Exploring the chemical space around the privileged pyrazolo[3,4-*d*]pyrimidine scaffold: towards novel allosteric inhibitors of T315I-mutated Abl

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Contents:

General Methods

All commercially available chemicals were used as supplied without further purification. THF was dried over Na/benzophenone prior to use, $POCl_3$ was freshly distilled, while DMF was bought already anhydrous. Anhydrous reactions were run under a positive pressure of dry Argon. Microwave irradiation experiments were conducted using a CEM Discover Synthesis Unit (CEM Corp., Matthews, NC). The machine consists of a continuous focused microwave power delivery system with operator-selectable power output from 0 to 300 W. The temperature of the contents of the vessels was monitored using a calibrated infrared temperature control located under the reaction vessel.

Merck pre-coated TLC plates (silica gel 60 F_{254}, 0.25 mm) were used. UV detection was monitored at 254 nm. Chromatographic purifications were performed on columns packed with Merk 60 silica gel, 23-400 mesh, for flash technique. ^{1}H NMR and ^{13}C NMR spectra were obtained using a Bruker Avance DPX400 (Bruker Biospin, Germany). Chemical shifts were reported in parts per million (ppm) from tetramethylsilane as internal standard. Multiplicities were indicated as follow: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), dt (doublet of triplet), td (triplet of doublet), bs (broad singlet). Coupling constants were reported in hertz (Hz). LC-MS was performed with the UV detection at 254 nm and a single quadrupole mass spectrometer using electrospray ionization (ESI) source.

1. Chemistry

2-(Methylthio)pyrimidine-4,6-diol (2)

A mixture of KOH (5.84 g, 104.1 mmol, 3.00 eq.), H_2O (50 mL), 2-thiobarbituric acid (**1**) (5.00 g, 34.7 mmol, 1.00 eq.) and MeI (2.4 mL, 38.2 mmol, 1.10 eq.) was stirred under reflux for 3h. The transparent solution was allowed to cool to r.t., then acidified with 3N HCl until pH~2. The product

was obtained as a light yellow precipitate, recovered from the reaction mixture by filtration under vacuum. Yield: 87%. Pale yellow solid. **^{1}H NMR** ($(CD_3)_2S=O$): 11.7 (bs, 2H), 5.12 (s, 1H); 2.06 (s, 3H). **^{13}C NMR** ($(CD_3)_2S=O$) : 167.6, 164.0, 86.0, 13.2. **MS** (ESI) *m/z*: 159 $[M + H]^+$, 181 $[M + Na]^+$.

4,6-Dichloro-2-(methylthio)pyrimidine-5-carbaldehyde (3a)

To an ice-cooled solution of freshly distilled $POCl_3$ (4.40 mL, 47.5 mmol, 7.50 eq.) was slowly added anhydrous DMF (1.60 mL, 19.0 mmol, 3.00 eq.). The mixture was stirred at r.t. for 1h, during this time it turned to bright yellow. 2-(methylthio)pyrimidine-4,6-diol (**2**) (1.00 g, 6.33 mmol, 1.00 eq.) was added in three times over 30 min and the reaction mixture was stirred under reflux o.n. Once cooled to r.t., H_2O (50 mL) was added and the suspension was stirred at r.t. o.n. After this time extraction with EtOAc (3 x 50 mL) was performed, the collected organic phases were dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The yellow oily residue obtained was purified by flash chromatography (Hex:EtOAc = 99:1) to obtain both products as a white solid. Yield: 42%. White solid. **^{1}H NMR** ($CDCl_3$): 10.3 (s, 1H), 2.55 (s, 3H). **^{13}C NMR** ($CDCl_3$): 185.2, 176.9, 162.2, 118.9, 14.7.

4,6-Dichloro-2-(methylthio)pyrimidine (3b)

2-(Methylthio)pyrimidine-4,6-diol (**2**) (1.00 g, 6.33 mmol, 1.00 eq.) was added to a solution of freshly distilled $POCl_3$ (4.40 mL, 47.5 mmol, 7.50 eq.). The yellow solution was reflux o.n., then allowed to cool to r.t. H_2O (50 mL) was added and extraction with EtOAc (3 x 50 mL) was performed, the collected organic phases were dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The yellow oily residue obtained was purified by flash chromatography (Hex:EtOAc = 99:1) to obtain the products as a yellow oil which solidified under vacuum. Yield:

80%. White solid. **^{1}H NMR** ($CDCl_3$): 6.89 (s, 1H); 2.57 (s, 3H). **^{13}C NMR** ($CDCl_3$): 174.1, 161.2, 115.6, 13.9. **MS** (ESI) *m/z*: 196 $[M + H]^+$.

4-Chloro-6-(methylthio)-1H-pyrazolo[3,4-*d*]pyrimidine (4)

Synthesized according **GP1**. Yield: 97%. Yellow solid.**^{1}H NMR** ($CDCl_3$): 8.09 (s, 1H), 2.65 (s, 3H). **^{13}C NMR** ($CDCl_3$): 171.1, 155.5, 154.4, 133.7, 110.3, 14.6. **MS** (ESI) *m/z*:199 $[M - H]^-$.

4,6-Dichloro-2-(methylthio)pyrimidin-5-yl)(phenyl)methanol (5)

Synthesized according to **GP4**. Yield: 40%. Light brown solid. **^{1}H NMR** ($CDCl_3$): 7.32 (m, 5H); 6.48 (s, 1H); 2.56 (s, 3H). **^{13}C NMR** ($CDCl_3$): 172.3, 161.1, 139.9, 128.5, 127.8, 127.0, 125.3, 70.5, 14.4.

