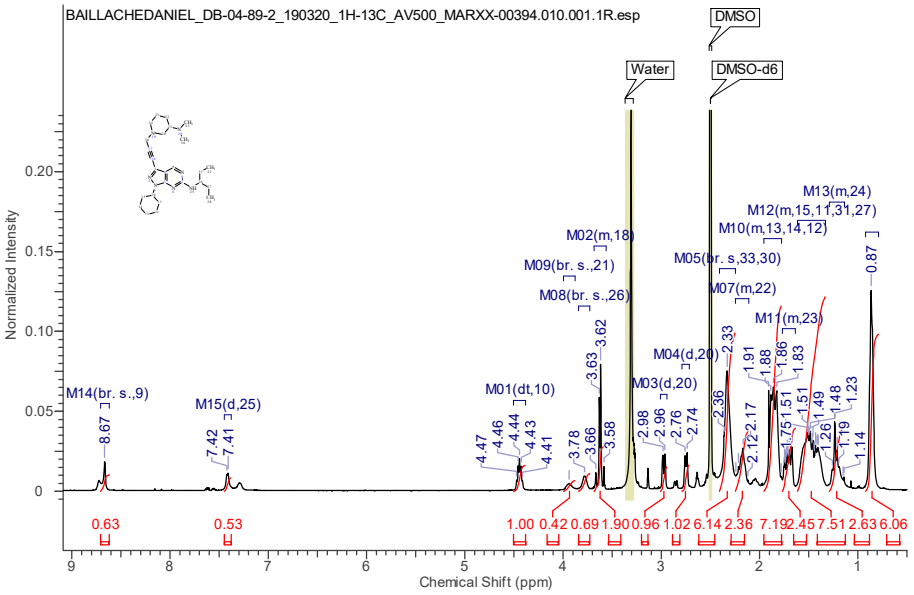
13C NMR (126 MHz, DMSO-d6) δ 161.4 (CH), 152.6 (2 x C), 126.7 (C), 107.7 (C), 89.4 (C), 76.2 (C), 60.9 (CH2), 54.8 (CH), 53.4 (CH), 51.9 (CH2), 51.6 (CH2), 47.3 (CH2), 41.7 (2 x CH2), 37.5 (CH), 30.1 (2 x CH2), 26.4 (CH2), 26.1 (CH2), 25.8 (CH2), 25.0 (2 x CH3), 24.0 (2 x CH2), 10.5 (2 x CH3).



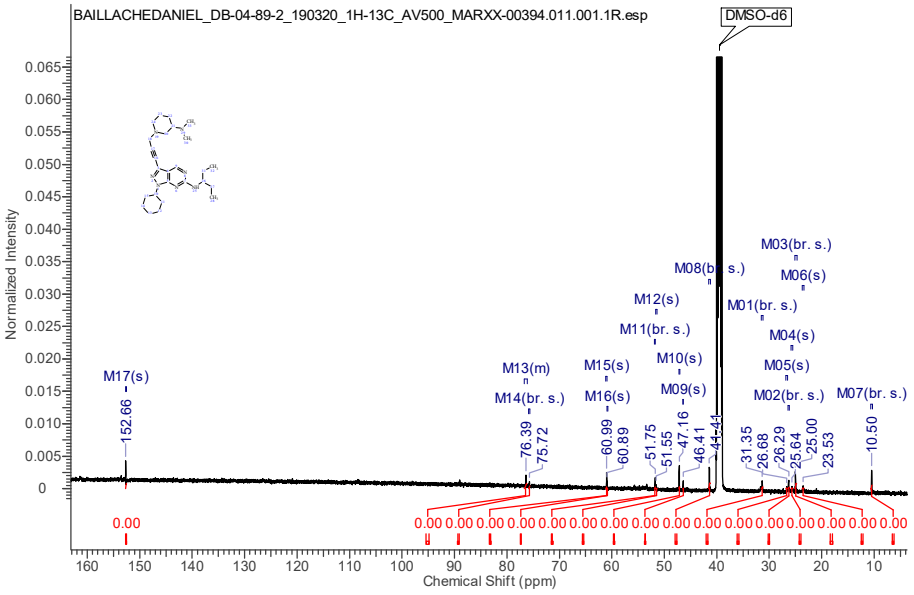
LRMS (ESI +ve) [M+H] 466.50. **HRMS** (ESI +ve) [M+H] 466.3652 ([C27H44N7]+, Calc. Exact Mass: 465.3580).

1-cyclohexyl-3-(3-(3-(dimethylamino)piperidine-1-yl)prop-1-yn-1-yl)-N-(pentan-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B45). Yield: 22.9 mg, 0.051 mmol, 32 %, as a golden brown solid.

1H NMR (500 MHz, DMSO-d6) δ 8.67 (br. s., 1H), 7.42 (d, J = 7.17 Hz, 1H), 4.44 (td, J = 7.64, 15.07 Hz, 1H), 3.94 (br. s., 1H), 3.78 (br. s., 1H), 3.56 - 3.69 (m, 2H), 2.97 (d, J = 9.22 Hz, 1H), 2.75 (d, J = 10.88 Hz, 1H), 2.35 (br. s, 6H), 2.11 - 2.24 (m, 2H), 1.78 - 1.95 (m, 6H), 1.63 - 1.77 (m, 2H), 1.33 - 1.62 (m, 8H), 1.14 - 1.29 (m, 2H), 0.87 (br. s., 6H).



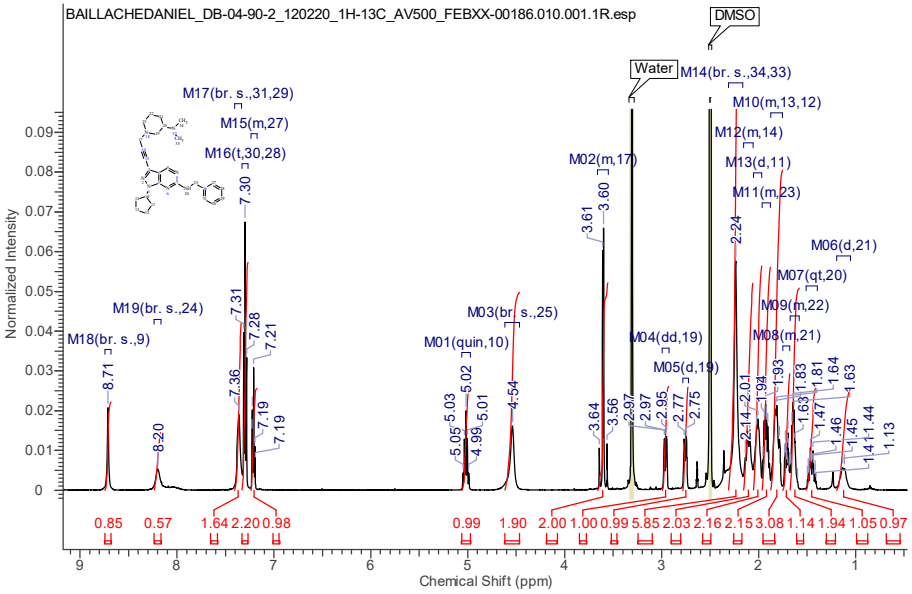
13C NMR (126 MHz, DMSO-d6) δ 172.4 (C), 161.1 (C), 153.5 (CH), 152.7 (C), 108.2 (C), 89.0 (C), 76.4 (C), 75.7 (CH), 61.0 (2 x CH2), 60.9 (CH2), 51.8 (CH2), 47.2 (2 x CH2), 46.4 (CH2), 41.4 (2 x CH2), 31.3 (CH2), 26.7 (CH2), 26.3 (CH2), 25.6 (CH2), 25.0 (2 x CH3), 23.5 (CH2), 10.5 (2 x CH3).



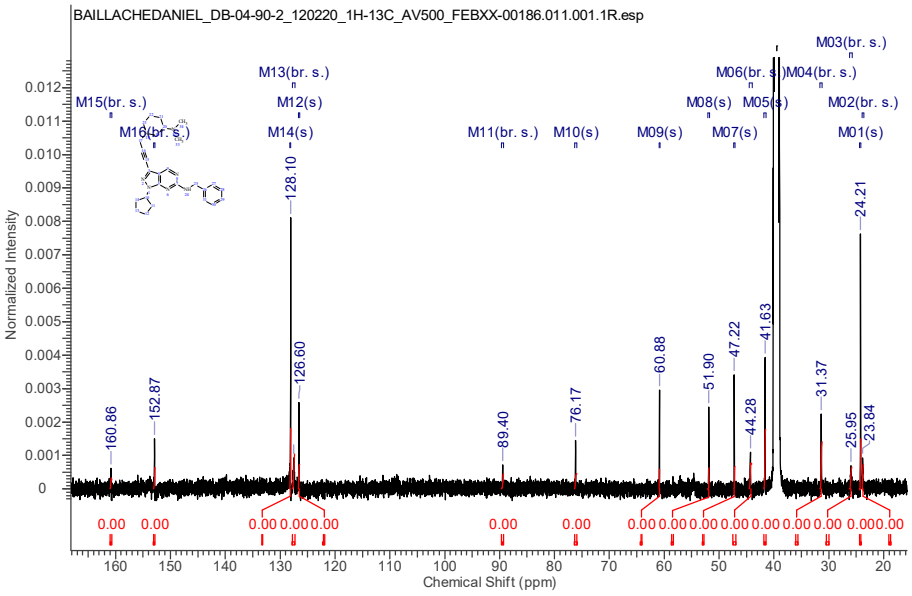
LRMS (ESI +ve) [M+H] 452.50.

N-benzyl-1-cyclopentyl-3-(3-(3-(dimethylamino)piperidine-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B46). Yield: 33.4 mg, 0.073 mmol, 38 %, as a golden brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.71 (br. s., 1H), 8.20 (br. s., 1H), 7.36 (br. s.,

2H), 7.30 (t, J = 7.57 Hz, 2H), 7.17 - 7.24 (m, 1H), 5.02 (quin, J = 7.33 Hz, 1H), 4.54 (br. s., 2H), 3.55 - 3.66 (m, 2H), 2.96 (dd, J = 1.42, 8.83 Hz, 1H), 2.76 (d, J = 10.88 Hz, 1H), 2.24 (br. s., 6H), 2.06 - 2.15 (m, 2H), 2.01 (d, J = 5.91 Hz, 2H), 1.88 - 1.96 (m, 2H), 1.75 - 1.88 (m, 4H), 1.68 - 1.75 (m, 1H), 1.58 - 1.67 (m, 2H), 1.45 (tq, J = 3.84, 12.36 Hz, 1H), 1.12 (d, J = 9.77 Hz, 1H).



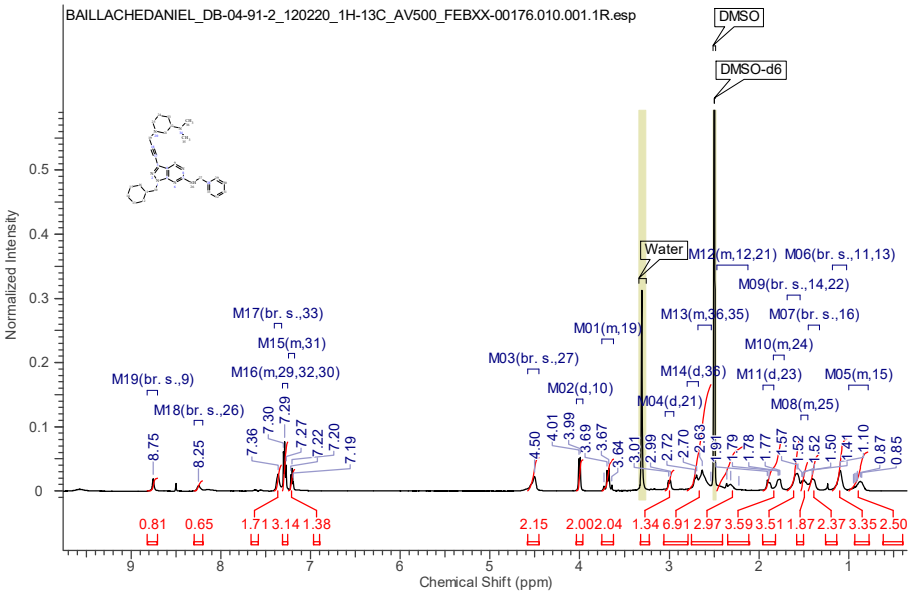
13C NMR (126 MHz, DMSO-d6) δ 160.9 (CH), 152.9 (2 x C), 139.8 (C), 128.1 (4 x CH), 127.5 (C), 126.6 (CH), 102.8 (C), 89.4 (C), 76.2 (C), 60.9 (2 x CH2), 54.6 (CH), 51.9 (CH2), 47.2 (2 x CH2), 44.3 (CH), 41.6 (2 x CH2), 31.4 (CH2), 26.0 (CH2), 24.2 (2 x CH3), 23.8 (CH2).



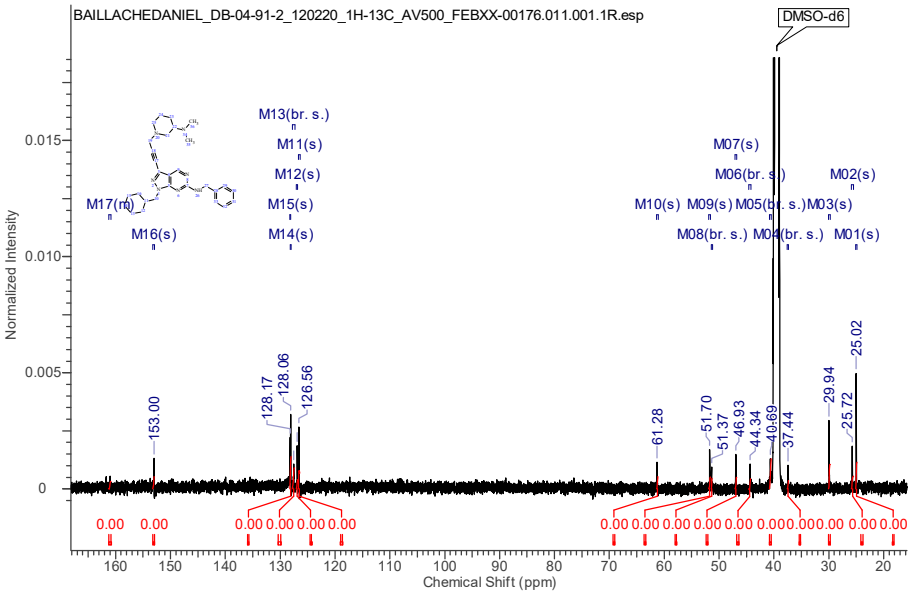
LRMS (ESI +ve) [M+H] 458.50.

N-benzyl-1-(cyclohexylmethyl)-3-(3-(3-(dimethylamino)piperidine-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B47). Yield: 24.3 mg, 0.050 mmol, 28 %, as a brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.75 (br. s., 1H), 8.25 (br. s., 1H), 7.36 (br. s., 1H), 7.26

- 7.31 (m, 3H), 7.18 - 7.24 (m, 1H), 4.50 (br. s., 2H), 4.00 (d, J = 7.01 Hz, 2H), 3.62 - 3.75 (m, 2H), 3.00 (d, J = 10.25 Hz, 1H), 2.71 (d, J = 10.72 Hz, 2H), 2.53 - 2.68 (m, 4H), 2.12 - 2.47 (m, 3H), 1.90 (d, J = 10.64 Hz, 2H), 1.72 - 1.84 (m, 2H), 1.57 (br. s., 3H), 1.46 - 1.53 (m, 2H), 1.41 (br. s., 2H), 1.10 (br. s., 3H), 0.78 - 1.00 (m, 2H).



13C NMR (126 MHz, DMSO-d6) δ 161.0 (CH), 153.0 (2 x C), 139.9 (C), 128.2 (CH), 128.1 (2 x CH), 127.5 (C), 126.9 (CH), 126.6 (2 x CH), 88.9 (C), 76.5 (C), 61.3 (CH2), 51.7 (2 x CH2), 51.4 (CH), 46.9 (CH2), 44.3 (CH2), 42.6 (CH), 40.7 (CH2), 37.4 (CH2), 29.9 (4 x CH2), 25.7 (CH2), 25.0 (2 x CH3).

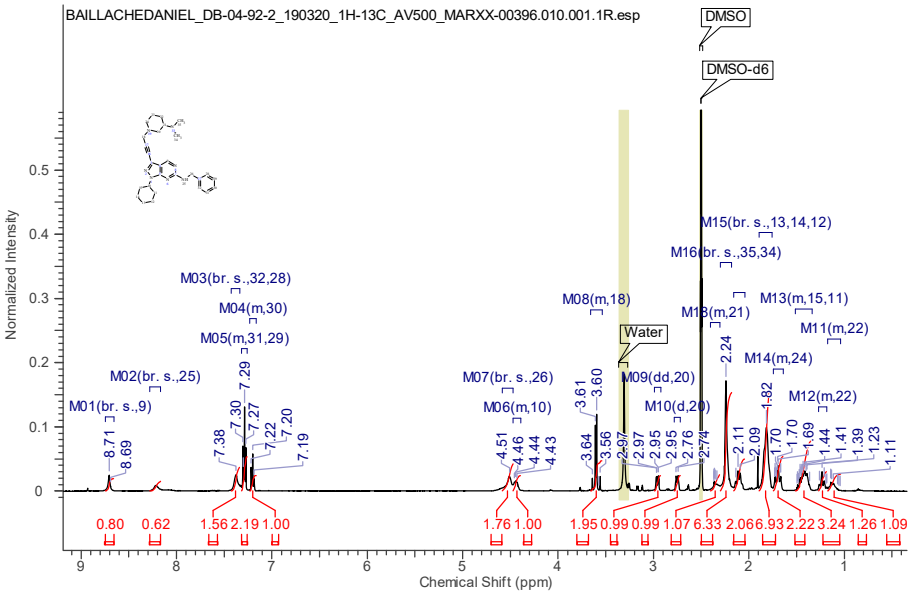


LRMS (ESI +ve) [M+H] 486.50.

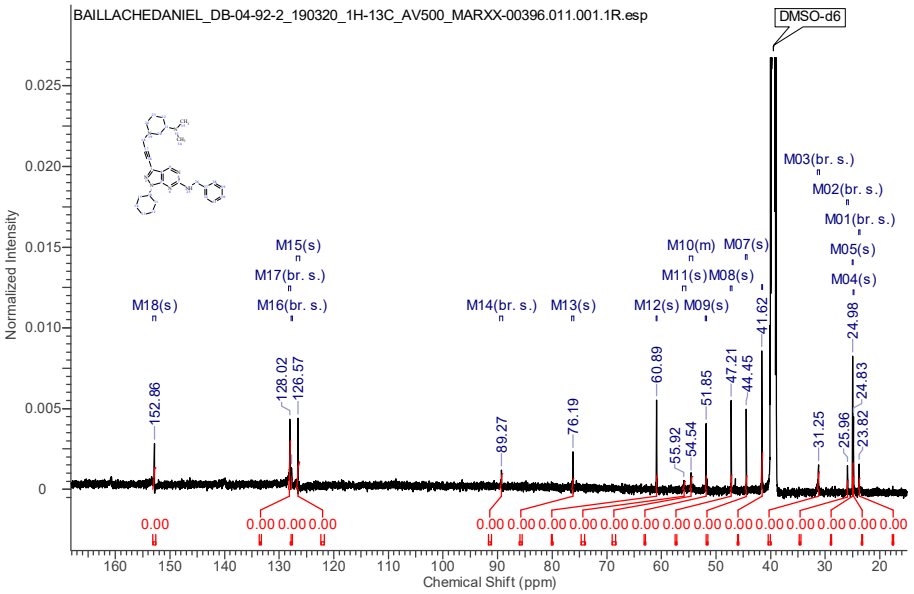
N-benzyl-1-cyclohexyl-3-(3-(3-(dimethylamino)piperidine-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B48). Yield: 39.5 mg, 0.084 mmol, 52 %, as a cream solid.