(4,6-Dichloro-2-(methylthio)pyrimidin-5-yl)(phenyl)methanone (6)

Synthesized according to **GP5.** Yield: 60%. White solid. **^{1}H NMR** ($CDCl_3$): 7.84 (d, J = 7.6 Hz, 2H); 7.67 (t, J = 7.6 Hz, 1H); 7.52 (t, J = 7.6 Hz, 2H); 2.62 (s, 3H). **^{13}C NMR** ($CDCl_3$): 189.3, 174.4, 158.0, 135.0, 129.6, 129.3, 129.2, 126.0, 14.5.

4-Chloro-6-(methylthio)-3-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (7)

Synthesized according to **GP1**. Yield: quant. Light yellow solid.**^{1}H NMR** (CD_2Cl_2+0.05 mL MeOD): 7.73 (m, 2H); 7.48 (m, 3H); 2.63 (s, 3H). **^{13}C NMR** (CD_2Cl_2+0.05 mL MeOD): 169.9, 156.8, 154.2, 146.1, 135.0, 129.9, 129.0, 128.1, 107.4, 13.9.

4-Chloro-6-(methylthio)-1-phenethyl-1H-pyrazolo[3,4-*d*]pyrimidine (8a)

Synthesized according to **GP2**. Yield: 71%. Light yellow oil. **^{1}H NMR** ($CDCl_3$): 7.97 (s, 1H), 7.16 (m, 5H), 4.63 (t, *J* = 7.4 Hz, 2H), 3.22 (t, *J* = 7.4 Hz, 2H), 2.55 (s, 3H). **^{13}C NMR** ($CDCl_3$): 169.5, 153.9, 153.7, 137.6, 132.3, 128.7,128.5, 126.7, 110.3, 48.7, 35.7, 14.4.

1-Benzyl-4-chloro-6-(methylthio)-1H-pyrazolo[3,4-*d*]pyrimidine (8b)

Synthesized according to **GP2**. Yield: 61%. Light yellow oil. **^{1}H NMR** ($CDCl_3$): 8.01 (s, 1H), 7.30 (m, 5H), 5.57 (s, 2H), 2.64 (s, 3H). **^{13}C NMR** ($CDCl_3$):170.3, 154.0, 153.9, 135.7, 132.7, 128.7, 128.2, 110.9, 51.3, 14.5.

4-Chloro-1-(2-methoxy-2-phenylethyl)-6-(methylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidine (8c)

Synthesized according to **GP2**. Yield: 64%. White solid. **^{1}H NMR** ($CDCl_3$): 8.01 (s, 1H), 7.30 (m, 5H), 4.81 (m, 1H), 4.73 (m, 1H), 4.48 (m, *J* = 4.8, 13.6 Hz, 1H), 3.19 (s, 3H), 2.58 (s, 3H). **^{13}C NMR** ($CDCl_3$): 169.9, 154.3, 153.7, 138.3, 132.4, 128.6, 128.4, 126.7, 110.3, 81.6, 57.0, 53.1, 14.4

4-Chloro-6-(methylthio)-1-(prop-2-ynyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (8d)

Synthesized according to **GP2**. Yield: 38%. White solid. **^{1}H NMR** ($CDCl_3$): 8.05 (s, 1H), 5.18 (s, 2H), 2.63 (s, 3H), 2.39 (s, 1H). **^{13}C NMR** ($CDCl_3$): 170.8, 154.0, 153.8, 133.3, 110.6, 76.4, 73.6, 36.8, 14.6.

4-Chloro-6-(methylthio)-1-phenethyl-3-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (8e)

Synthesized according to **GP2**. Yield: 72%. White solid. **^{1}H NMR** ($CDCl_3$): 7.77 (m, 2H); 7.50 (m, 3H); 7.22 (m, 5H); 4.70 (t, *J* = 7.6 Hz, 2H); 3.28 (t, *J* = 7.6 Hz, 2H); 2.60 (s, 3H). **^{13}C NMR** ($CDCl_3$): 169.6, 155.0, 154.1, 145.4, 137.8, 131.3, 129.9, 129.1, 128.8, 128.6, 128.2, 126.8, 107.8, 43.7, 35.8, 14.4.

4-Chloro-1-(2-methoxy-2-phenylethyl)-6-(methylthio)-3-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (8f)

Synthesized according to **GP2**. Yield: 69%. White solid. **^{1}H NMR** ($CDCl_3$): 7.77 (m, 2H); 7.49 (m, 3H); 7.32 (m, 5H); 4.90 (m, 1H); 4.83 (m, 1H); 4.51 (dd, *J* = 4.8 Hz, 8.8 Hz, 1H); 3.22 (s, 3H); 2.61 (s, 3H). **^{13}C NMR** ($CDCl_3$): 169.6, 155.6, 154.0, 145.5, 138.5, 131.33, 130.0, 129.1, 128.6, 128.5, 128.2, 126.9, 107.8, 81.5, 57.1, 53.0, 14.4.

***N*-(3-chlorophenyl)-6-(methylthio)-1-phenethyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (9a)**

Synthesized according to **GP3** from **8a**; purified by crystallization from EtOH. Yield: 92%.White solid. **^{1}H NMR** ($CDCl_3$+0.05mL CD_3OD): 7.62 (s, 1H), 7.20 (m, 9H), 4.46 (t, *J* = 7.6 Hz, 2H), 3.11 (t, *J* = 7.4 Hz, 2H), 2.50 (s, 1H). **^{13}C NMR** ($CDCl_3$+0.05mL CD_3OD): 168.4, 154.1, 153.7, 138.7, 137.9, 134.9, 131.6, 130.2, 128.7, 128.5, 126.6, 125.9, 123.6, 121.4, 98.1, 48.3, 35.7, 14.2. **MS** (ESI) *m/z*: 396 $[M+H]^+$.