1H NMR (500 MHz, DMSO-d6) δ 8.71 (br. s., 1H), 8.21 (br. s., 1H), 7.38 (br. s., 2H), 7.26 - 7.32

(m, 2H), 7.16 - 7.23 (m, 1H), 4.51 (br. s., 2H), 4.39 - 4.47 (m, 1H), 3.54 - 3.66 (m, 2H), 2.96 (dd, J = 1.50, 8.83 Hz, 1H), 2.75 (d, J = 10.88 Hz, 1H), 2.30 - 2.40 (m, 1H), 2.24 (br. s., 6H), 2.04 - 2.16 (m, 2H), 1.82 (br. s., 6H), 1.64 - 1.74 (m, 2H), 1.34 - 1.51 (m, 4H), 1.18 - 1.27 (m, 1H), 1.04 - 1.17 (m, 1H).



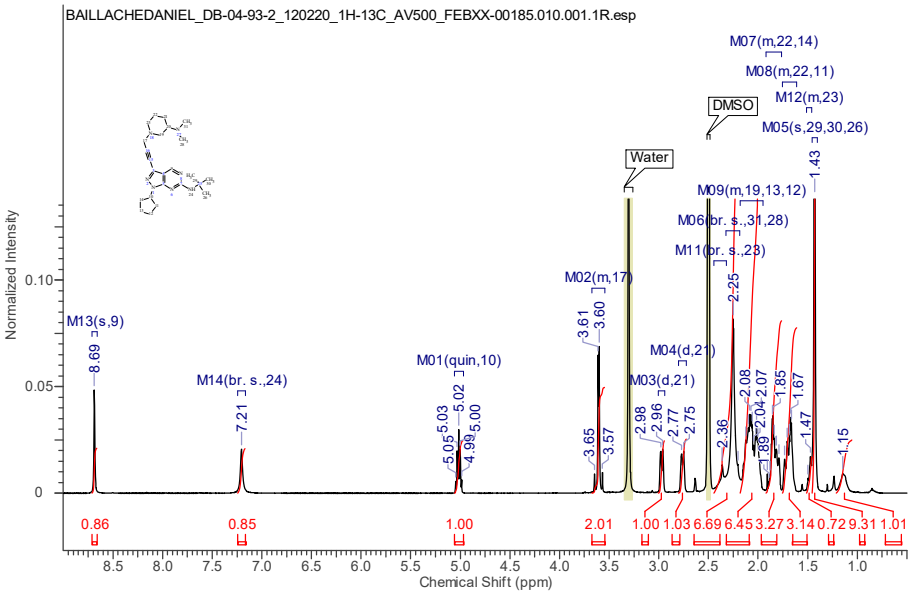
13C NMR (126 MHz, DMSO-d6) δ 160.7 (CH), 152.9 (2 x C), 139.9 (C), 128.0 (2 x CH), 127.7 (CH), 126.6 (2 x CH), 108.6 (C), 96.5 (C), 89.3 (C), 76.2 (C), 60.9 (CH2), 55.9 (CH), 54.6 (CH2), 51.8 (CH2), 47.2 (CH2), 46.5 (CH), 44.5 (CH2), 41.6 (2 x CH2), 31.2 (CH2), 26.0 (CH2), 25.0 (2 x CH3), 24.8 (CH2), 23.8 (CH2).



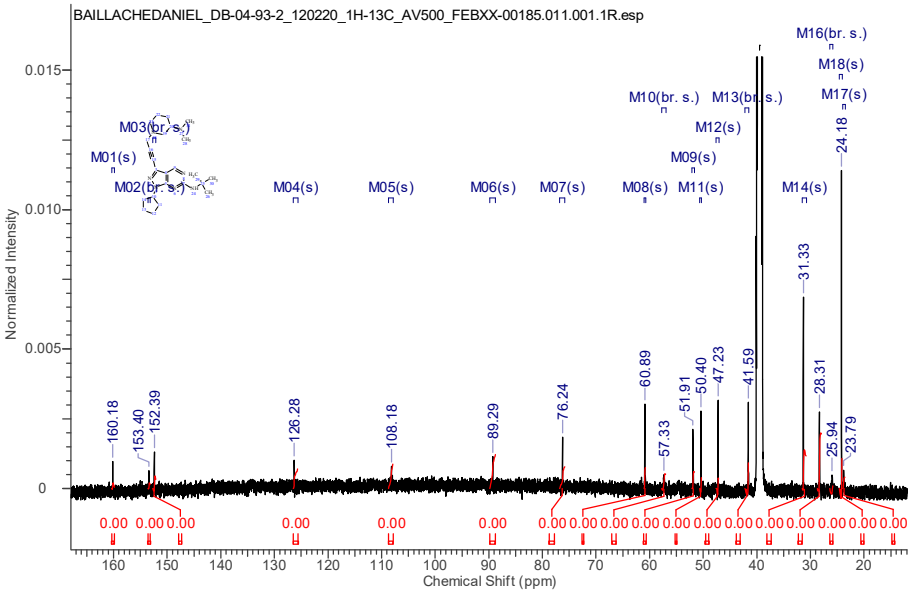
LRMS (ESI +ve) [M+H] 472.50. **HRMS** (ESI +ve) [M+H] 472.3197 ([C28H38N7]+, Calc. Exact Mass: 471.3110).

N-(tert-butyl)-1-cyclopentyl-3-(3-(3-(dimethylamino)piperidine-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B49). Yield: 27.9 mg, 0.066 mmol, 30%, as a golden

brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.69 (s, 1H), 7.21 (br. S., 1H), 5.02 (quin, J = 7.39 Hz, 1H), 3.55 – 3.67 (m, 2H), 2.97 (d, J = 9.62 Hz, 1H), 2.76 (d, J = 10.72 Hz, 1H), 2.36 (br. S., 1H), 2.25 (br. S., 6H), 1.95 – 2.18 (m, 6H), 1.76 – 1.92 (m, 3H), 1.61 – 1.75 (m, 3H), 1.46 – 1.51 (m, 1H), 1.43 (s, 9H), 1.15 (br. S., 1H).



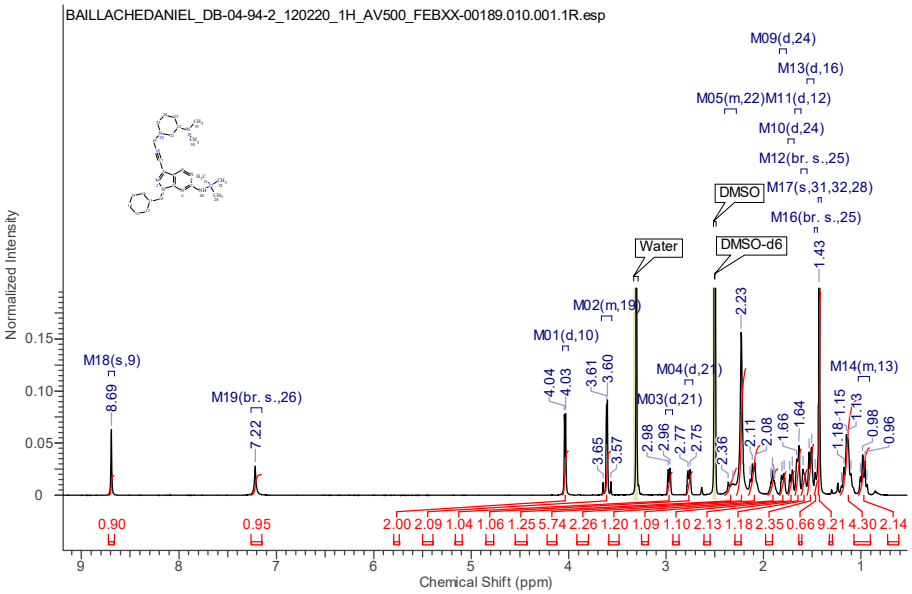
13C NMR (126 MHz, DMSO-d6) δ 160.2 (CH), 153.4 (C), 152.4 (C), 126.3 (C), 108.2 (C), 89.3 (C), 76.2 (C), 60.9 (CH2), 57.3 (C), 51.9 (CH2), 50.4 (CH2), 47.2 (CH2), 41.6 (CH2), 31.3 (4 x CH2), 28.3 (2 x CH3), 25.9 (CH), 24.2 (3 x CH3), 23.8 (CH).



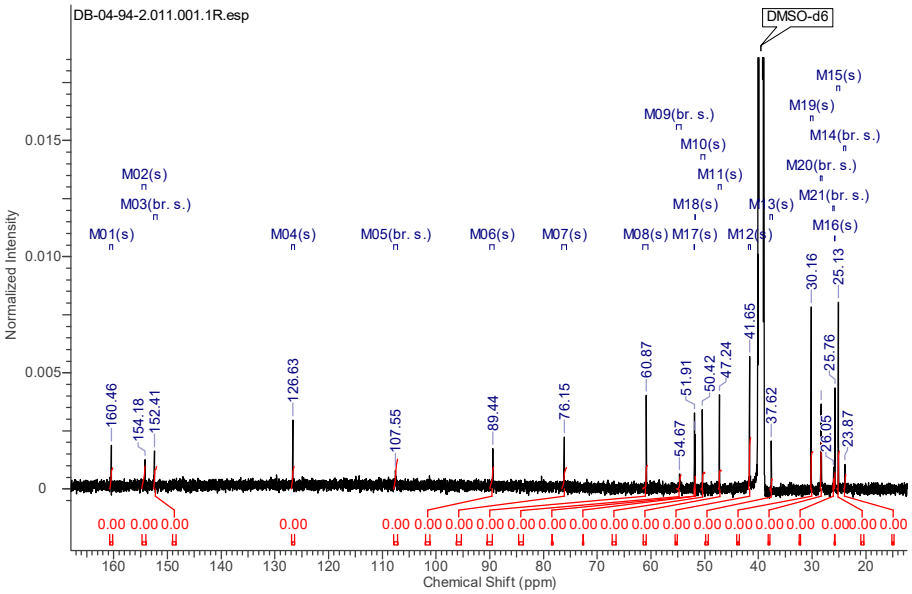
LRMS (ESI +ve) [M+H] 424.20. **HRMS** (ESI +ve) [M+H] 424.3189 ([C24H38N7]+, Calc. Exact Mass: 423.3110).

N-(tert-butyl)-1-(cyclohexylmethyl)-3-(3-(3-(dimethylamino)piperidine-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B50). Yield: 36.3 mg, 0.080 mmol, 38%, as a golden solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.69 (s, 1H), 7.22 (br. S., 1H), 4.04 (d, J = 6.86

Hz, 2H), 3.56 – 3.66 (m, 2H), 2.97 (d, J = 9.93 Hz, 1H), 2.76 (d, J = 10.56 Hz, 1H), 2.28 – 2.40 (m, 1H), 2.23 (br. S., 6H), 2.03 – 2.16 (m, 2H), 1.85 – 1.96 (m, 1H), 1.80 (d, J = 11.35 Hz, 1H), 1.72 (d, J = 13.16 Hz, 1H), 1.65 (d, J = 11.51 Hz, 2H), 1.59 (br. S., 1H), 1.52 (d, J = 12.85 Hz, 2H), 1.47 (br. S., 1H), 1.43 (s, 9H), 1.04 – 1.21 (m, 4H), 0.91 – 1.03 (m, 2H).



13C NMR (126 MHz, DMSO-d6) δ 160.5 (CH), 154.2 (C), 152.4 (C), 126.6 (C), 107.6 (C), 89.4 (C), 76.2 (C), 60.9 (CH2), 54.7 (C), 51.9 (CH2), 51.8 (CH2), 50.4 (CH2), 47.2 (CH2), 41.7 (2 x CH2), 37.6 (CH), 30.2 (2 x CH2), 28.3 (CH2), 26.0 (CH2), 25.8 (2 x CH3), 25.1 (3 x CH3), 23.9 (CH).

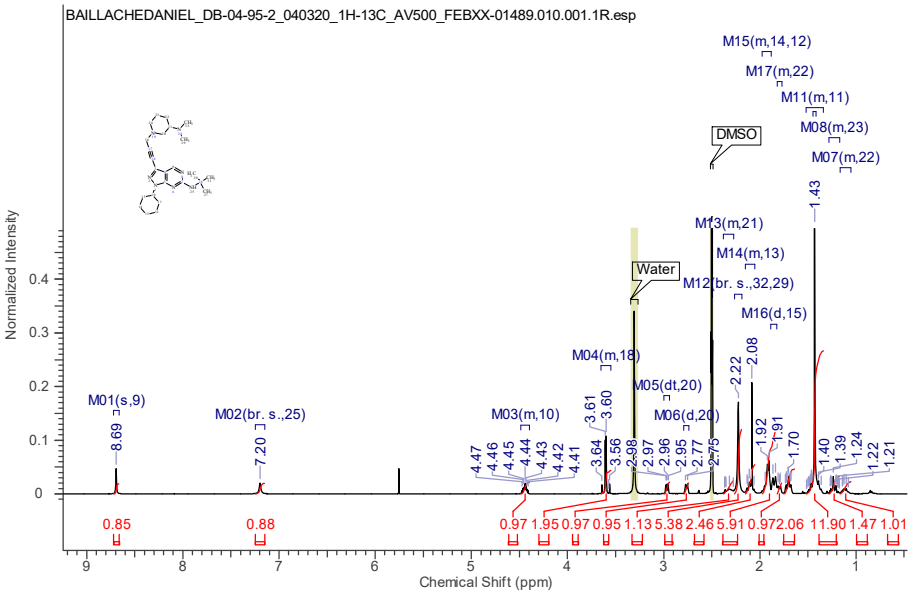


LRMS (ESI +ve) [M+H] 452.30.

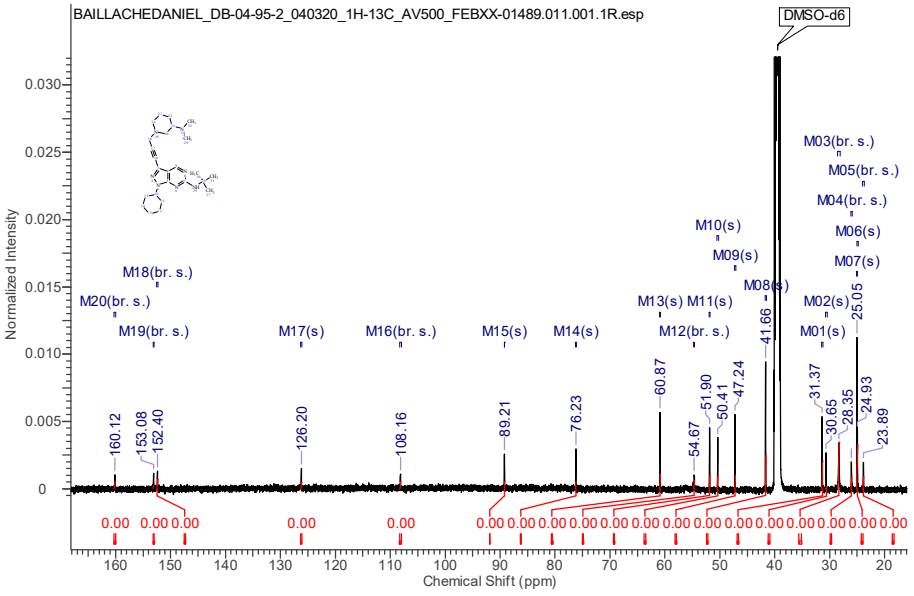
N-(tert-butyl)-1-cyclohexyl-3-(3-(3-(dimethylamino)piperidine-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B51). Yield: 37.8 mg, 0.086 mmol, 40%, as a brown solid.

1H NMR (500 MHz, DMSO-d6) δ 8.69 (s, 1H), 7.20 (br. S., 1H), 4.40 – 4.49 (m, 1H), 3.55 –

3.65 (m, 2H), 2.97 (td, J = 1.62, 10.40 Hz, 1H), 2.76 (d, J = 10.80 Hz, 1H), 2.27 – 2.38 (m, 1H), 2.22 (br. S., 6H), 2.05 – 2.15 (m, 2H), 1.88 – 1.98 (m, 4H), 1.85 (d, J = 13.40 Hz, 2H), 1.77 – 1.82 (m, 1H), 1.64 – 1.75 (m, 2H), 1.43 (s, 9H), 1.34 – 1.52 (m, 2H), 1.17 – 1.28 (m, 2H), 1.05 – 1.17 (m, 1H).



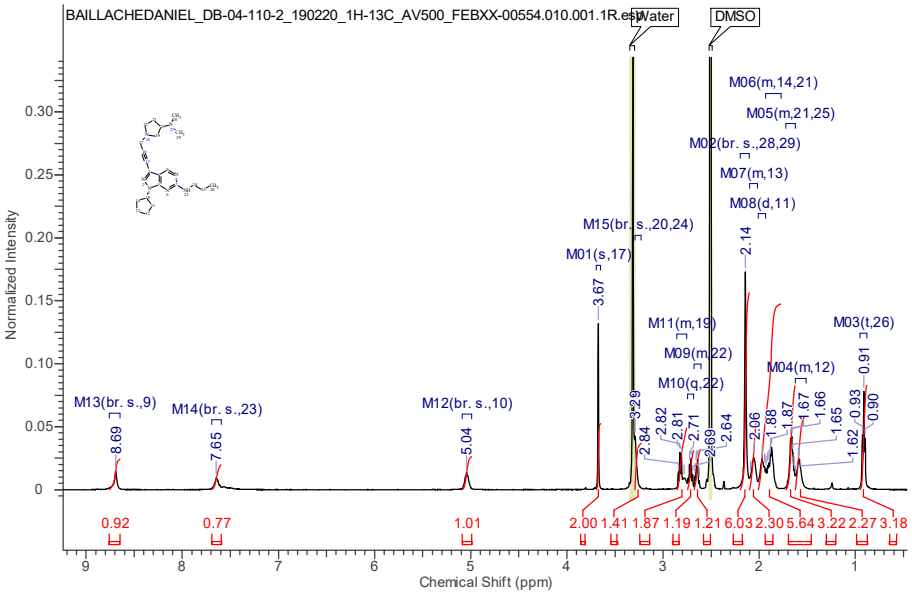
13C NMR (126 MHz, DMSO-d6) δ 160.1 (CH), 153.1 (C), 152.4 (C), 126.2 (C), 108.2 (C), 89.2 (C), 76.2 (C), 60.9 (CH2), 54.7 (C), 51.9 (CH2), 50.4 (CH2), 47.2 (CH2), 41.7 (3 x CH2), 31.4 (CH2), 30.7 (CH2), 28.3 (2 x CH3), 26.0 (CH), 25.0 (CH2), 24.9 (3 x CH3), 23.9 (CH).



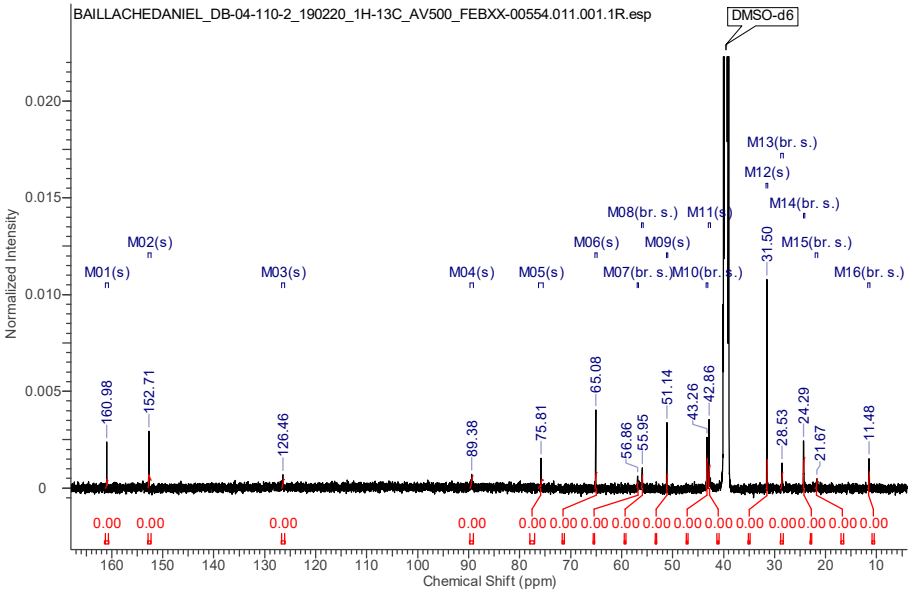
LRMS (ESI +ve) [M+H] 438.20.

1-cyclopentyl-3-(3-(3-(dimethylamino)pyrrolidine-1-yl)prop-1-yn-1-yl)-N-propyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B52). Yield: 30.1 mg, 0.076 mmol, 29 %, as a golden solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.69 (br. S., 1H), 7.65 (br. S., 1H), 5.04 (br. S., 1H), 3.67 (s, 2H), 3.29 (br. S., 3H), 2.75 – 2.86 (m, 2H), 2.72 (q, J = 7.25 Hz, 1H), 2.61 – 2.68 (m,

1H), 2.14 (br. S., 6H), 2.02 – 2.10 (m, 2H), 1.96 (d, J = 13.32 Hz, 2H), 1.77 – 1.93 (m, 3H), 1.63 – 1.72 (m, 3H), 1.51 – 1.63 (m, 2H), 0.91 (t, J = 7.09 Hz, 3H).

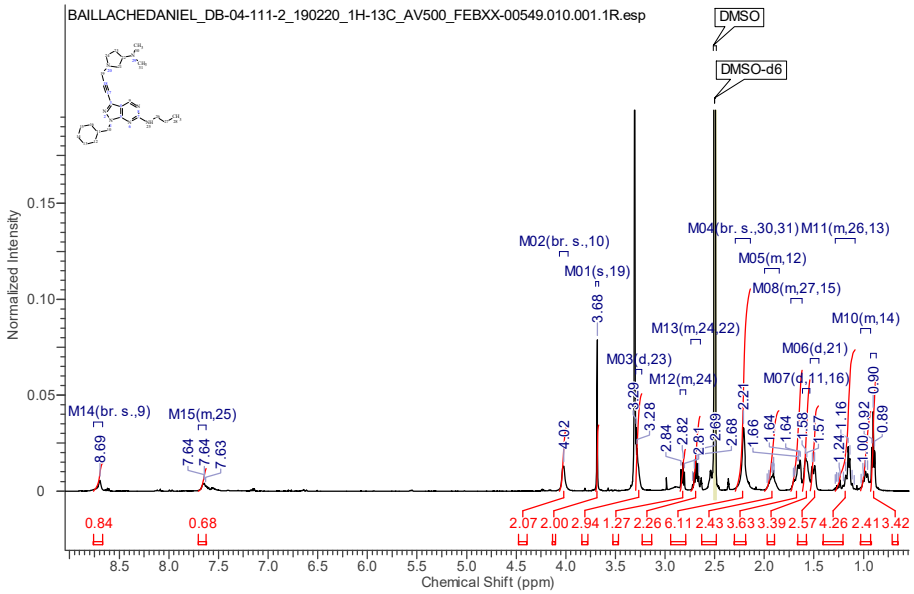


13C NMR (126 MHz, DMSO-d6) δ 161.0 (2 x C), 152.7 (2 x C), 126.5 (CH), 89.4 (C), 75.8 (C), 65.1 (2 x CH2), 56.9 (CH), 56.0 (CH2), 51.1 (CH2), 43.3 (CH2), 42.9 (CH2), 31.5 (3 x CH2), 28.5 (CH2), 24.3 (2 x CH3), 21.7 (CH), 11.5 (CH3).

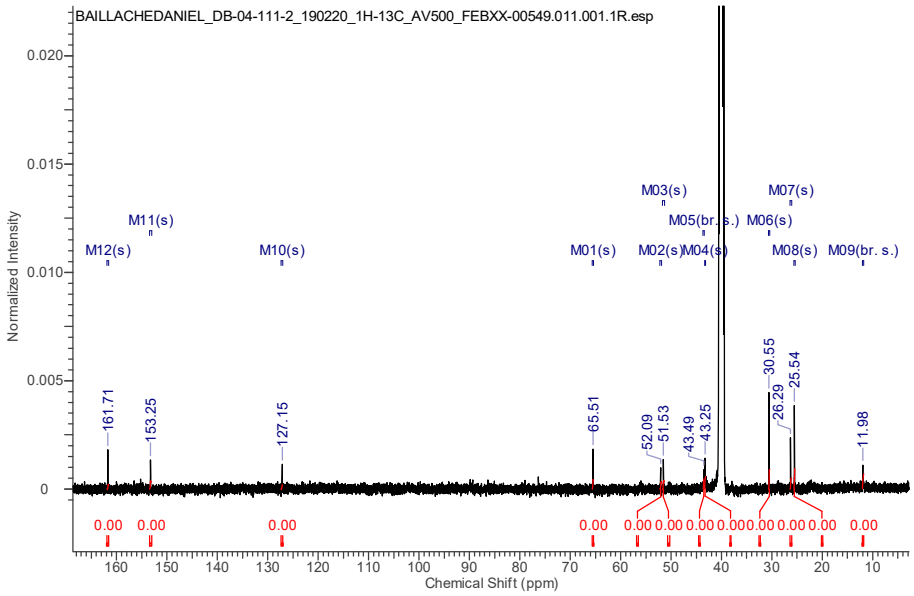


LRMS (ESI +ve) [M+H] 396.20.

1-(cyclohexylmethyl)-3-(3-(3-(dimethylamino)pyrrolidine-1-yl)prop-1-yn-1-yl)-N-propyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B53). Yield: 16.2 mg, 0.038 mmol, 25 %, as a brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.69 (br. S., 1H), 7.63 – 7.70 (m, 1H), 4.02 (br. S., 2H), 3.68 (s, 2H), 3.29 (d, J = 4.81 Hz, 2H), 2.79 – 2.85 (m, 1H), 2.65 – 2.74 (m, 2H), 2.21 (br. S., 6H), 1.85 – 2.00 (m, 2H), 1.62 – 1.73 (m, 4H), 1.58 (d, J = 6.78 Hz, 3H), 1.50 (d, J = 12.14 Hz, 2H), 1.09 – 1.29 (m, 4H), 0.93 – 1.03 (m, 2H), 0.90 (t, J = 7.33 Hz, 3H).

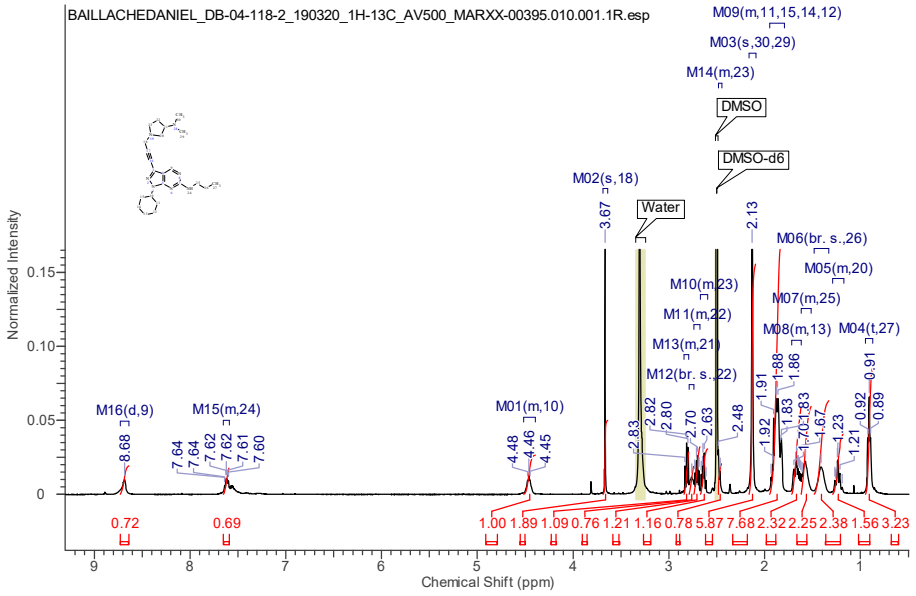


13C NMR (126 MHz, DMSO-d6) δ 161.7 (2 x C), 153.2 (CH), 127.1 (2 x C), 89.3 (C), 75.8 (C), 65.5 (2 x CH2), 55.5 (CH), 52.1 (CH2), 51.5 (CH2), 43.5 (CH2), 43.3 (CH2), 30.6 (4 x CH2), 26.3 (3 x CH2), 25.5 (2 x CH3), 12.0 (CH3).

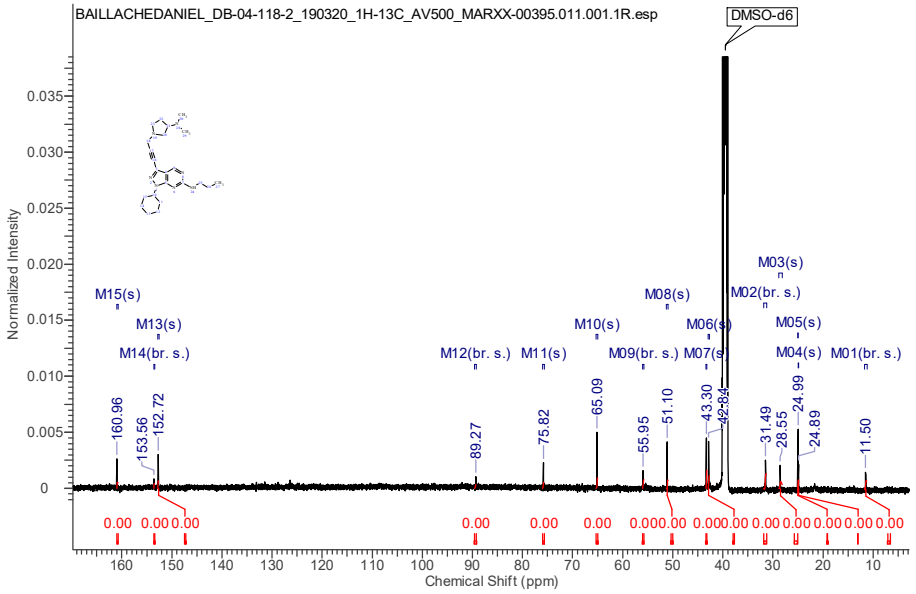


LRMS (ESI +ve) [M+H] 424.30.

1-cyclohexyl-3-(3-(3-(dimethylamino)pyrrolidine-1-yl)prop-1-yn-1-yl)-N-propyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B54). Yield: 27.1 mg, 0.066 mmol, 26 %, as a golden solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.69 (d, J = 7.01 Hz, 1H), 7.59 – 7.65 (m, 1H), 4.40 – 4.52 (m, 1H), 3.67 (s, 2H), 2.79 – 2.84 (m, 1H), 2.76 (br. S., 1H), 2.68 – 2.74 (m, 1H), 2.59 – 2.67 (m, 1H), 2.45 – 2.48 (m, 1H), 2.13 (s, 6H), 1.79 – 1.95 (m, 8H), 1.62 – 1.72 (m, 2H), 1.51 – 1.62 (m, 2H), 1.41 (br. S., 2H), 1.17 – 1.29 (m, 2H), 0.91 (t, J = 7.09 Hz, 3H).



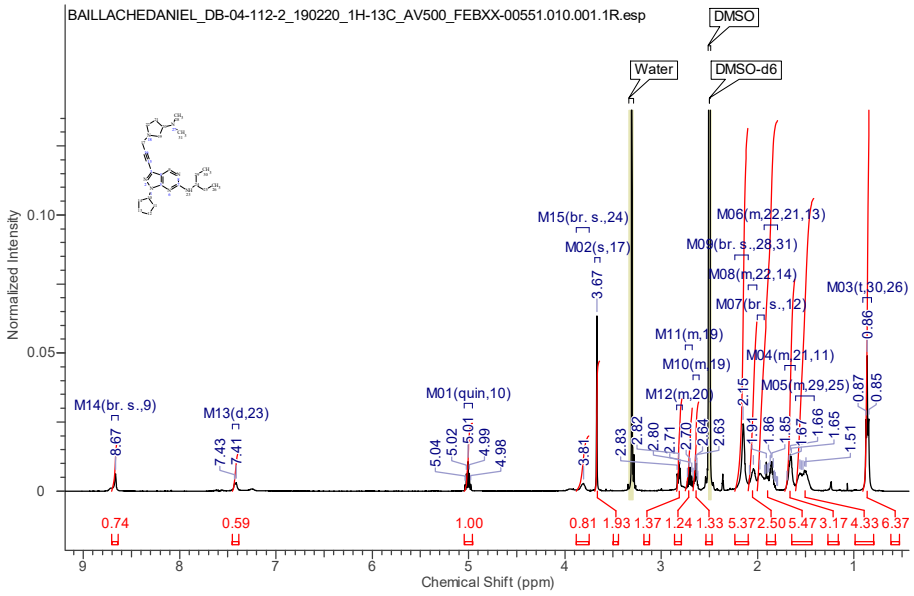
13C NMR (126 MHz, DMSO-d6) δ 161.0 (C), 153.6 (CH), 152.7 (C), 131.5 (C), 126.4 (C), 89.3 (C), 75.8 (C), 65.1 (2 x CH2), 55.9 (CH), 51.1 (2 x CH2), 43.3 (2 x CH2), 42.8 (2 x CH2), 31.5 (CH2), 28.6 (CH2), 25.0 (2 x CH3), 24.9 (CH2), 21.7 (CH), 11.5 (CH3).



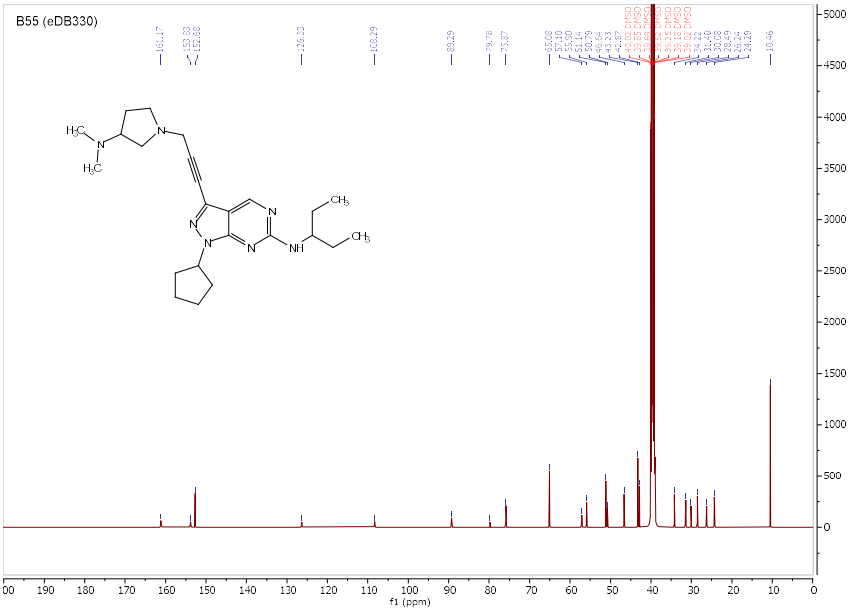
LRMS (ESI +ve) [M+H] 410.20.

1-cyclopentyl-3-(3-(3-(dimethylamino)pyrrolidine-1-yl)prop-1-yn-1-yl)-N-(pentan-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B55). Yield: 26.2 mg, 0.062 mmol, 29 %, as a golden solid.

1H NMR (500 MHz, DMSO-d6) δ 8.67 (br. S., 1H), 7.42 (d, J = 7.80 Hz, 1H), 5.01 (quin, J = 7.31 Hz, H1H), 3.81 (br. S., 1H), 3.67 (s, 2H), 2.78 – 2.84 (m, 1H), 2.68 – 2.75 (m, 1H), 2.61 – 2.67 (m, 1H), 2.15 (br. S., 6H), 2.01 – 2.10 (m, 3H), 1.98 (br. S., 2H), 1.79 – 1.93 (m, 4H), 1.61 – 1.72 (m, 3H), 1.41 – 1.60 (m, 4H), 0.86 (t, J = 7.21 Hz, 6H).