***N*-Cyclohexyl-6-(methylthio)-1-phenethyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (9b)**

Synthesized according to **GP3** from **8a**; purified by crystallization from EtOH. Yield: 40%. White solid. **^{1}H NMR** ($(CD_3)_2CO$): 7.87 (s, 1H), 7.20 (m, 5H), 4.50 (t, *J* = 7.4 Hz, 2H), 4.14 (bs, 1H), 3.19 (t, *J* = 7.4 Hz, 2H), 2.50 (s, 1H), 2.04 (m, 3H), 1.77 (d, *J* = 12.0 Hz, 2H), 1.65 (d, *J* = 12.8 Hz, 1H), 1.32 (m, 5H). **^{13}C NMR** ($(CD_3)_2CO$):168.9, 155.0, 153.9, 138.7, 130.9, 128.8, 128.3, 126.6, 98.4, 47.6, 35.3, 32.6, 25.5, 24.5, 13.6. **MS** (ESI) *m/z*: 368 $[M+H]^+$, 390 $[M+Na]^+$.

3-(6-(Methylthio)-1-phenethyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)phenol (9c)

Synthesized according to **GP3** from **8a**; the oily residue was dissolved in EtOAc and the organic phase was extracted two times with 0.5 N HCl. The organic layer was dried over Na_2SO_4, filtered then the solvent evaporated under reduced pressure to obtain the product as a light brown solid.

Yield: 84%. Light brown solid. **^{1}H NMR** ($CDCl_3$+0.05mL CD_3OD): 7.04 (m, 10H), 4.51 (t, *J* = 7.6 Hz, 2H), 3.13 (t, *J* = 7.6 Hz, 2H), 2.52 (s, 1H). **^{13}C NMR** ($CDCl_3$+0.05mL CD_3OD):169.2, 157.3, 155.2, 154.2, 138.4, 130.4, 128.7, 128.5, 126.6, 116.6, 114.2, 112.2, 97.9, 48.2, 35.7, 14.1. **MS** (ESI) *m/z*: 378 $[M+H]^+$.

6-(Methylthio)-1-phenethyl-*N*-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (9d)

Synthesized according to **GP3** from **8a**; purified by flash chromatography using DCM:MeOH = 99:1 as eluent. Yield: 91%. White solid. **^{1}H NMR** ($CDCl_3$): 7.60 (s, 1H), 7.44 (m, 4H), 7.22 (m, 4H), 4.54 (t, *J* = 7.6, 2H), 3.18 (t, *J* = 7.6, 2H), 2.57 (s, 3H). **^{13}C NMR** ($CDCl_3$): 169.3, 155.2, 154.5, 138.2, 137.7, 132.0, 131.8, 129.2, 128.8, 128.6, 126.5, 124.8, 97.9, 48.1, 35.7, 14.1. **MS** (ESI) *m/z*: 362 $[M+H]^+$.

6-(Methylthio)-1-phenethyl-*N*-(4-(trifluoromethoxy)phenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (9e)

Synthesized according to **GP3** from **8a**; purified by flash chromatography using DCM:MeOH = 99:1 as eluent. Yield: 75%. White solid. **^{1}H NMR** ($CDCl_3$): 7.50 (d, *J* = 8.4 Hz, 2H), 7.16 (m, 8H), 4.48 (t, *J* = 7.4 Hz, 2H), 3.11 (t, *J* = 7.6 Hz, 2H), 2.48 (s, 3H). **^{13}C NMR** ($CDCl_3$): 170.1, 155.3, 155.2, 146.2, 138.8, 137.2, 131.9, 129.5, 129.3, 127.4, 125.7, 122.7, 99.0, 49.1, 36.5, 15.0. **MS** (ESI) *m/z*: 446 $[M+H]^+$.

1-Benzyl-*N*-cyclohexyl-6-(methylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (9f)

Synthesized according to **GP3** from **8b**; the oily residue was dissolved in EtOAc and the organic phase was extracted twice with 0.5 N HCl. The organic layer was dried over Na_2SO_4, filtered then the solvent evaporated under reduced pressure to obtain the product as a light brown solid. Yield: 70%. Light brown solid. **^{1}H NMR** ($CDCl_3$):7.75 (s, 1H), 5.49 (s, 2H), 2.58 (s, 3H), 2.10 (d, *J* = 10.0 Hz, 2H), 1.80 (d, *J* = 12.4 Hz, 2H), 1.67 (d, *J* = 12.0 Hz, 1H), 1.36 (m, 7H). **^{13}C NMR**

($(CD_3)_2CO$): 170.2, 155.9, 154.9, 138.4, 132.2, 129.2, 128.7, 128.2, 99.3, 50.8, 50.2, 33.4, 26.2, 25.7, 14.0. **MS** (ESI) *m/z*: 354 $[M+H]^+$

3-(1-Benzyl-6-(methylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)phenol (9g)

Synthesized according to **GP3** from **8b**; the oily residue was dissolved in EtOAc and the organic phase was extracted twice with 0.5 N HCl. The organic layer was dried over Na_2SO_4, filtered then the solvent evaporated under reduced pressure to obtain the product as a light brown solid. Yield: 56%. Light brown solid. **^{1}H NMR** (CD_3OD): 7.89 (s, 1H), 7.25 (m, 6H), 7.16 (d, *J* = 5.2Hz, 2H), 6.67 (m, 1H)5.47 (s, 2H), 2.57 (s, 3H). **^{13}C NMR** ($(CD_3)_2S=O$): 169.2, 158.0, 154.2, 153.8, 140.2, 137.6, 132.9, 129.8, 129.0, 128.1, 128.0, 112.6, 111.4, 108.9, 99.2, 50.3, 14.2. **MS** (ESI) *m/z*: 364 $[M+H]^+$.