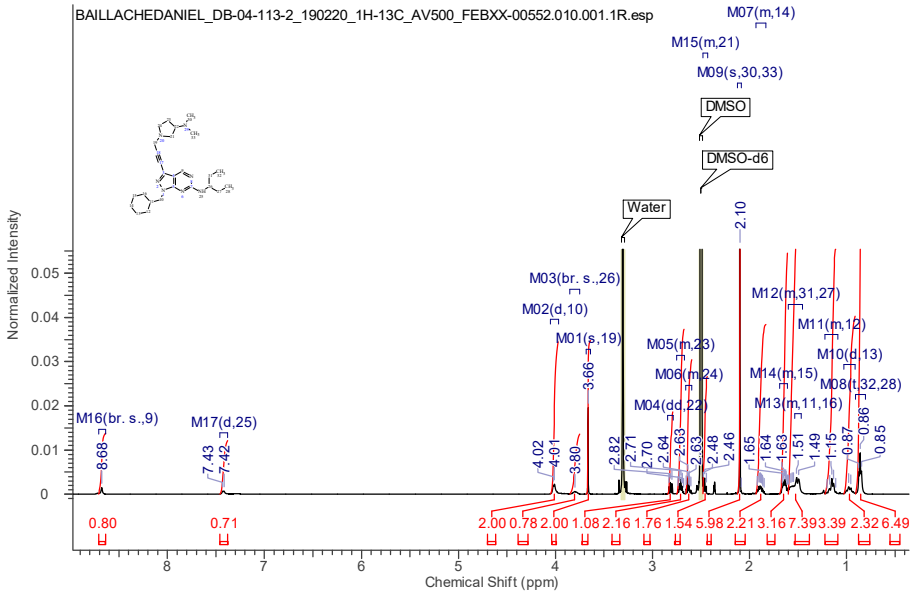
13C NMR (126 MHz, DMSO-d6) δ 161.2 (C), 153.8 (CH), 152.7 (C), 126.3 (C), 108.3 (C), 89.3 (C), 79.8 (C), 75.9 (CH), 65.1 (CH2), 57.1 (CH), 55.9 (CH2), 51.1 (CH2), 50.8 (CH), 46.6 (CH2), 43.2 (CH2), 42.9 (CH2), 34.2 (CH2), 31.4 (CH2), 30.1 (CH2), 28.5 (CH2), 26.2 (CH3), 24.3 (CH3), 10.5 (2 x CH3).



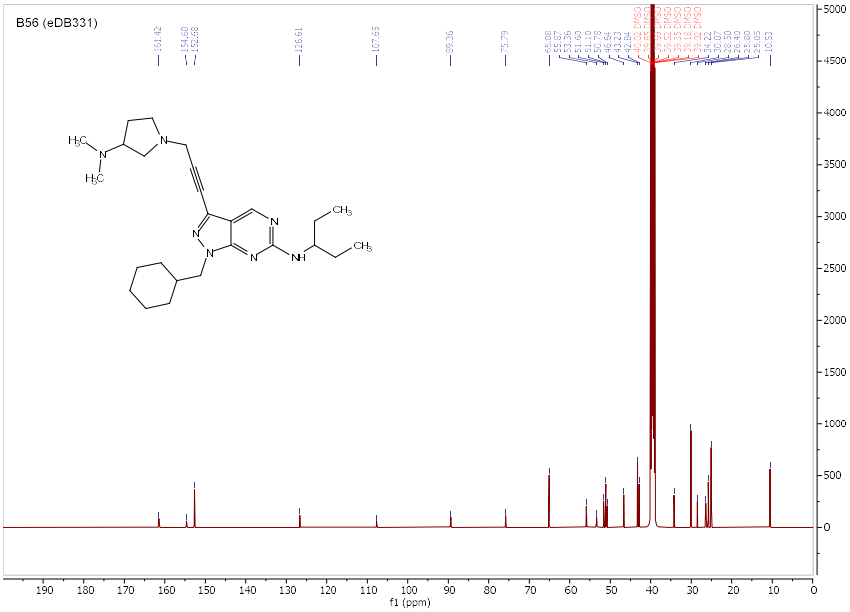
LRMS (ESI +ve) [M+H] 424.20. **HRMS** (ESI +ve) [M+H] 424.3183 ([C24H37N7]+, Calc. Exact Mass: 423.3110).

1-(cyclohexylmethyl)-3-(3-(3-(dimethylamino)pyrrolidine-1-yl)prop-1-yn-1-yl)-N-(pentan-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B56). Yield: 26.1 mg, 0.058 mmol, 28 %, as a golden solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.68 (br. S., 1H), 7.42 (d, J = 8.04 Hz, 1H), 4.02 (d, J = 5.91 Hz, 2H), 3.80 (br. S., 1H), 3.66 (s, 2H), 2.81 (dd, J = 7.29, 8.63 Hz, 1H), 2.67 – 2.75 (m, 2H), 2.59 – 2.66 (m, 2H), 2.43 – 2.48 (m, 2H), 2.09 (s, 6H), 1.83 – 1.93 (m, 2H), 1.61 – 1.69

(m, 2H), 1.47 – 1.54 (m, 3H), 1.45 – 1.60 (m, 4H), 1.09 – 1.22 (m, 2H), 0.97 (d, J = 11.03 Hz, 2H), 0.86 (t, J = 7.05 Hz, 6H).



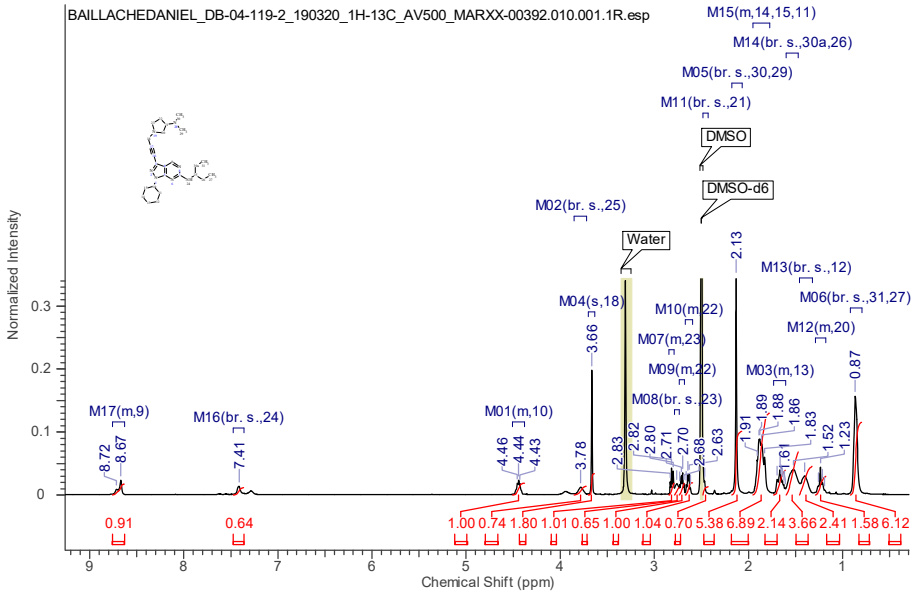
13C NMR (126 MHz, DMSO-d6) δ 161.4 (CH), 154.6 (C), 152.7 (C), 126.6 (C), 107.6 (C), 89.4 (C), 75.8 (C), 65.1 (CH2), 55.9 (CH2), 53.4 (CH), 51.6 (CH), 51.1 (CH2), 50.8 (CH), 46.6 (CH2), 43.2 (CH2), 42.8 (CH2), 34.2 (CH2), 30.1 (2 x CH2), 28.5 (CH2), 26.4 (CH2), 25.8 (CH2), 25.0 (2 x CH3), 10.5 (2 x CH3).



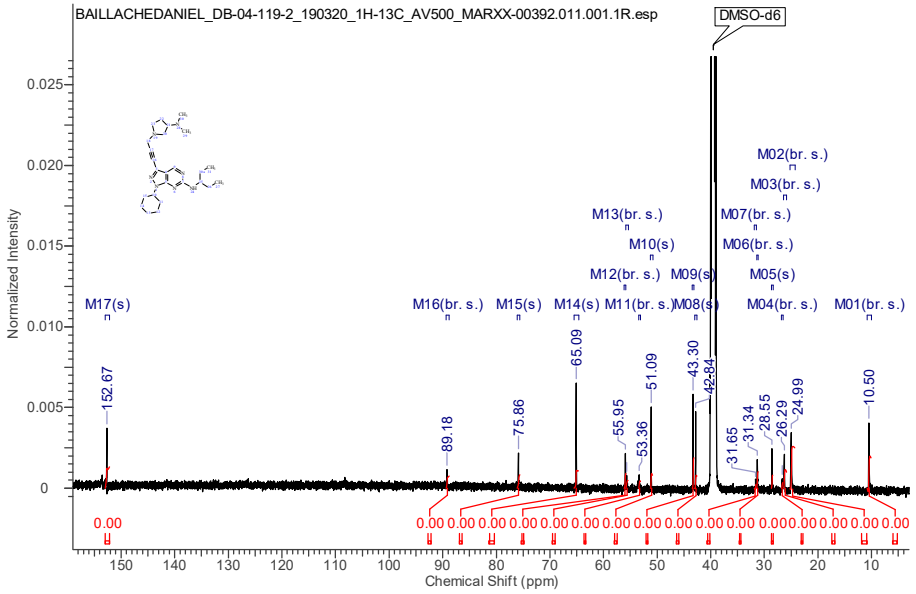
LRMS (ESI +ve) [M+H] 452.20. **HRMS** (ESI +ve) [M+H] 452.3486 ([C26H41N7]+, Calc. Exact Mass: 451.3420).

1-cyclohexyl-3-(3-(3-(dimethylamino)pyrrolidine-1-yl)prop-1-yn-1-yl)-N-(pentan-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B57). Yield: 13.7 mg, 0.031 mmol, 18 %, as a golden solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.63 – 8.76 (m, 1H), 7.41 (br. S., 1H), 4.38 – 4.51 (m, 1H), 3.78 (br. S., 1H), 3.66 (s, 2H), 2.79 – 2.85 (m, 1H), 2.76 (br. S., 1H), 2.68 – 2.74 (m, 1H),

2.59 – 2.67 (m, 1H), 2.47 (br. S., 1H), 2.13 (br. S., 6H), 1.78 – 1.95 (m, 6H), 1.61 – 1.73 (m, 2H), 1.52 (br. S., 4H), 1.40 (br. S., 2H), 1.18 – 1.29 (m, 2H), 0.87 (br. S., 6H).



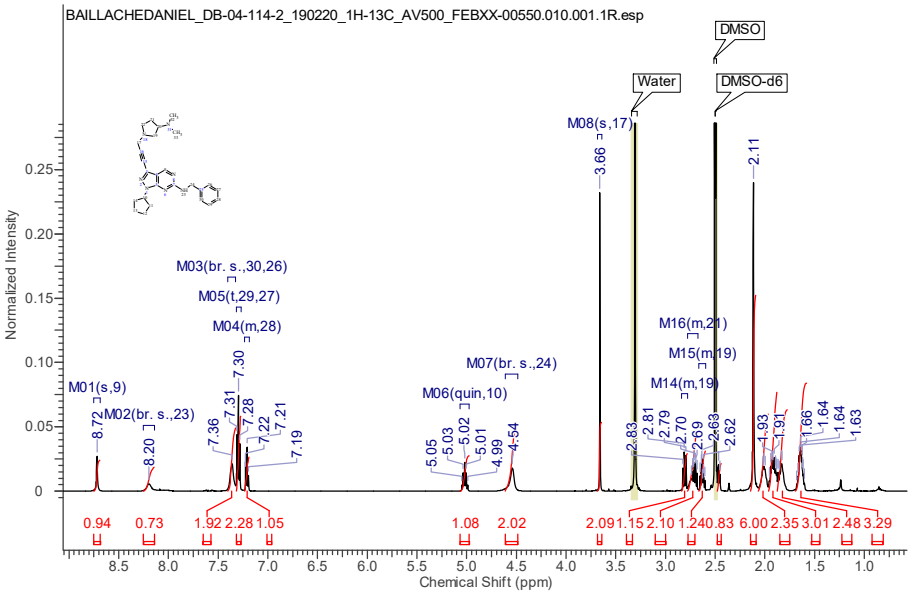
13C NMR (126 MHz, DMSO-d6) δ 153.5 (CH), 152.7 (3 x C), 108.2 (C), 89.2 (C), 75.9 (C), 65.1 (2 x CH2), 55.9 (CH2), 55.7 (CH), 53.4 (CH), 51.1 (CH2), 43.3 (CH2), 42.8 (2 x CH2), 31.6 (CH), 31.3 (CH2), 28.6 (CH2), 26.6 (CH2), 26.3 (CH2), 25.0 (2 x CH3), 10.5 (2 x CH3).



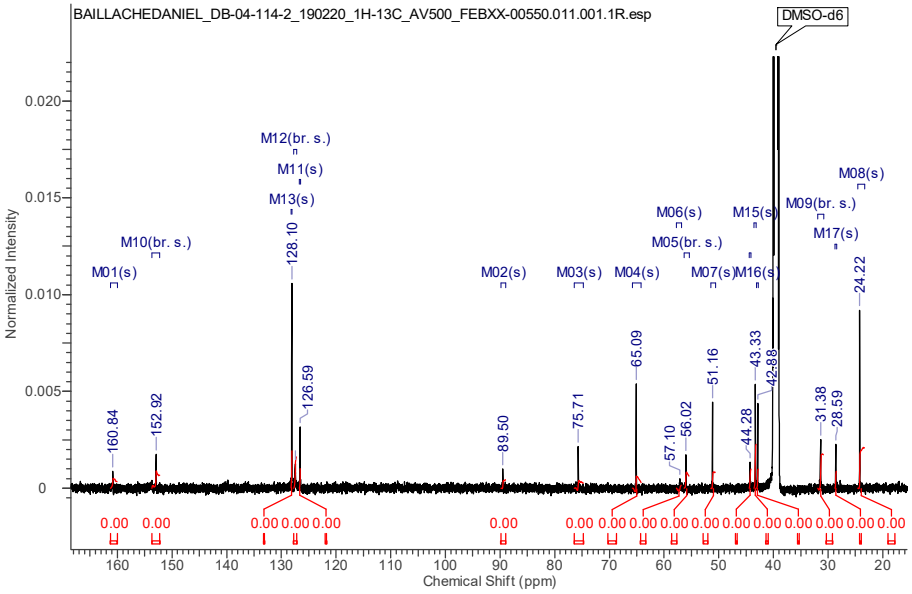
LRMS (ESI +ve) [M+H] 438.30. **HRMS** (ESI +ve) [M+H] 438.3333 ([C25H39N7]+, Calc. Exact Mass: 437.3270).

N-benzyl-1-cyclopentyl-3-(3-(3-(dimethylamino)pyrrolidine-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B58, eDB333). Yield: 28.3 mg, 0.065 mmol, 38 %, as a light brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.72 (s, 1H), 8.20 (br. S., 1H), 7.36 (br. S., 2H), 7.30 (t, J = 7.57 Hz, 2H), 7.19 – 7.23 (m, 1H), 5.02 (quin, J = 7.33 Hz, 1H), 4.54 (br. S., 2H), 3.66 (s, 2H), 2.78 – 2.84 (m, 1H), 2.67 – 2.78 (m, 2H), 2.60 – 2.66 (m, 1H), 2.44 – 2.48

(m, 1H), 2.11 (s, 6H), 1.97 – 2.07 (m, 2H), 1.88 – 1.96 (m, 3H), 1.77 – 1.87 (m, 2H), 1.58 – 1.69 (m, 3H).



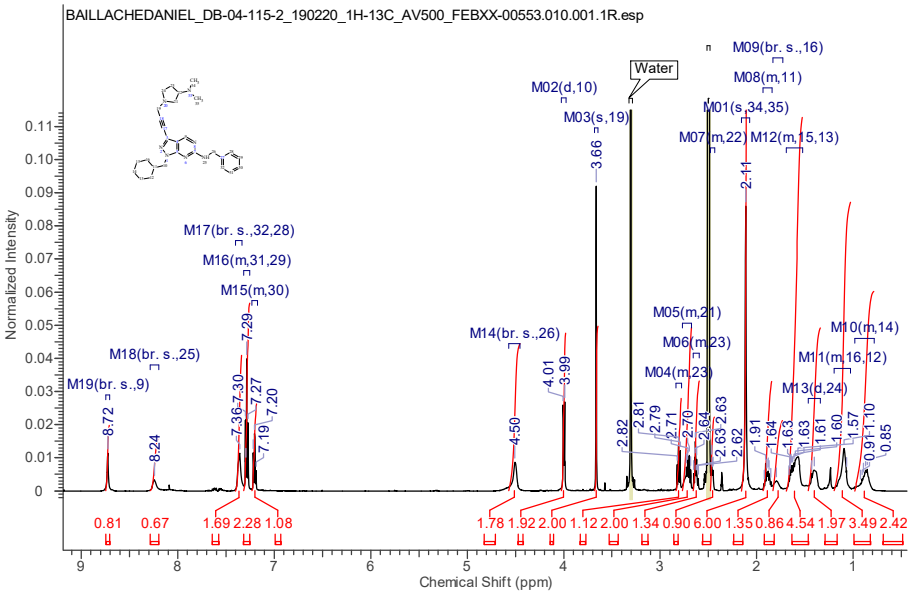
13C NMR (126 MHz, DMSO-d6) δ 160.8 (CH), 152.9 (2 x C), 139.8 (C), 128.1 (4 x CH), 127.5 (2 x C), 126.6 (CH), 89.5 (C), 75.7 (C), 65.1 (2 x CH2), 57.1 (CH), 56.0 (CH2), 51.2 (2 x CH2), 44.3 (CH), 43.3 (CH2), 42.9 (CH2), 31.4 (CH2), 28.6 (CH2), 24.2 (2 x CH3).



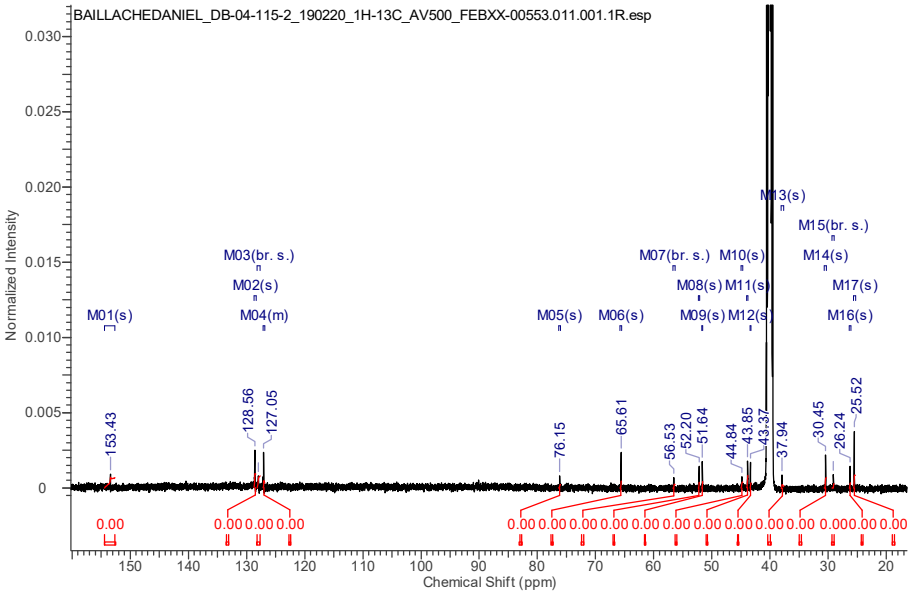
LRMS (ESI +ve) [M+H] 444.20. **HRMS** (ESI +ve) [M+H] 444.2870 ([C26H33N7]+, Calc. Exact Mass: 443.2800).