3-(1-(2-Methoxy-2-phenylethyl)-6-(methylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)phenol (9h)

Synthesized according to **GP3** from **8c**; the oily residue was dissolved in EtOAc and the organic phase was extracted twice with 0.5 N HCl. The organic layer was dried over Na_2SO_4, filtered then the solvent evaporated under reduced pressure to obtain the product as a light brown solid. Yield: 98%. Light brown solid. **^{1}H NMR** (CD_3OD+0.05mL $CDCl_3$): 7.87 (s, 1H), 7.19 (m, 8H), 6.60 (m, 1H), 4.73 (dd, *J* = 4.8, 8.2 Hz, 1H), 4.56 (dd, *J* = 8.4, 14 Hz, 1H), 4.32 (dd, *J* = 4.8, 14 Hz, 1H), 3,11 (s, 3H), 2.51 (s, 3H). **^{13}C NMR** (CD_3OD+0.05mL $CDCl_3$): 169.9, 157.4, 154.4, 154.1, 139.7, 138.5, 131.9, 129.1, 128.2, 128.0, 126.6, 113.2, 111.2, 109.1, 98.6, 81.8, 55.9, 52.2, 13.2. **MS** (ESI) *m/z*: 408 $[M+H]^+$.

1-(2-Methoxy-2-phenylethyl)-6-(methylthio)-*N*-(pyridin-4-ylmethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (9i)

Synthesized according to **GP3** from **8c**; the oily residue was dissolved in EtOAc and the organic phase was extracted twice with 0.5 N HCl. The organic layer was dried over Na_2SO_4, filtered then the solvent evaporated under reduced pressure to obtain the product as a light yellow solid. Yield: 81%. Light yellow solid. **^{1}H NMR** ($CDCl_3$): 8.40 (d, *J* = 5.6 Hz, 2H), 7.86 (s, 1H), 7.27 (m, 6H), 4.73 (m, 1H), 4.56 (dd, *J* = 8.8, 14 Hz, 1H), 4.32 (dd, *J* = 4.8, 14 Hz, 1H), 4.08 (s, 2H), 3.11 (s, 3H), 2.41 (s, 3H). **^{13}C NMR** ($CDCl_3$): 169.8, 156.0, 154.4, 149.1, 148.9, 138.5, 131.4, 128.4, 128.2, 126.7, 122.7, 98.9, 81.7, 56.7, 52.7, 43.1, 13.9. **MS** (ESI) *m/z*: 407 $[M+H]^+$.

1-(2-Methoxy-2-phenylethyl)-6-(methylthio)-*N*-(4-(trifluoromethoxy)phenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (9j)

Synthesized according to **GP3** from **8c**; purified by flash chromatography using DCM:MeOH = 99:1 as eluent. Yield: 88%. White solid. **^{1}H NMR** ($CDCl_3$): 7.69 (s, 1H), 7.60 (d, *J* = 8.8 Hz, 2H), 7.31 (m, 5H), 7.24 (d, *J* = 8.4 Hz, 2H), 4.80 (dd, *J* = 4.8, 8.4 Hz, 1H), 4.64 (dd, *J* = 8.8, 14 Hz, 1H), 4.39 (dd, *J* = 4.4, 14 Hz, 1H), 3.15 (s, 3H), 2.54 (s, 3H). **^{13}C NMR** ($CDCl_3$): 169.4, 154.6, 153.9, 145.8, 138.5, 136.8, 131.4, 128.4, 128.2, 126.7, 123.7, 121.6, 98.4, 81.6, 56.8, 52.7, 14.1. **MS** (ESI) *m/z*: 476 $[M+H]^+$.

3-(6-(Methylthio)-1-(prop-2-ynyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)phenol (9k)

Synthesized according to **GP3** from **8d**; the oily residue was dissolved in EtOAc and the organic phase was extracted two times with 0.5 N HCl. The organic layer was dried over Na_2SO_4, filtered then the solvent evaporated under reduced pressure to obtain the product as a light brown solid. Yield: quant. Light brown solid. **^{1}H NMR** ($CDCl_3$+0.05 mL CD_3OD): 7.53 (s, 1H), 7.09 (m, 3H), 6.61 (d, *J* = 7.6 Hz, 1H), 5.00 (d, *J* = 1.6 Hz, 2H), 2.51 (s, 3H), 2.32 (m, 1H). **^{13}C NMR**

($CDCl_3$+0.05 mL CD_3OD): 170.1, 157.2, 154.0, 153.9, 138.8, 133.1, 129.6, 114.5, 112.4, 110.2, 98.5, 72.8, 35.8, 29.4, 13.9. **MS** (ESI) *m/z*: 312 $[M+H]^+$, 334 $[M+Na]^+$.

_N_-Methyl-6-(methylthio)-1-(prop-2-ynyl)-_N_-(pyridin-3-yl)-1_H_-pyrazolo[3,4-_d_]pyrimidin-4-amine (9l)

Synthesized according to **GP3** from **8d**; the oily residue was dissolved in EtOAc and the organic phase was extracted twice with 0.5 N HCl. The organic layer was dried over Na_2SO_4, filtered then the solvent evaporated under reduced pressure to obtain the product as a light yellow solid. Yield: 92%. Light yellow solid. **^{1}H NMR** ($CDCl_3$): 8.56 (d, *J* = 3.6 Hz, 1H), 7.89 (s, 1H), 7.69 (t, *J* = 7.4 Hz, 1H), 7.24 (m, 2H), 5.09 (s, 1H), 4.86 (d, *J* = 4.4 Hz, 3H), 2.57 (s, 3H), 2.34 (m, 1H). **^{13}C NMR** ($CDCl_3$): 170.0, 156.1, 155.3, 153.9, 148.7, 137.2, 131.8, 122.7, 98.8, 72.8, 36.0, 29.7, 14.2. **MS** (ESI) *m/z*: 311 $[M+H]^+$, 333 $[M+Na]^+$.

6-(Methylthio)-1-(prop-2-yn-1-yl)-_N_-(4-(trifluoromethoxy)phenyl)-1_H_-pyrazolo[3,4-_d_]pyrimidin-4-amine (9m)

Synthesized according to **GP3** from **8d**. Yield: 89%. White solid. **^{1}H NMR** ($CDCl_3$): 7.70 (s, 1H); 7.61 (d, *J* = 9.2 Hz, 2H); 7.24 (m, 5H); 5.17 (d, *J* = 2.4 Hz, 1H); 2.62 (s, 3H); 2.38 (m, 1H). **^{13}C NMR** ($CDCl_3$): 169.5, 154.6, 154.0, 145.8, 136.9, 131.5, 128.3, 123.8, 121.6, 98.5, 73.0, 35.8, 29.4, 14.1. **MS (ESI)** *m/z*: 380 $[M+H]^+$.

_N_-Methyl-6-(methylthio)-1-phenethyl-3-phenyl-1_H_-pyrazolo[3,4-_d_]pyrimidin-4-amine (9n)

Synthesized according to **GP3** from **8e**; the oily residue was dissolved in EtOAc and the organic phase was extracted twice with 0.5 N HCl. The organic layer was dried over Na_2SO_4, filtered then the solvent evaporated under reduced pressure to obtain the product as a white solid. Yield: 81%.

White solid. **^{1}H NMR** ($CDCl_3$): 7.62 (d, *J* = 7.2 Hz, 2H); 7.50 (m, 3H); 7.25 (m, 5H); 5.34 (bs, 1H); 4.62 (t, *J* = 7.6 Hz, 2H); 3.25 (t, *J* = 7.6 Hz, 2H); 3.06 (s, 3H); 2.59 (s, 3H). **^{13}C NMR** ($CDCl_3$): 170.1, 157.2, 155.2, 144.9, 139.1, 134.5, 130.2, 129.8, 129.7, 129.3, 129.2, 127.4, 97.0, 49.2, 36.7, 28.8, 15.0. **MS (ESI)** *m/z*: 376 $[M+H]^+$.

1-(2-Methoxy-2-phenylethyl)-*N*-methyl-6-(methylthio)-3-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (9o)

Synthesized according to **GP3** from **8f**; the oily residue was dissolved in EtOAc and the organic phase was extracted twice with 0.5 N HCl. The organic layer was dried over Na_2SO_4, filtered then the solvent evaporated under reduced pressure to obtain the product as a white solid. Yield: quant. White solid. **^{1}H NMR** ($CDCl_3$): 7.62 (d, *J* = 7.2 Hz, 2H); 7.48 (m, 3H); 7.32 (m, 5H); 5.26 (bs, 1H); 4.91 (dd, *J* = 5.2, 8 Hz, 1H); 4.70 (dd, *J* = 8.0, 9.6 Hz, 1H); 4.44 (dd, *J* = 5.2, 14 Hz, 1H); 3.21 (s, 3H); 3.04 (d, *J* = 4.8 Hz, 3H); 2.58 (s, 3H). **^{13}C NMR** ($CDCl_3$): 169.5, 156.6, 155.3, 144.0, 139.0, 133.8, 129.3, 128.9, 128.5, 128.4, 128.2, 127.0, 96.3, 81.7, 57.1, 52.7, 27.8, 14.2. **MS (ES)** *m/z*: 406 $[M+H]^+$.

1-(2-Methoxy-2-phenylethyl)-6-(methylthio)-3-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (9p)

In a sealed tube equipped with a rubber septum, to a suspension of **8f** (44.7 mg, 0.11 mmol, 1.00 eq.) in EtOH (1 mL) at -78 °C was added NH_3 7 N in MeOH (1 mL). The tube was sealed and the reaction was heated to reflux o.n. The solvent was removed under reduced pressure, to obtain a solid residue, purified by flash chromatography using PE:EtOAc = 85:15 as eluent. Yield: 92%. White solid. **^{1}H NMR** ($CDCl_3$): 7.66 (d, *J* = 6.8 Hz, 2H); 7.48 (m, 3H); 7.31 (m, 5H); 5.71 (bs, 2H); 4.90 (dd, *J* = 5.2, 8 Hz, 1H); 4.71 (dd, *J* = 8.4, 14 Hz, 1H); 4.46 (dd, *J* = 5.2, 14 Hz, 1H); 3.22 (s, 3H); 2.56 (s, 3H). **^{13}C NMR** ($CDCl_3$): 169.2, 156.8, 155.7, 144.6, 138.9, 133.4, 129.3, 129.0, 128.5,

128.4, 128.2, 126.9, 96.0, 81.7, 57.1, 52.7, 14.0. **MS** (ESI) *m/z*: [392 [M+H], 414 [M+Na], 430 [M+K]]$^+$.