N-benzyl-1-(cyclohexylmethyl)-3-(3-(3-(dimethylamino)pyrrolidine-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B59). Yield: 15.1 mg, 0.032 mmol, 30 %, as a brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.72 (br. S., 1H), 8.24 (br. S., 1H), 7.36 (br. S., 2H), 7.25 – 7.32 (m, 2H), 7.17 – 7.23 (m, 1H), 4.50 (br. S., 2H), 4.00 (d, J = 7.09 Hz, 2H), 3.66 (s, 2H), 2.78 – 2.84 (m, 1H), 2.67 – 2.77 (m, 2H), 2.59 – 2.66 (m, 1H), 2.43 – 2.48 (m, 1H), 2.11

(s, 6H), 1.84 – 1.93 (m, 1H), 1.80 (br. S., 1H), 1.52 – 1.69 (m, 4H), 1.42 (d, J = 13.71 Hz, 2H), 1.03 – 1.20 (m, 3H), 0.78 – 0.98 (m, 2H).



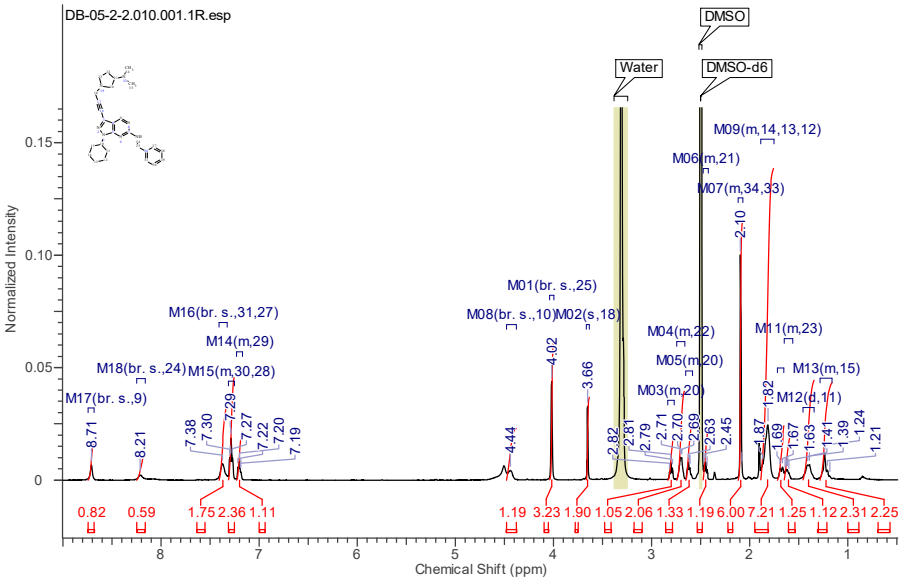
13C NMR (126 MHz, DMSO-d6) δ 161.0 (CH), 153.4 (C), 139.9 (C), 128.6 (2 x CH), 128.0 (C), 127.6 (CH), 127.0 (2 x CH), 125.9 (C), 103.7 (C), 89.6 (C), 76.1 (C), 65.6 (2 x CH2), 56.5 (CH), 52.2 (CH2), 51.6 (CH2), 44.8 (CH2), 43.9 (CH2), 43.4 (CH2), 37.9 (CH), 30.5 (2 x CH2), 29.1 (CH2), 26.2 (CH2), 25.5 (2 x CH3).



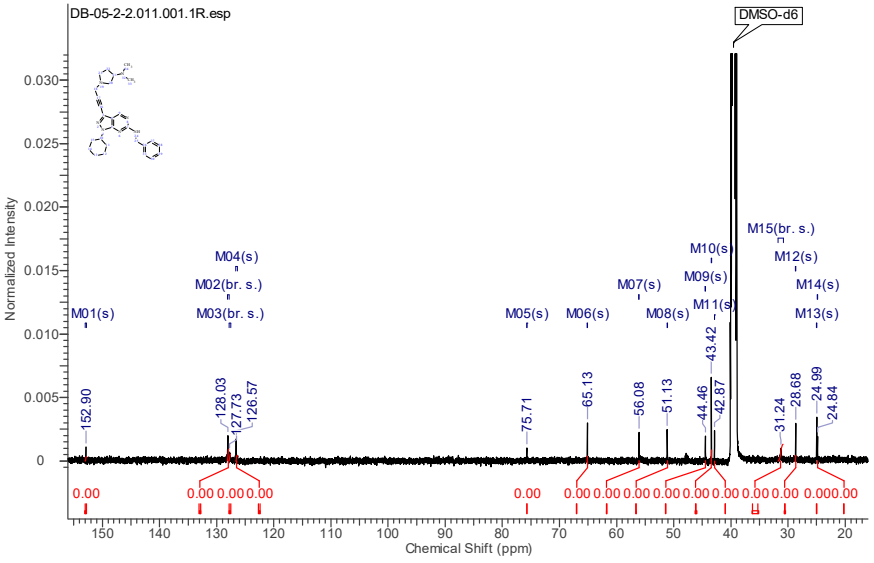
LRMS (ESI +ve) [M+H] 472.30.

N-benzyl-1-cyclohexyl-3-(3-(3-(dimethylamino)pyrrolidine-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B60). Yield: 24.3 mg, 0.053 mmol, 31 %, as a golden solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.71 (br. S., 1H), 8.21 (br. S., 1H), 7.38 (br. S., 2H), 7.26 – 7.32 (m, 2H), 7.17 – 7.23 (m, 1H), 4.44 (br. S., 1H), 4.02 (br. S., 2H), 3.66 (s, 2H), 2.77 – 2.84 (m, 1H), 2.66 – 2.74 (m, 2H), 2.58 – 2.65 (m, 1H), 2.42 – 2.48 (m, 1H), 2.07 – 2.12 (m,

6H), 1.75 – 1.89 (m, 6H), 1.68 (d, J = 12.69 Hz, 1H), 1.56 – 1.65 (m, 1H), 1.40 (d, J = 9.46 Hz, 2H), 1.16 – 1.29 (m, 2H).

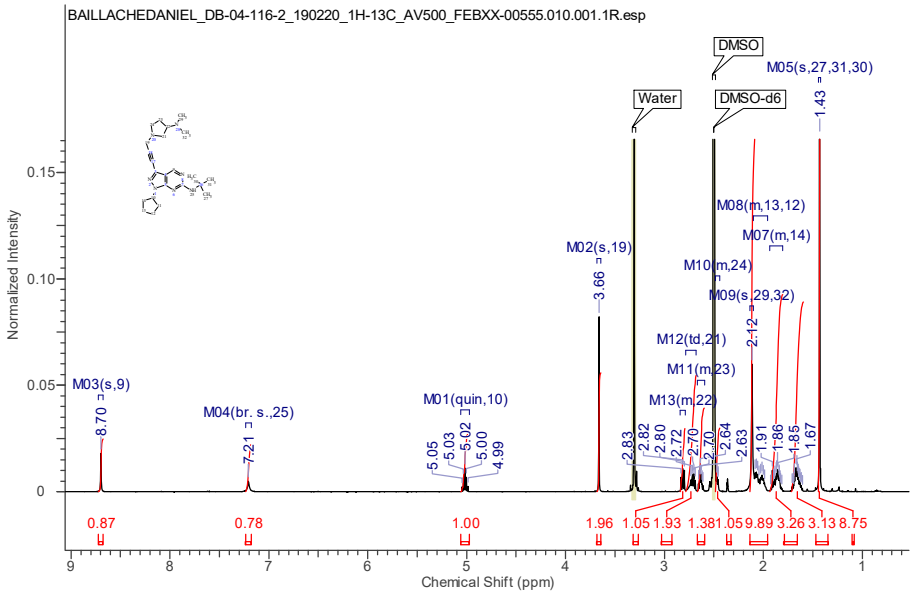


13C NMR (126 MHz, DMSO-d6) δ 161.0 (CH), 152.9 (C), 139.9 (C), 128.0 (2 x CH), 127.7 (C), 127.6 (CH), 126.6 (2 x CH, C), 103.7 (C), 87.7 (C), 75.7 (C), 65.1 (2 x CH2), 56.1 (CH2), 51.1 (CH2), 47.8 (CH), 44.5 (CH2), 43.4 (2 x CH2), 42.9 (CH2), 31.2 (CH), 28.7 (2 x CH2), 25.0 (CH3), 24.8 (CH3).

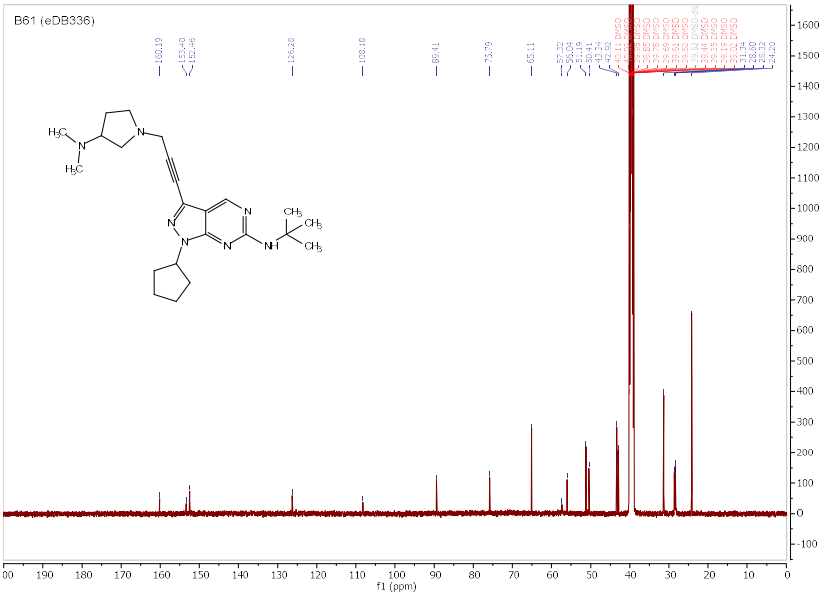


LRMS (ESI +ve) [M+H] 458.20.

N-(tert-butyl)-1-cyclopentyl-3-(3-(3-(dimethylamino)pyrrolidine-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B61). Yield: 36.9 mg, 0.090 mmol, 32 %, as a golden brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.70 (s, 1H), 7.21 (br. S., 1H), 5.02 (quin, J = 7.41 Hz, 1H), 3.66 (s, 2H), 2.79 – 2.84 (m, 1H), 2.71 (dt, J = 6.07, 8.24 Hz, 2H), 2.59 – 2.67 (m, 2H), 2.44 – 2.48 (m, 2H), 2.12 (s, 6H), 1.96 – 2.10 (m, 4H), 1.80 – 1.94 (m, 2H), 1.59 – 1.72 (m, 2H), 1.43 (s, 9H).

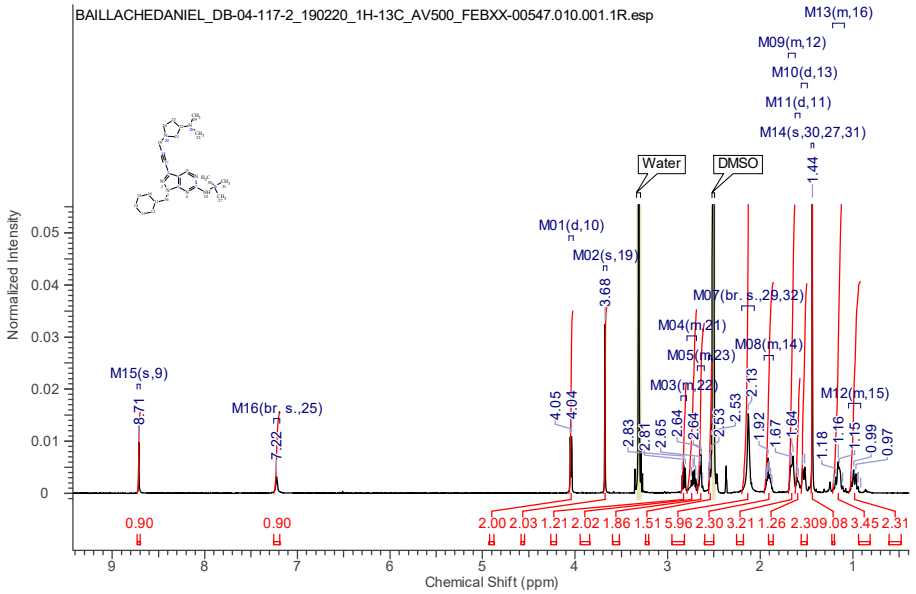


13C NMR (126 MHz, DMSO-d6) δ 160.2 (C), 153.4 (CH), 152.5 (C), 126.3 (C), 108.2 (C), 89.4 (C), 75.8 (C), 65.1 (2 x CH2), 57.3 (CH), 56.0 (C), 51.2 (CH2), 50.4 (CH), 43.3 (2 x CH2), 42.9 (CH2), 31.3 (2 x CH3), 28.6 (CH2), 28.3 (CH2), 24.2 (3 x CH3).

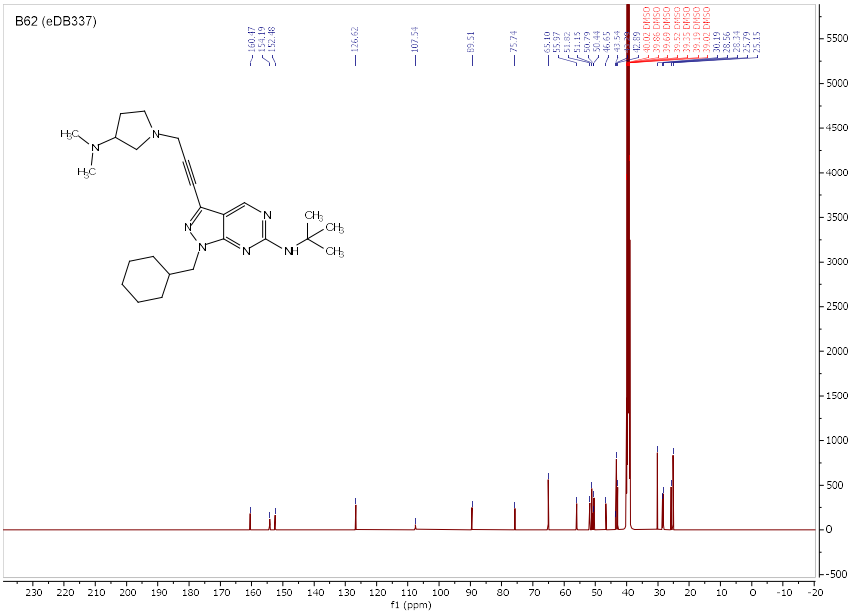


LRMS (ESI +ve) [M+H] 410.20. **HRMS** (ESI +ve) [M+H] 410.3021 ([C23H36N7]+, Calc. Exact Mass: 409.2950).

N-(tert-butyl)-1-(cyclohexylmethyl)-3-(3-(3-(dimethylamino)pyrrolidine-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B62). Yield: 25.2 mg, 0.058 mmol, 30 %, as a brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.71 (s, 1H), 7.22 (br. S., 1H), 4.04 (d, J = 6.94 Hz, 2H), 3.68 (s, 2H), 2.80 – 2.85 (m, 1H), 2.69 – 2.79 (m, 2H), 2.60 – 2.68 (m, 2H), 2.53 – 2.56 (m, 2H), 2.13 (br. S., 6H), 1.86 – 1.96 (m, 2H), 1.62 – 1.70 (m, 2H), 1.60 (d, J = 7.65 Hz, 1H), 1.53 (d, J = 11.35 Hz, 2H), 1.44 (s, 9H), 1.09 – 1.22 (m, 2H), 0.92 – 1.05 (m, 2H).

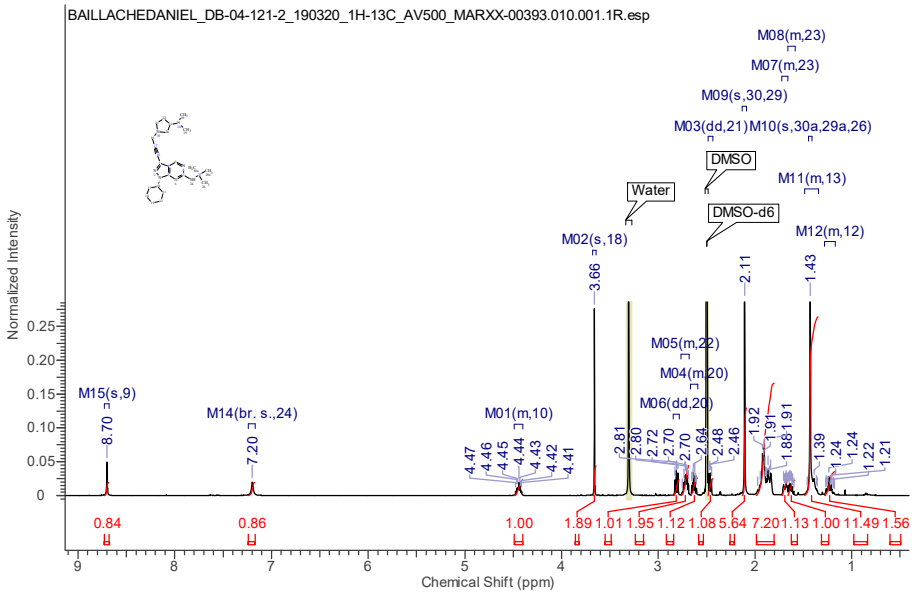


13C NMR (126 MHz, DMSO-d6) δ 160.5 (C), 154.2 (CH), 152.5 (C), 126.6 (C), 107.5 (C), 89.5 (C), 75.7 (C), 65.1 (CH2), 56.0 (CH2), 51.8 (CH2), 51.1 (CH2), 50.8 (C), 50.4 (CH), 46.6 (CH2), 43.5 (CH), 43.3 (CH2), 42.9 (CH2), 30.2 (CH2), 28.6 (2 x CH3), 28.3 (CH2), 25.8 (CH2), 25.1 (3 x CH3).

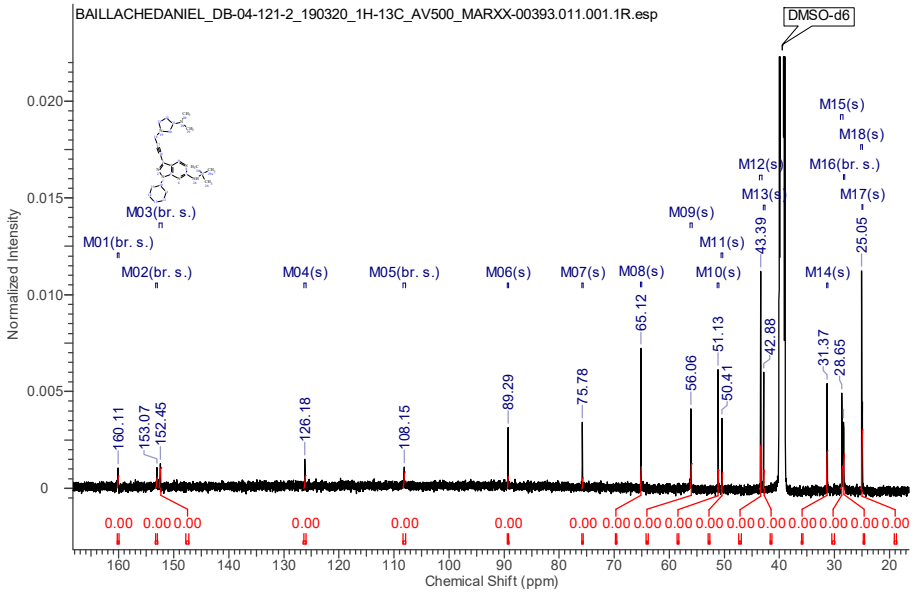


LRMS (ESI +ve) [M+H] 424.20.