N^4-(3-Chlorophenyl)-1-phenethyl-N^6-propyl-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (10a)

Synthesized according to **GP6** starting from **9a**, using *n*-propylamine as nucleophile. Yield: 50%. White solid. **^{1}H NMR** ($CDCl_3$): 7.85 (s, 1H), 7.26 (m, 9H), 5.12 (s, 1H), 4.52 (t, *J* = 7.6 Hz, 2H), 3.42 (q, *J* = 6.5 Hz, 2H), 3.18 (t, *J* = 7.8 Hz, 2H), 1.66 (q, *J* = 7.2 Hz, 2H), 1.00 (t, *J* = 7.4 Hz, 3H). **^{13}C NMR** ($CDCl_3$): 161.2, 156.2, 154.9, 139.8, 138.4, 134.8, 130.6, 129.9, 128.8, 128.4, 126.4, 124.3, 122.1, 119.7, 96.2, 47.8, 43.6, 35.6, 23.0, 11.6. **MS** (ESI) *m/z*: 407 $[M+H]^+$.

N^4-(3-chlorophenyl)-N^6-(2-morpholinoethyl)-1-phenethyl-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (10b)

Synthesized according to **GP6** starting from **9a**, using 4-(2-aminoethyl)morpholine (5.0 eq.) as nucleophile. Yield: 89%. White solid. **^{1}H NMR** ($CDCl_3$): 7.81 (s, 1H), 7.51 (s, 1H); 7.38 (m, 1H); 7.17 (m, 7H), 5.56 (bs, 1H), 4.42 (t, *J* = 8 Hz, 2H), 3.70 (t, *J* = 4.4 Hz, 4H), 3.55 (m, 2H), 3.15 (t, *J* = 8 Hz, 2H), 2.59 (t, *J* = 6 Hz, 2H), 2.48 (s, 4H). **^{13}C NMR** ($CDCl_3$): 161.0, 156.2, 155.0, 139.9, 138.4, 134.6, 130.9, 129.9, 128.8, 128.5, 126.4, 124.3, 122.2, 119.9, 96.3, 66.9, 57.5, 53.5, 47.8, 38.1, 35.7. **MS** (ESI) *m/z*: 478 $[M+H]^+$, 500 $[M+Na]^+$.

***N*-cyclohexyl-6-(2-morpholinoethoxy)-1-phenethyl-1H-pyrazolo[3,4-*d*]pyrimidin-4-amine (10c)**

Synthesized according to **GP6** starting from **9b**, using 2-morpholinoethanol (5.0 eq.) as nucleophile. Yield: 48%. White solid. **^{1}H NMR** ($(CD_3)_2CO$): 7.72 (s, 1H); 7.07 (m, 5H); 4.31 (t, *J* = 7.2 Hz, 4H); 3.46 (m, 4H); 3.04 (t, *J* = 7.4 Hz, 2H); 2.04 (m, 4H); 1.77 (m, 2H); 1.67 (m, 1H); 1.32

(m, 4H). **^{13}C NMR** ($(CD_3)_2CO$): 165.2, 158.8, 156.1, 139.5, 131.7, 129.5, 129.1, 127.0, 98.4, 67.3, 64.5, 58.1, 54.8, 48.3, 40.8, 36.1, 32.2, 20.7, 14.0. **MS** (ESI) *m/z*: 451 $[M+H]^+$.

1-Phenethyl-*N*^{6}-propyl-*N*^{4}-(4-(trifluoromethoxy)phenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (10d)

Synthesized according to **GP6** starting from **9e**, using *n*-propylamine as nucleophile. Yield: 30%. White solid. **^{1}H NMR** ($CDCl_3$): 7.61 (d, *J* = 8.8 Hz, 2H), 7.40 (s, 1H), 7.22 (m, 7H), 5.06 (t, *J* = 5.2 Hz, 1H), 4.44 (t, *J* = 8 Hz, 2H), 3.40 (q, *J* = 6.8 Hz, 2H), 3.17 (t, *J* = 7.6 Hz, 2H), 1.64 (q, *J* = 7.6 Hz, 2H), 0.99 (t, *J* = 7.4 Hz, 3H). **^{13}C NMR** ($CDCl_3$): 161.0, 156.2, 155.0, 145.7, 138.5, 137.1, 130.7, 128.8, 128.4, 126.4, 123.6, 121.8, 119.3, 95.9, 47.8, 43.5, 35.6, 23.0, 11.6. **MS** (ESI) *m/z*: 457 $[M+H]^+$, 479 $[M+Na]^+$.

***N*^{4}-(3-chlorophenyl)-*N*^{6}-methyl-1-phenethyl-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (10e)**

Synthesized according to **GP6** starting from **9a**, using methylamine 1M in THF (5.0 eq.) as nucleophile. Yield: 58%. Light brown solid. **^{1}H NMR** ($CDCl_3$): 7.85 (s, 1H), 7.53 (s, 1H); 7.38 (d, *J* = 6.8 Hz, 1H); 7.22 (m, 6H); 7.09 (d, *J* = 8 Hz, 1H), 5.07 (d, *J* = 4.4 Hz, 1H), 4.44 (t, *J* = 7.6 Hz, 2H), 3.17 (t, *J* = 7.6 Hz, 2H), 3.02 (d, *J* = 4.4 Hz, 2H). **^{13}C NMR** ($CDCl_3$): 161.8, 156.2, 154.8, 139.8, 138.4, 134.6, 130.7, 129.9, 128.8, 128.5, 126.5, 124.2, 122.0, 119.7, 96.3, 47.8, 35.7, 28.6. **MS** (ESI) *m/z*: 379 $[M+H]^+$, 401 $[M+Na]^+$.