N-(tert-butyl)-1-cyclohexyl-3-(3-(3-(dimethylamino)pyrrolidine-1-yl)prop-1-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-amine (B63). Yield: 21.5 mg, 0.051 mmol, 22 %, as a light brown solid. **1H NMR** (500 MHz, DMSO-d6) δ 8.70 (s, 1H), 7.20 (br. S., 1H), 4.40 – 4.49 (m, 1H), 3.66 (s, 2H), 2.81 (dd, J = 7.29, 8.55 Hz, 1H), 2.68 – 2.76 (m, 2H), 2.59 – 2.67 (m, 1H), 2.47 (dd, J = 7.05, 8.63 Hz, 1H), 2.11 (s, 6H), 1.80 – 1.99 (m, 6H), 1.66 – 1.72 (m, 1H), 1.59 – 1.66 (m, 1H), 1.43 (s, 9H), 1.34 – 1.49 (m, 2H), 1.17 – 1.28 (m, 2H).



13C NMR (126 MHz, DMSO-d6) δ 160.1 (CH), 153.1 (C), 152.5 (C), 126.2 (C), 108.2 (C), 89.3 (C), 75.8 (C), 65.1 (2 x CH2), 56.1 (CH), 51.1 (2 x CH2), 50.4 (C), 43.4 (3 x CH2), 42.9 (2 x CH2), 31.4 (CH2), 28.6 (CH2), 28.3 (CH), 24.9 (3 x CH3).



LRMS (ESI +ve) [M+H] 424.30. **HRMS** (ESI +ve) [M+H] 424.3184 ([C24H38N7]+, Calc. Exact Mass: 423.3110).

10. Biological protocols

Seven-Point Dose-Response Cell Viability Assay. Cells (T98, U87 and bEnd.3) were plated in a 96-well plate at 500, 1,000 and 10,000 cells/well respectively in 100 µL of DMEM medium containing 10 % FBS and 1 % L-glutamine and incubated for 48 h at 37 °C and 5 % CO2. After 48 hours, media was replaced (95 µL) and cells were dosed (5 µL) with compounds in triplicate at 30, 10, 3, 1, 0.3, 0.1 and 0.03 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 assessed using PrestoBlueTM and fluorescence data was collected, normalised and curves plotted using GraphPadTM.

Full protocol of high-content Cell "Painting" Assay. Cells were seeded (30 µL per well in complete media) into 384-well, CELLSTAR Cell Culture Microplates (Grenier), which had been pre-coated with laminin (10 µg/mL) in complete media (20 µL per well) and incubated for 2 hours under standard conditions, to a final volume of 50 µL. Cells were incubated under standard conditions for 24 hours before compound treatment. Cells were seeded at 1000 cells/well (E13, E21, E31, E34 and FT3469), 1500 cells/well (E28) and 400 cells/well (E57). Compound dilution plates were made at 1000-fold assay concentration and added to the cells with an overall dilution in complete media of 1:1000 from source to assay plate. Compounds were dosed at 30, 15, 10, 7.5, 5, 2.5, 1, 0.3, 0.1 and 0.03 µM using a TECN D300 Digital Dispenser to a final concentration 0.1% v/v DMSO in triplicate. Treated plates were incubated for 72 hours under standard conditions. After this time, cells were further treated with MitoTracker Deep Red (5 µL, 3 µM solution) to a final concentration of 300 nM using a Biomek Liquid Handling Robot. MitoTracker treated cells were incubated for 30 minutes at 37 °C prior to fixing. Cells were fixed by the addition of formaldehyde (20 µL, 15% in PBS) and incubated for 30 minutes at room temperature and washed twice with PBS using a Biotek plate washer. Cells were permeabilised with Triton-X100 (50µL, 0.1% in PBS) and washed with PBS twice again. Staining solution of Hoescht 33342 (4 µgmL-1, Molecular Probes), STYO 14 (3 µM, Invitrogen), Phalloidin 594 (0.14 x, AbCam), Wheat germ agglutinin Alexa Fluor 594 (1 µgmL-1, Invitrogen) and Concanavalin A Alexa Fluor 488 (20 µgmL-1, Invitrogen) was prepared in bovine serum albumin solution (1%) and was added to each well (25 µL) and incubated for 30 minutes in the dark at room temperature, followed by three washes of PBS and no final aspiration. Plates were foil sealed. Plates were imaged on an ImageXpress micro XLS (Molecular Devices, USA) equipped with a robotic plate loader (Scara4, UK). Four fields of view were captured per well using a 20x objective and five filters (CY3, CY5, DAPI, FITC, TxRED). Each field of view typically contained 300 – 400 cells. Images were analysed by two methods: Cell nuclei counts were determined by ImageXpress MetaXpress software and access images. CellProfiler v3.0.0 image analysis software was used to segment the cells and extract 733 features per cell per image. The pipeline first identified nuclei from the DAPI channel, using these as seeds to aid segmentation of cells via an algorithm identifying cell boundaries from the TxRed channel. These two masks were subtracted to identify the cytoplasm. These three masks; nuceli, cell membrane and cytoplasm, were used to measure morphological features including size, shape, texture, and intensity of object across the five channels. Cell-level data was aggregated to image-level by taking the median of each feature per image. CellProfiler quality metrics were used to remove low-quality images and image artifacts. Images with fewer than 20 cells were also removed from the final analysis. For the remaining images, features were normalised on a plate-by-plate basis relative to median DMSO control. Features with NA values or zero or near-zero variance were removed. All remaining features were scaled and centred globally prior to aggregation to the compound level. Data was analysed using HC StratoMineRTM to carry out multiparametric analysis and principal component analysis.

11. Supporting Figures and Tables

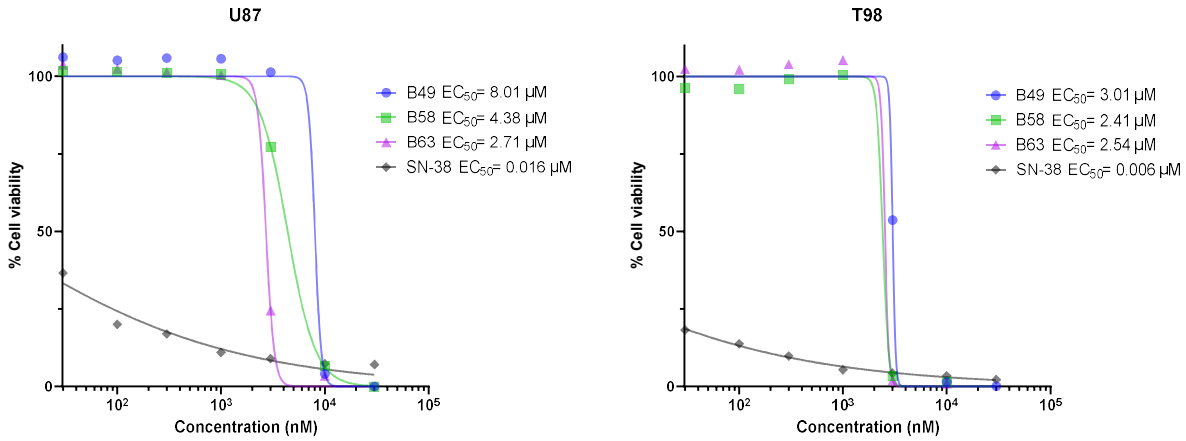


Figure S1. Seven-point dose response study. EC_{50} values calculated for **B49**, **B58**, **B63** and positive control SN38 against U87 and T98. Data obtained from a cell viability assay where PrestoBlue® reagent was added after 5 days drug treatment at 0.03, 0.1, 0.3, 1, 3, 10 and 30 µM doses. Error bars: ±SD from n =3.

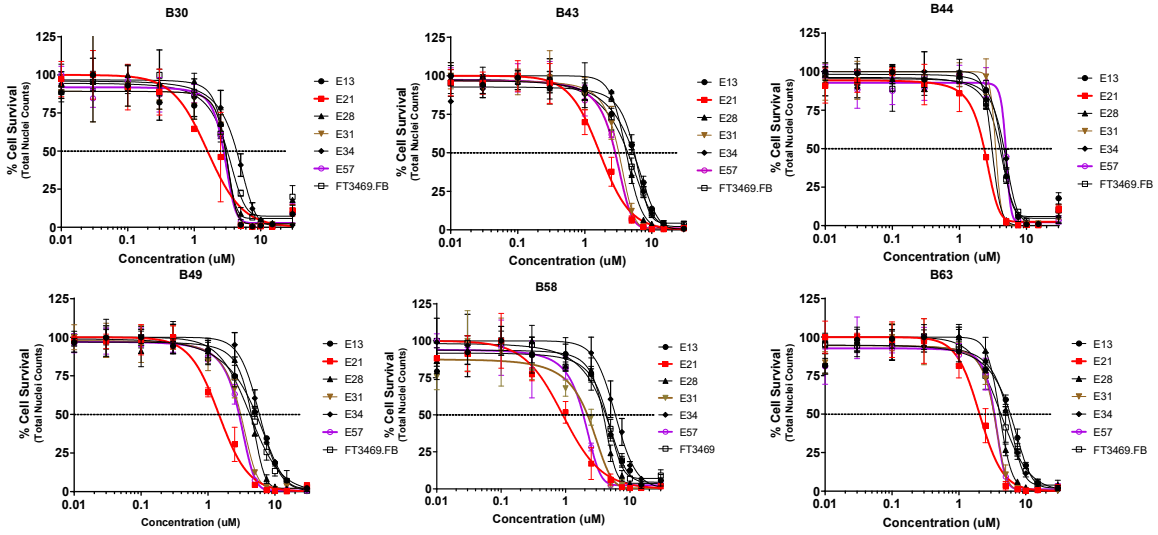


Figure S2. Dose response curves for **B44**, **B30**, **B43**, **B49**, **B63** and **B58** across GSCs tested, including non-transformed neural stem cells FT3469.

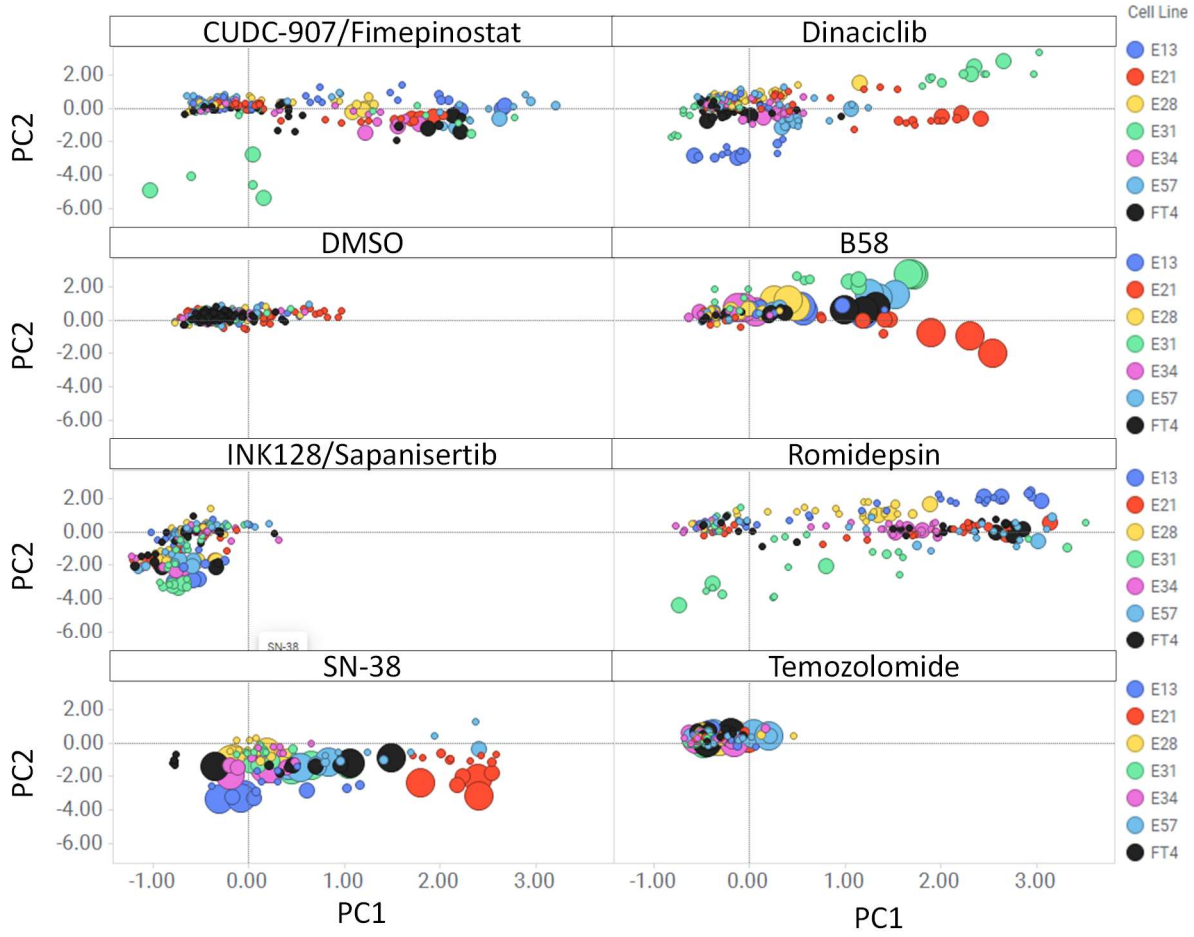


Figure S3. Principle component analysis (PC1 vs PC2) of a set of compounds with varied targets from Cell Painting image analysis. Compounds were treated by dose response across the glioma stem cell panel (colored). Scatter plots are sized by doses from 2.5 μM (max) to 4 nM vs DMSO controls. Data was normalized to DMSO.

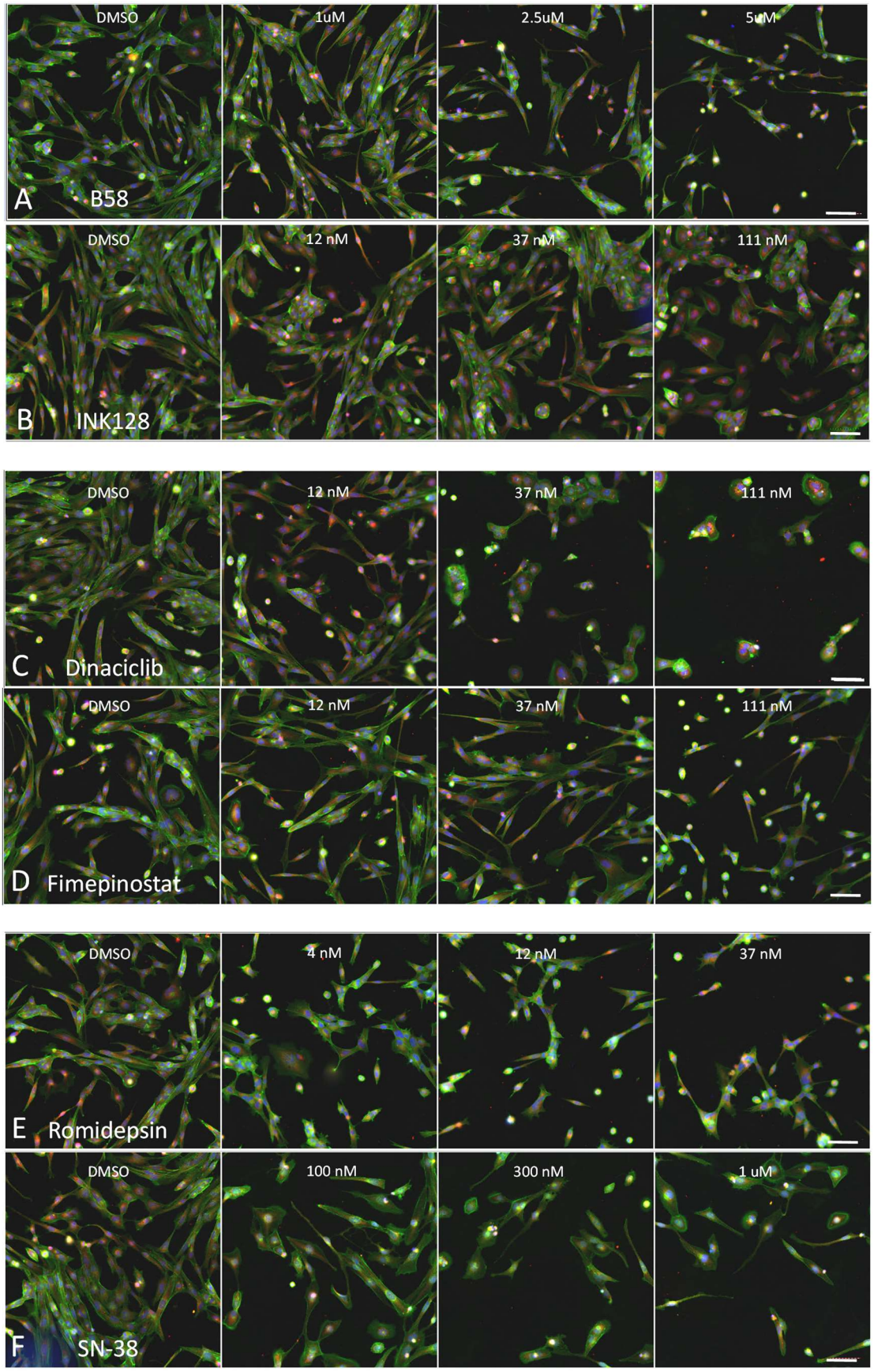


Figure S4. Representative Images of compound phenotypes on GCGR-E13 cells. Doses at approximate EC50 value vs DMSO. Scale bar 100 µm.

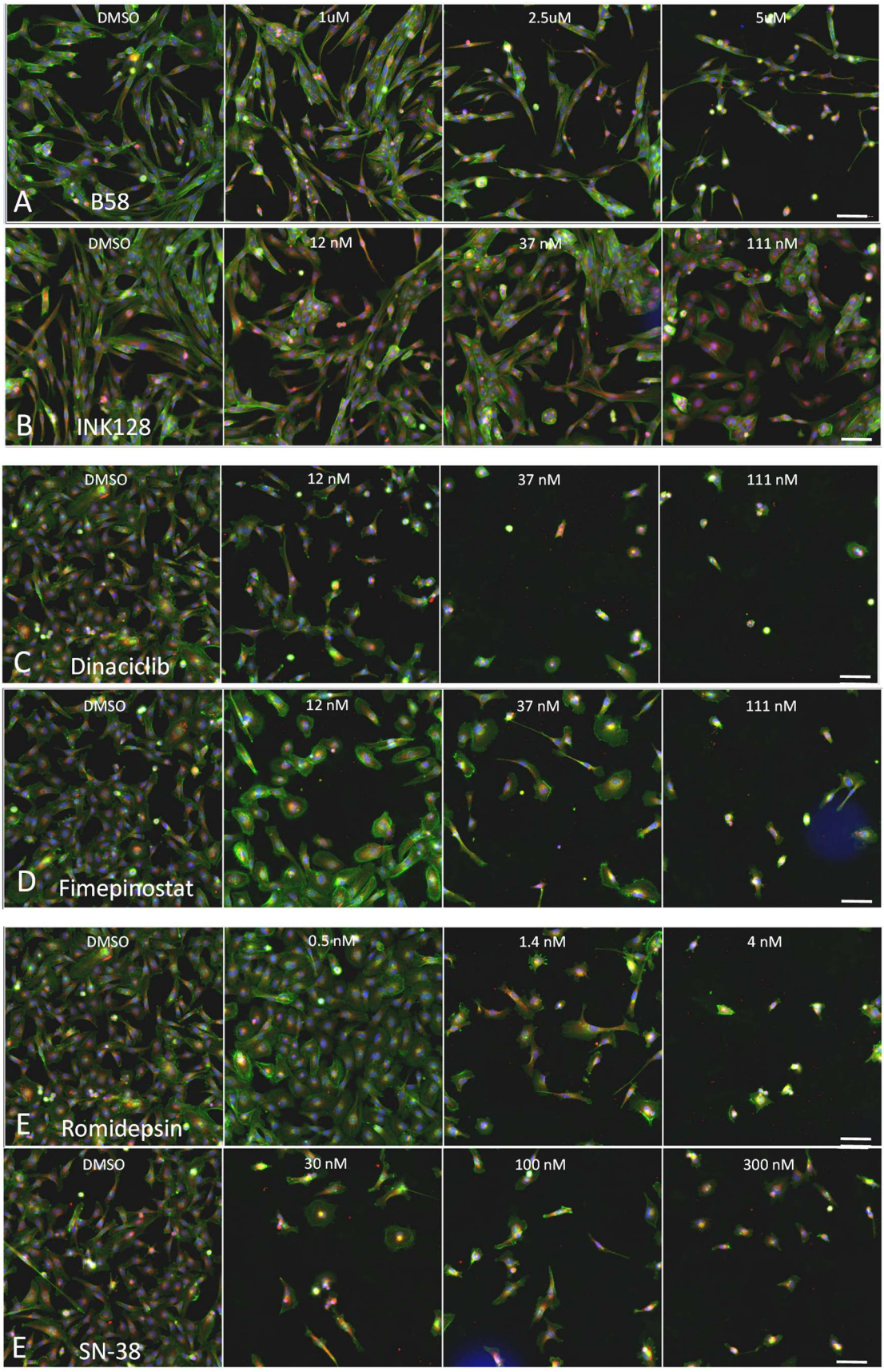


Figure S5. Representative Images of compound phenotypes on GCGR-E21 cells. Doses at approximate EC50 value vs DMSO. Scale bar 100 µm.

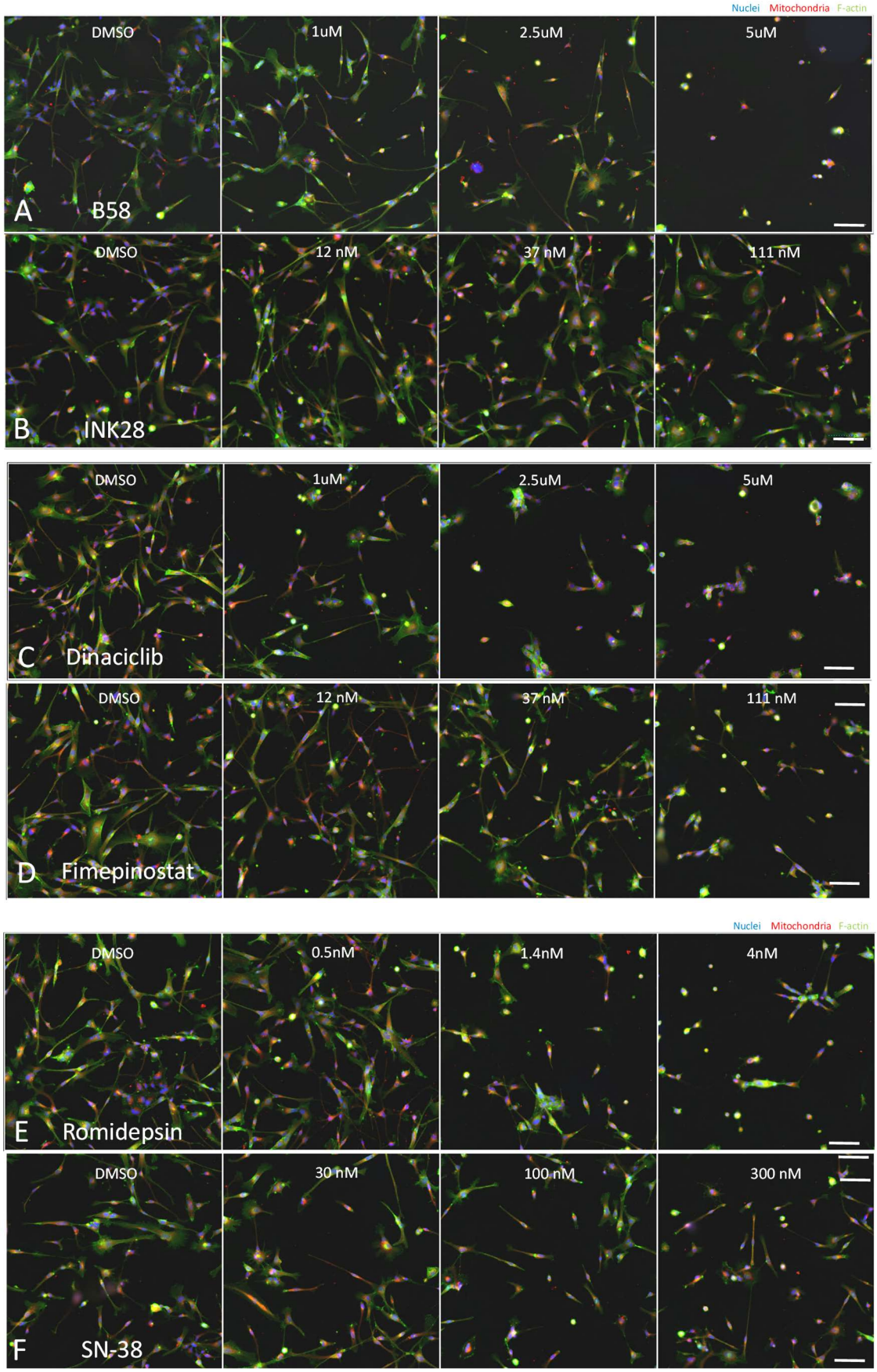


Figure S6. Representative Images of compound phenotypes on GCGR-E28 cells. Doses at approximate EC50 value vs DMSO. Scale bar 100 µm.

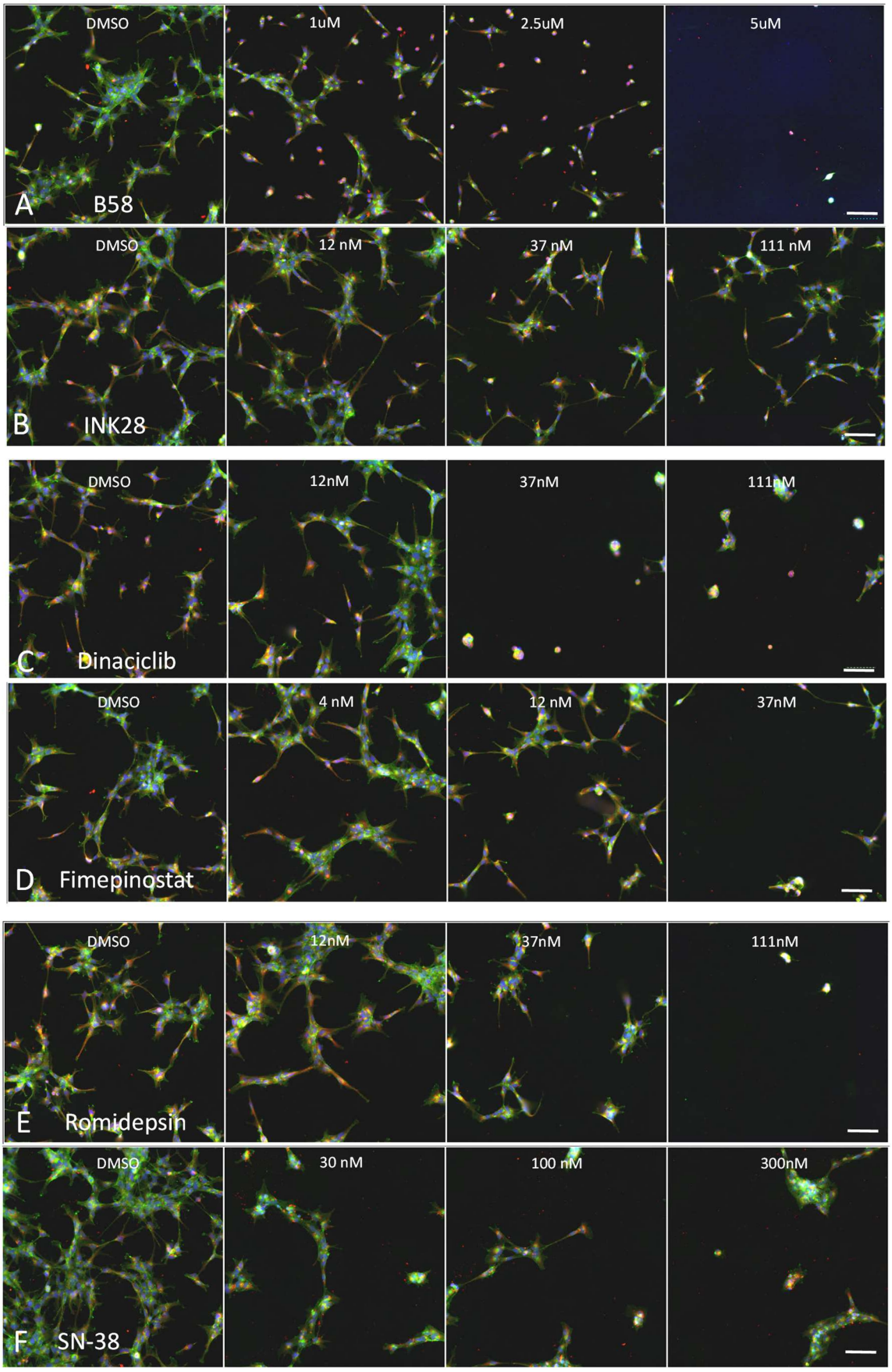


Figure S7. Representative Images of compound phenotypes on GCGR-E31 cells. Doses at approximate EC50 value vs DMSO. Scale bar 100 µm.

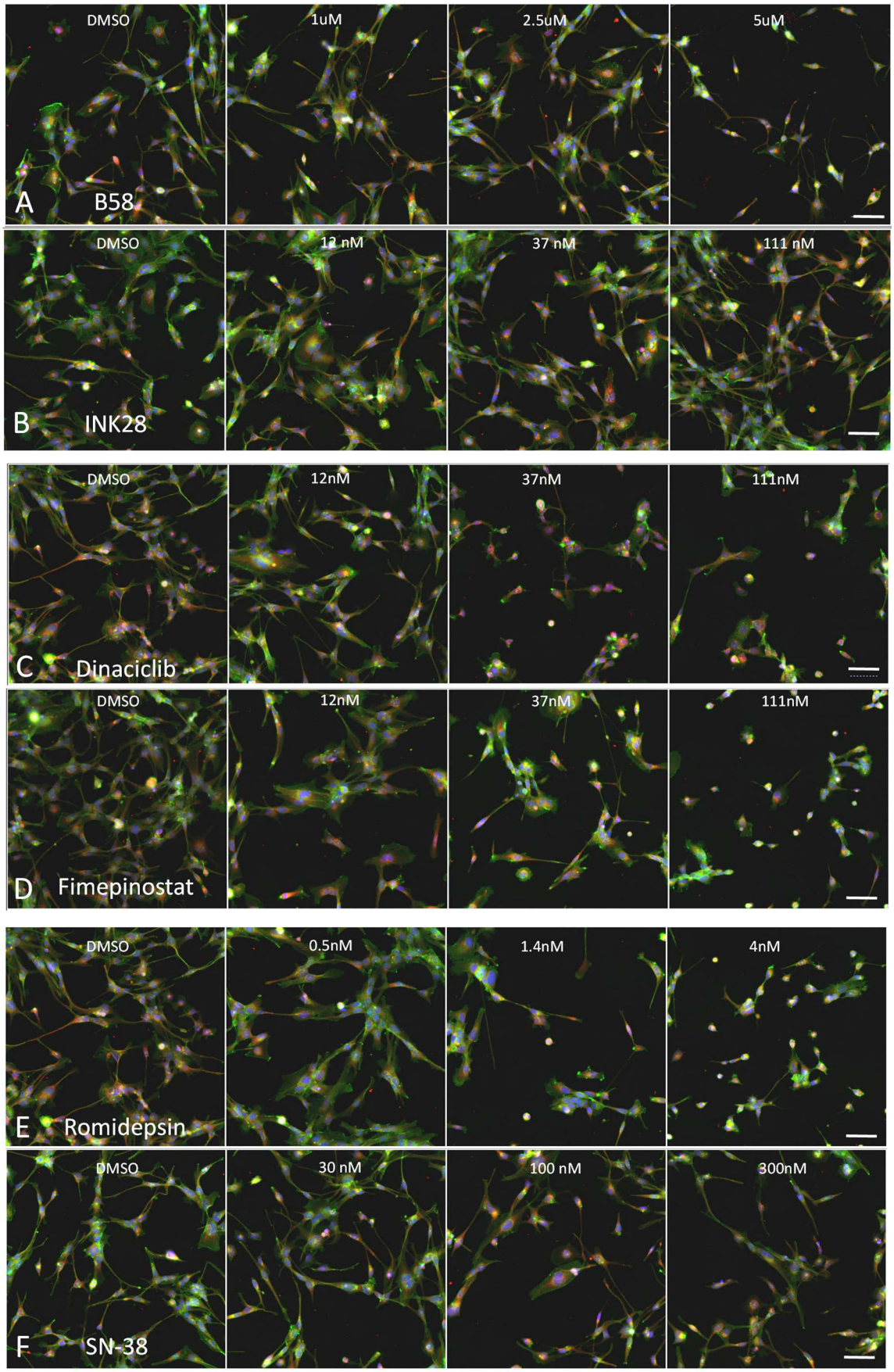


Figure S8. Representative Images of compound phenotypes on GCGR-E34 cells. Doses at approximate EC50 value vs DMSO. Scale bar 100 µm.

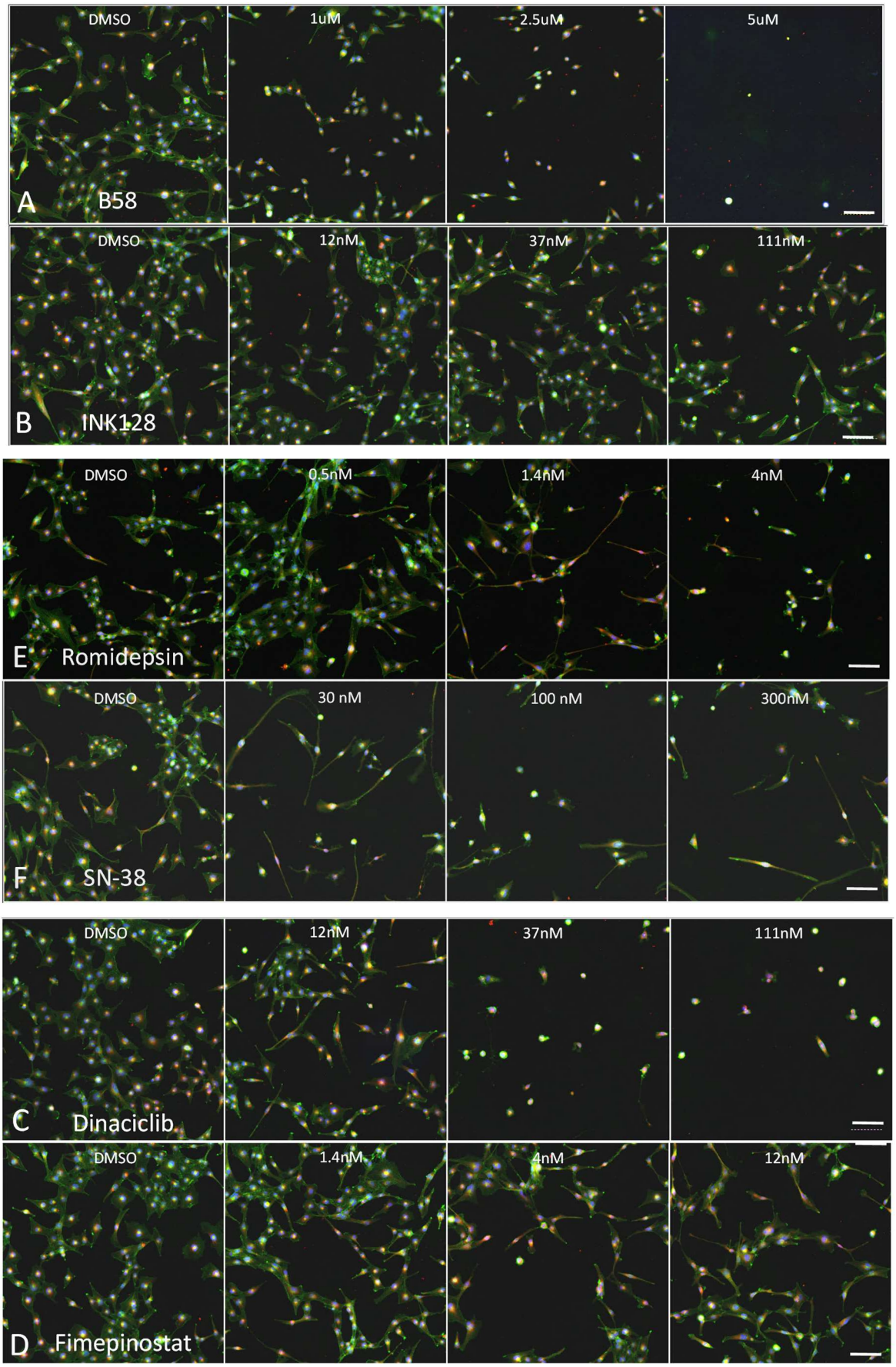


Figure S9. Representative Images of compound phenotypes on GCGR-E57 cells. Doses at approximate EC50 value vs DMSO. Scale bar 100 µm.