***N*^{4},*N*^{6}-dimethyl-1-phenethyl-3-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (10f)**

Synthesized according to **GP6** starting from **9n** using methylamine sol 40 % in H_2O (15.00 eq.) as nucleophile. The crude was purified by flash chromatography using DCM:MeOH = 99:1 as eluent. Yield: 49%. White solid. **^{1}H NMR** ($CDCl_3$): 7.63 (d, *J* = 7.6 Hz, 2H); 7.50 (t, *J* = 8 Hz, 2H); 7.44 (m, 1H); 7.28 (m, 4H); 7.22 (m, 1H); 5.10 (bs, 1H); 5.00 (bs, 1H); 4.50 (t, *J* = 8 Hz, 2H); 3.24 (t, *J*

= 8 Hz, 2H); 3.04 (d, *J* = 4.8 Hz, 3H); 2.99 (d, *J* = 4.8 Hz, 3H). **^{13}C NMR** ($CDCl_3$): 162.1, 157.9, 156.6, 144.0, 138.8, 134.4, 129.2, 128.9, 128.9, 128.6, 128.3, 126.4, 94.0, 47.8, 35.8, 28.5, 27.5. **MS (ES)** *m/z*: 359 $[M+H]^+$.

1-(2-Methoxy-2-phenylethyl)-*N*^{4},*N*^{6}-dimethyl-3-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (10g)

Synthesized according to **GP6** starting from **9o** using methylamine sol 40 % in H_2O (15.00 eq.) as nucleophile. The crude was purified by flash chromatography using DCM:MeOH = 99:1 as eluent. Yield: 40%. White solid. **^{1}H NMR** ($CDCl_3$): 7.61 (d, *J* = 7.6 Hz, 2H); 7.49 (t, *J* = 7.2 Hz, 2H); 7.42 (m, 3H); 7.32 (m, 3H); 5.10 (bs, 2H); 4.92 (dd, *J* = 8.4, 4.8Hz, 1H); 4.63 (dd, *J* = 8.4, 5.2 Hz, 1H); 4.32 (dd, *J* = 4.8, 14 Hz, 1H); 3.22 (s, 3H); 3.02 (d, *J* = 5.2 Hz, 3H); 2.99 (d, *J* = 4.8 Hz, 3H). **^{13}C NMR** ($CDCl_3$): 161.7, 157.7, 156.9, 144.1, 139.4, 134.3, 129.2, 128.6, 128.4, 128.0, 127.1, 93.9, 81.7, 57.1, 52.5, 28.5, 27.6. **MS (ES)** *m/z*: 389 $[M+H]^+$.

***N*-(3-chlorophenyl)-1-phenethyl-1*H*-pyrazolo[3,4-d]pyrimidin-4-amine (11a)**

Synthesized starting from **9a**, according to **GP8**. Yield: 67%. White solid. **^{1}H NMR** ($(CD_3)_2S=O$): 8.27 (s, 1H), 7.97 (s, 1H), 7.20 (m, 9H), 4.23 (t, *J* = 7.6 Hz, 2H), 3.20 (t, *J* = 7.6 Hz, 2H). **^{13}C NMR** ($CDCl_3$): 158.1, 154.8, 153.1, 152.8, 140.5, 131.9, 129.7, 128.5, 125.9, 122.2, 121.7, 112,6, 111.1, 108.9, 100.1, 48.6, 35.8. **MS** (ESI) *m/z*: 350 $[M+H]^+$.

1-Phenethyl-*N*-(4-(trifluoromethoxy)phenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (11b)

Synthesized starting from **9e**, according to **GP8**. Yield: 67%. Light brown solid. **^{1}H NMR** ($CDCl_3$): 8.37 (s, 1H), 7.56 (d, *J* = 8.8 Hz, 2H), 7.23 (m, 8H), 4.62 (t, *J* = 7.6 Hz, 2H), 3.20 (t, *J* = 7.6 Hz, 2H). **^{13}C NMR** ($CDCl_3$): 154.8, 153.1, 152.8, 147.5, 135.7, 131.9, 128.7, 128.5, 126.7, 125.9, 122.2, 121.7, 119.1, 100.1, 48.6, 35.8. **MS** (ESI) *m/z*: 400 $[M+H]^+$, 422 $[M+Na]^+$.

1-(2-Methoxy-2-phenylethyl)-3-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (11c)

Synthesized starting from **9p**, according to **GP8**. Yield: 87% White solid. **^{1}H NMR** ($CDCl_3$): 8.30 (s, 1H), 7.65 (m, 2H), 7.57 (m, 3H), 7.34 (m, 5H), 4.81 (m, 2H); 4.44 (m, 1H); 3.16 (s, 3H). **^{13}C NMR** ($CDCl_3$): 157.4, 155.6, 153.8, 141.6, 138.6, 133.0, 129.2, 128.7, 128.6, 127.6, 127.5, 127.4, 98.6, 84.6, 67.9, 56.4. **MS (ES)** *m/z*: 346 $[M+H]^+$.

1-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-4-chloro-6-(methylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidine (12)

Synthesized according to **GP7**, starting from **8d**. Yield: 40%. White solid. **^{1}H NMR** ($(CD_3)_2CO$): 8.05 (s, 1H), 7.89 (s, 1H), 7.25 (m, 5H), 5.62 (s, 2H), 5.49 (s, 2H), 2.51 (s, 3H). **^{13}C NMR** ($(CD_3)_2CO$): 170.8, 154.8, 154.2, 143.4, 136.7, 133.2, 129.6, 129.0, 128.8, 124.3, 111.2, 54.3, 43.5, 14.4. **MS** (ESI) *m/z*: 372 $[M+H]^+$, 394 $[M+Na]^+$.

3-(1-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-6-(methylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)phenol (13a)

Synthesized according to **GP3**, starting from **12** (Yield: quant.) or alternatively, according **GP7** starting from **9k** (Yield: 45%). Light brown solid. **^{1}H NMR** ($(CD_3)_2CO$): 7.49 (s, 1H), 7.30 (s, 1H), 7.10 (m, 5H), 6.20 (m, 4H), 5.00 (s, 2H), 4.76 (s, 2H), 2.49 (s, 3H). **^{13}C NMR** ($(CD_3)_2CO$): 171.0, 159.0, 154.2, 152.0, 144.5, 143.2, 136.3, 134.4, 131.0, 129.1, 128.7, 125.8, 123.2, 108.9, 105.9, 103.2, 99.8, 57.1, 53.4, 14.3. **MS** (ESI) *m/z*: 445 $[M+H]^+$.

1-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-*N*-methyl-6-(methylthio)-*N*-(pyridin-3-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (13b)

Synthesized according to **GP3**, starting from **12** (Yield: 85%) or alternatively, according **GP7** starting from **9l** (Yield: 45%). Light brown solid. **^{1}H NMR** ($CDCl_3$): 8.56 (d, *J* = 3.6 Hz, 1H), 7.89 (s, 1H), 7.69 (t, *J* = 7.4 Hz, 1H), 7.24 (m, 2H), 5.40 (s, 2H); 5.09 (s, 2H), 4.86 (d, *J* = 4.4 Hz, 3H), 2.57 (s, 3H). **^{13}C NMR** ($CDCl_3$): 170.8, 156.3, 155.7, 154.0, 148.7, 143.4, 137.5, 124.3, 122.7, 111.3, 54.3, 43.7, 36.5, 14.1. **MS** (ESI) *m/z*: 444 $[M+H]^+$.

1-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-6-(methylthio)-*N*-(4-(trifluoromethoxy)phenyl)-1*H*-pyrazolo[3,4-d]pyrimidin-4-amine (13c)

Synthesized according to **GP3**, starting from **12** (Yield: quant.) or alternatively, according **GP7** starting from **9m**(Yield: 42%). Light brown solid. **^{1}H NMR** ($CDCl_3$): 8.35 (bs, 1H); 7.69 (d, *J* = 8.8, 2H); 7.49 (s, 1H), 7.35 (m, 1H), 7.21 (m, 5H), 5.53 (s, 2H), 5.47 (s, 2H), 2.41 (s, 3H). **^{13}C NMR** ($CDCl_3$): 169.8, 154.3, 153.7, 146.0, 143.6, 137.0, 134.1, 132.2, 129.2, 128.9, 128.2, 123.9, 123.0, 121.8, 121.6, 98.3, 54.4, 41.6, 14.1. **MS** (ESI) *m/z*: 514 $[M+H]^+$, 536 $[M+Na]^+$.

2. Enzymatic Assays

Active recombinant kinases were purchased from ProQuinase (Germany). Specific peptide substrates (Abltide, cat. 12-493; Src Substrate Peptide, cat 12-140) were purchased from Merk-Millipore. Kinase assays were performed as follow: active Src and Abl *wt* reactions were performed in presence of 100 μM ATP and 50 μM peptide substrate. All inhibition assays were conducted with 0.01 μg active kinase, 0.33 pmol [$^{\gamma 32}$P]ATP, 60 mM HEPES-NaOH pH 7.5, 3 μM Na-orthovanadate, 1.2 mM DTT, 50 μg/ml $PEG_{20.000}$, 10 mM magnesium acetate, 0,004%NP40 and 10 % DMSO in a final volume of 10 μL. Enzymes and inhibitors were preincubated in ice for 5 min; after addition of the substrates the reaction was conducted at 30 °C for 10 min. The reaction was stopped by adding 5 μL of 0,8 % phosphoric acid. Aliquots (10 μL) were then transferred into a P30 Filtermat (PerkinElmer), washed five times with 75 mM phosphoric acid and once with acetone for 5 mins. The filter was dried and transferred to a sealable plastic bag, and scintillation cocktail (4 mL) was added. Spotted reactions were read in a scintillation counter. The ID_{50} values were obtained according to Equation (1), where v is the measured reaction velocity, V is the apparent maximal velocity in the absence of inhibitor, I is the inhibitor concentration, and the ID_{50} is the 50% inhibitory dose.

$$(1) \qquad v = V/(1+(I/ID_{50}))$$

K_i values, expressed as a concentration (μM), toward recombinant enzymes were calculated according to Equation (2) for competitive inhibition toward ATP and peptide substrates, where $[S_{ATP}]$ and $[S_{pep}]$ are the concentration of competing substrate (ATP and peptide, respectively). Curve fitting was performed with the program GraphPad Prism version 5.00.

(2) $K_i=(ID_{50}/(1+K_{mATP}/[S_{ATP}]))/(1+K_{mpep}/[S_{pep}])$