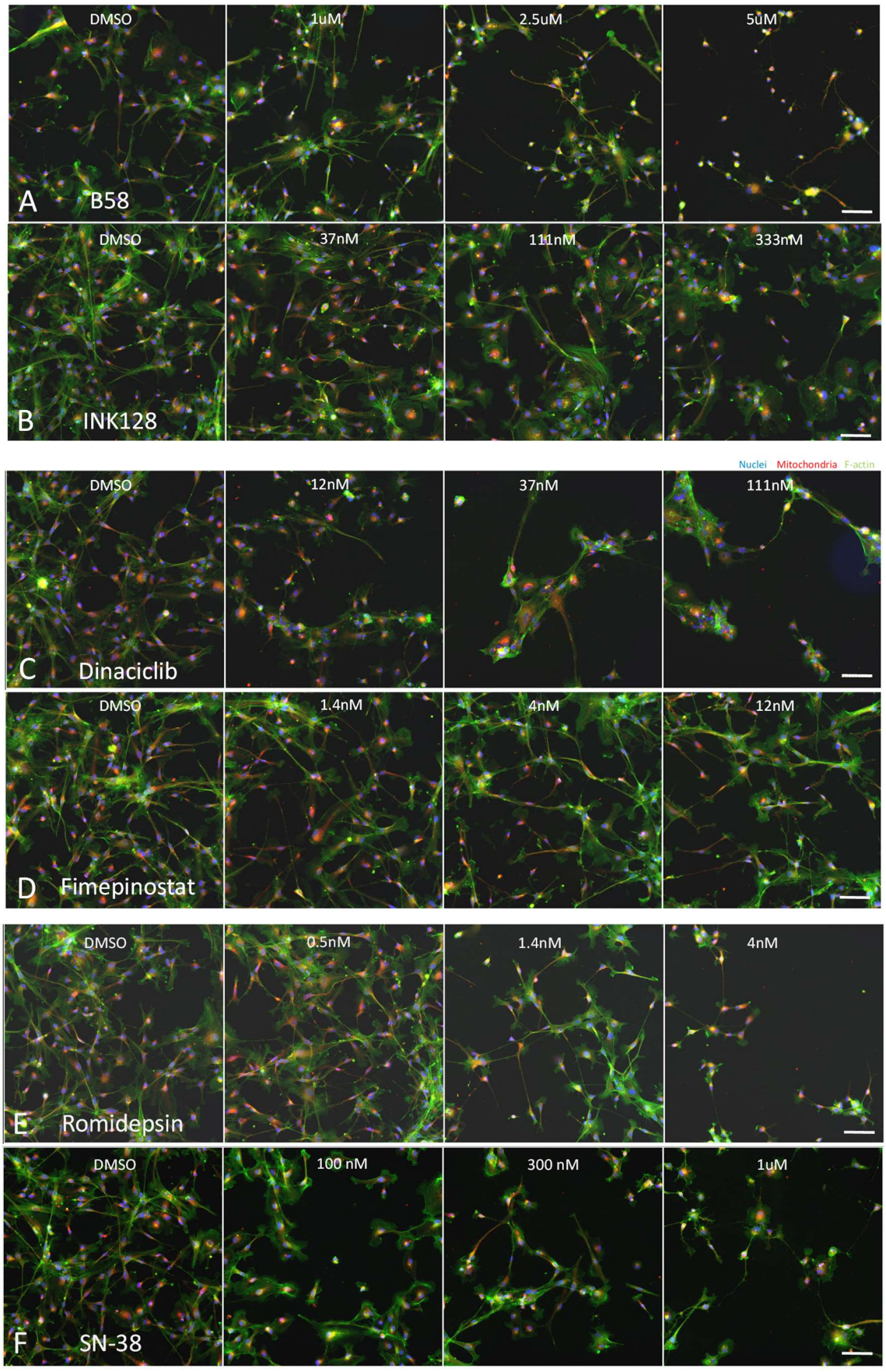


Figure S10. Representative Images of compound phenotypes on NSC FT3469 cells. Doses at approximate EC50 value vs DMSO. Scale bar 100 µm.

Table S1. Kinase inhibition activity of **B58** at three concentrations (100, 1000 and 10000 nM) across a broad panel of 74 protein kinases.

		% Inhibition		
Concentration (nM)	**Kinase Tested**	**Point 1**	**Point 2**	**Mean**
100	ABL1	2	-1	1
100	ACVR1B (ALK4)	-2	3	0
100	AKT1 (PKB alpha)	4	5	4
100	AMPK A1/B1/G1	-1	1	0
100	AURKB (Aurora B)	9	8	8
100	BMX	15	6	11
100	BRAF	-4	-2	-3
100	BTK	5	11	8
100	CAMK4 (CaMKIV)	3	4	4
100	CDK2/cyclin A	6	8	7
100	CDK5/p25	1	1	1
100	CDK7/cyclin H/MNAT1	-8	-3	-6
100	CDK9/cyclin T1	6	4	5
100	CHEK1 (CHK1)	0	4	2
100	CHEK2 (CHK2)	10	1	6
100	CLK2	8	8	8
100	**CSF1R (FMS)**	**48**	**40**	**44**
100	CSNK1A1 (CK1 alpha 1)	2	1	2
100	DAPK1	-1	4	1
100	DYRK1A	4	5	4
100	EGFR (ErbB1)	6	21	14
100	EPHA3	0	-11	-6
100	EPHB2	7	-1	3
100	FGFR2	1	-1	0
100	FLT3	15	10	13
100	GRK4	7	7	7
100	GSK3B (GSK3 beta)	-3	-5	-4
100	HCK	2	1	2
100	IKBKB (IKK beta)	-2	-1	-1
100	INSR	5	10	7
100	IRAK1	4	7	6
100	IRAK4	-5	6	0
100	ITK	4	-3	1
100	JAK1	2	2	2
100	KDR (VEGFR2)	14	11	12
100	KIT	1	0	1
100	LCK	16	19	17
100	LRRK2	0	-3	-1
100	MAP2K1 (MEK1)	4	13	9
100	MAP2K6 (MKK6)	8	-7	0
100	MAPK1 (ERK2)	-2	5	2

100	MAPK13 (p38 delta)	1	0	1
100	MAPK14 (p38 alpha) Direct	6	11	8
100	MAPK8 (JNK1)	-1	3	1
100	MAPKAPK2	1	-6	-2
100	MARK2	4	3	4
100	MET (cMet)	8	5	6
100	MINK1	10	12	11
100	MST4	9	8	9
100	NEK2	0	4	2
100	NEK4	8	11	9
100	NTRK1 (TRKA)	6	6	6
100	NUAK1 (ARK5)	7	-8	0
100	PAK1	1	4	2
100	PDK1 Direct	-3	2	0
100	PIK3CA/PIK3R1 (p110 alpha/p85 alpha)	5	-12	-3
100	PIM1	3	0	1
100	PKN1 (PRK1)	4	-5	-1
100	PLK1	0	-1	-1
100	PRKACA (PKA)	2	3	3
100	PRKCI (PKC iota)	11	8	9
100	PRKCQ (PKC theta)	-2	1	-1
100	PRKG2 (PKG2)	4	9	7
100	PTK6 (Brk)	0	-3	-2
100	RAF1 (cRAF) Y340D Y341D	1	-10	-5
100	ROCK1	0	-11	-5
100	RPS6KA3 (RSK2)	2	8	5
100	RPS6KB1 (p70S6K)	5	8	6
100	SGK (SGK1)	7	5	6
100	SRC	-1	-1	-1
100	STK22D (TSSK1)	5	2	4
100	SYK	0	5	2
100	TAOK2 (TAO1)	-1	-5	-3
100	TBK1	0	3	2

		% Inhibition		
Concentration (nM)	**Kinase Tested**	**Point 1**	**Point 2**	**Mean**
1000	ABL1	6	6	6
1000	ACVR1B (ALK4)	4	6	5
1000	AKT1 (PKB alpha)	5	5	5
1000	AMPK A1/B1/G1	12	20	16
1000	AURKB (Aurora B)	21	22	21
1000	BMX	-5	12	4
1000	BRAF	1	0	1
1000	BTK	5	6	5
1000	CAMK4 (CaMKIV)	9	5	7
1000	CDK2/cyclin A	8	9	9
1000	CDK5/p25	1	6	3
1000	CDK7/cyclin H/MNAT1	-4	6	1
1000	CDK9/cyclin T1	-1	3	1
1000	CHEK1 (CHK1)	1	-10	-5
1000	CHEK2 (CHK2)	9	5	7
1000	CLK2	11	11	11
1000	**CSF1R (FMS)**	**82**	**81**	**82**
1000	CSNK1A1 (CK1 alpha 1)	4	0	2
1000	DAPK1	-6	2	-2
1000	DYRK1A	4	4	4
1000	EGFR (ErbB1)	8	7	7
1000	EPHA3	-13	-5	-9
1000	EPHB2	7	10	8
1000	FGFR2	9	8	8
1000	FLT3	26	22	24
1000	GRK4	18	4	11
1000	GSK3B (GSK3 beta)	-1	-1	-1
1000	HCK	1	3	2
1000	IKBKB (IKK beta)	1	1	1
1000	INSR	12	4	8
1000	IRAK1	13	13	13
1000	IRAK4	-8	14	3
1000	ITK	10	0	5
1000	JAK1	2	-1	0
1000	KDR (VEGFR2)	14	15	14
1000	KIT	7	8	7
1000	LCK	31	32	31
1000	LRRK2	4	-4	0
1000	MAP2K1 (MEK1)	9	8	9
1000	MAP2K6 (MKK6)	5	8	6
1000	MAPK1 (ERK2)	3	10	7
1000	MAPK13 (p38 delta)	-1	0	0
1000	MAPK14 (p38 alpha) Direct	4	2	3
1000	MAPK8 (JNK1)	3	7	5
1000	MAPKAPK2	-2	-2	-2

1000	MARK2	3	3	3
1000	MET (cMet)	30	25	28
1000	MINK1	21	13	17
1000	MST4	24	16	20
1000	NEK2	-4	11	4
1000	NEK4	7	9	8
1000	NTRK1 (TRKA)	20	15	17
1000	NUAK1 (ARK5)	7	14	10
1000	PAK1	5	3	4
1000	PDK1 Direct	5	2	4
1000	PIK3CA/PIK3R1 (p110 alpha/p85 alpha)	2	7	4
1000	PIM1	1	2	2
1000	PKN1 (PRK1)	-1	3	1
1000	PLK1	-2	-9	-5
1000	PRKACA (PKA)	7	4	6
1000	PRKCI (PKC iota)	7	4	5
1000	PRKCQ (PKC theta)	11	28	19
1000	PRKG2 (PKG2)	5	8	6
1000	PTK6 (Brk)	4	-1	1
1000	RAF1 (cRAF) Y340D Y341D	-4	-4	-4
1000	ROCK1	3	-6	-1
1000	RPS6KA3 (RSK2)	-1	5	2
1000	RPS6KB1 (p70S6K)	1	6	4
1000	SGK (SGK1)	11	8	9
1000	SRC	11	13	12
1000	STK22D (TSSK1)	-1	-4	-2
1000	SYK	-1	2	0
1000	TAOK2 (TAO1)	2	3	2
1000	TBK1	4	3	3

		% Inhibition		
Concentration (nM)	**Kinase Tested**	**Point 1**	**Point 2**	**Mean**
10000	ABL1	28	34	31
10000	ACVR1B (ALK4)	-14	-3	-8
10000	AKT1 (PKB alpha)	8	7	7
10000	AMPK A1/B1/G1	67	70	68
10000	AURKB (Aurora B)	52	50	51
10000	BMX	8	20	14
10000	BRAF	4	-2	1
10000	BTK	27	18	22
10000	CAMK4 (CaMKIV)	26	21	23
10000	CDK2/cyclin A	5	3	4
10000	CDK5/p25	4	3	3
10000	CDK7/cyclin H/MNAT1	-7	19	6
10000	CDK9/cyclin T1	-14	-10	-12
10000	CHEK1 (CHK1)	8	22	15
10000	CHEK2 (CHK2)	14	19	17
10000	CLK2	35	31	33
10000	**CSF1R (FMS)**	**110**	**104**	**107**
10000	CSNK1A1 (CK1 alpha 1)	-6	8	1
10000	DAPK1	7	8	7
10000	DYRK1A	4	-1	2
10000	EGFR (ErbB1)	10	24	17
10000	EPHA3	-5	-1	-3
10000	EPHB2	34	28	31
10000	FGFR2	45	46	46
10000	FLT3	55	62	59
10000	GRK4	-8	-4	-6
10000	GSK3B (GSK3 beta)	7	7	7
10000	HCK	21	18	19
10000	IKBKB (IKK beta)	2	2	2
10000	INSR	33	28	31
10000	IRAK1	37	38	37
10000	IRAK4	-2	1	0
10000	ITK	19	11	15
10000	JAK1	3	1	2
10000	KDR (VEGFR2)	56	56	56
10000	KIT	9	12	11
10000	LCK	72	84	78
10000	LRRK2	6	9	7
10000	MAP2K1 (MEK1)	10	17	13
10000	MAP2K6 (MKK6)	13	3	8
10000	MAPK1 (ERK2)	-2	6	2
10000	MAPK13 (p38 delta)	2	2	2
10000	MAPK14 (p38 alpha) Direct	6	2	4
10000	MAPK8 (JNK1)	6	10	8

10000	MAPKAPK2	-5	5	0
10000	MARK2	18	15	16
10000	MET (cMet)	70	74	72
10000	MINK1	52	57	55
10000	MST4	79	76	78
10000	NEK2	9	21	15
10000	NEK4	14	10	12
10000	NTRK1 (TRKA)	65	65	65
10000	NUAK1 (ARK5)	50	46	48
10000	PAK1	0	3	1
10000	PDK1 Direct	-1	8	3
10000	PIK3CA/PIK3R1 (p110 alpha/p85 alpha)	3	-7	-2
10000	PIM1	0	3	2
10000	PKN1 (PRK1)	8	1	4
10000	PLK1	-7	-3	-5
10000	PRKACA (PKA)	14	11	12
10000	PRKCI (PKC iota)	11	1	6
10000	PRKCQ (PKC theta)	11	15	13
10000	PRKG2 (PKG2)	2	7	5
10000	PTK6 (Brk)	5	7	6
10000	RAF1 (cRAF) Y340D Y341D	-6	12	3
10000	ROCK1	-5	-3	-4
10000	RPS6KA3 (RSK2)	19	12	15
10000	RPS6KB1 (p70S6K)	9	11	10
10000	SGK (SGK1)	22	19	20
10000	SRC	40	41	41
10000	STK22D (TSSK1)	-6	-9	-8
10000	SYK	-1	0	-1
10000	TAOK2 (TAO1)	7	4	5
10000	TBK1	10	11	10

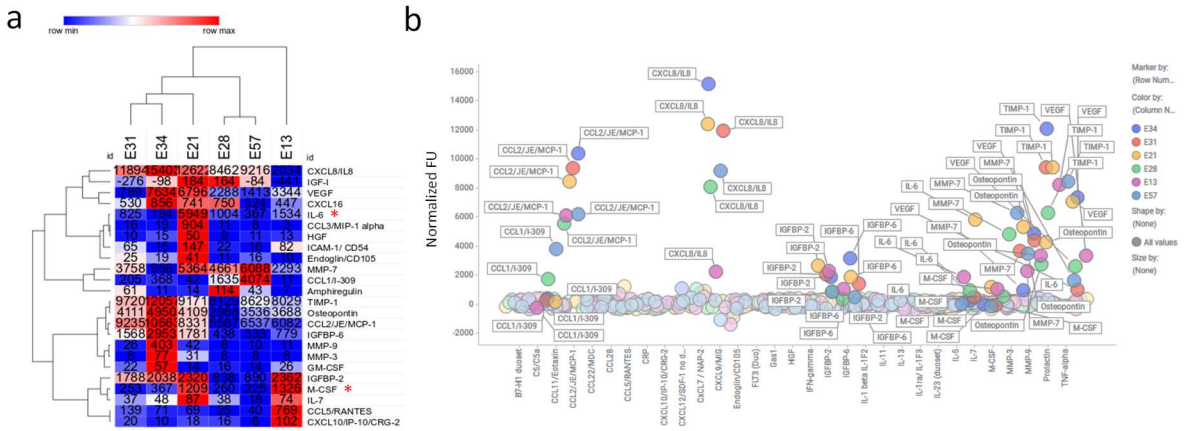


Figure S11. Forward-phase protein array [basal] cytokine analysis. **a)** Heatmap and hierarchical clustering of the most significant cytokines expressed at the basal level. Normalized FU values shown. IL-6 and M-CSF marked with *. **b)** Scatter plot of normalized FU across all 64 antibodies assayed against the glioma stem cell panel.

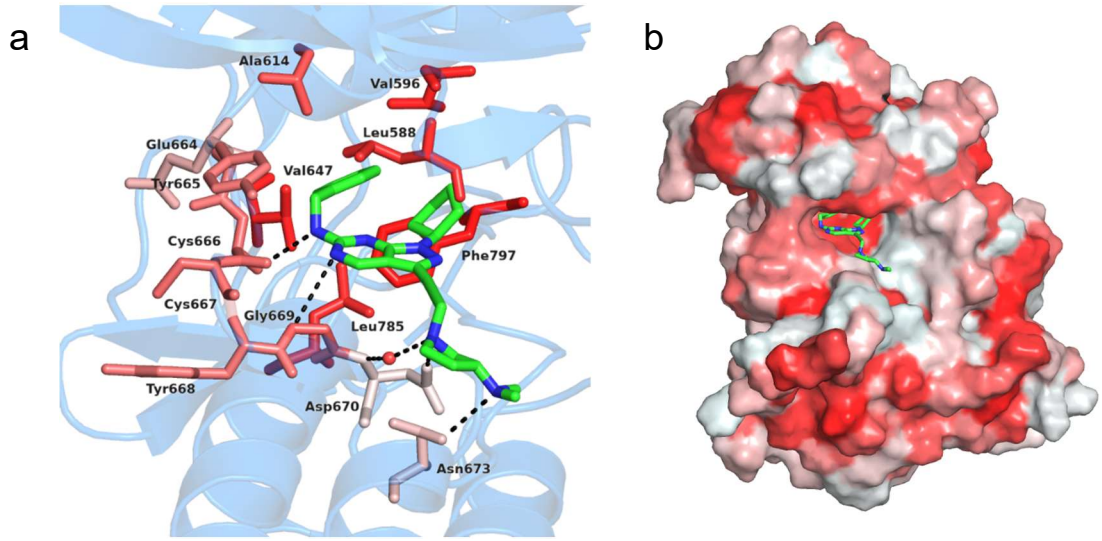


Figure S12. *In silico* docking of **B58** in the crystalized kinase domain of CSF-1R. **a)** Docking pose of **B58** in the ATP binding pocket of CSF-1R. The benzyl and cyclopentyl groups of B58 occupy a large hydrophobic pocket formed by Leu588, Val596, Ala614, Val747, Leu785 and Phe797. **b)** Surface representation of CSF-1R with **B58** docked in the ATP pocket. The displayed key residues and the protein surface are color-coded according to the Eisenberg hydrophobicity scale (red: hydrophobic; white: hydrophilic).