Supporting Information

Discovery of N-(2-Chloro-6-methylphenyl)-2-(6-(4-(2-hydroxyethyl)piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide (BMS-354825), a Dual Src/Abl Kinase Inhibitor with Potent Anti-Tumor Activity in Preclinical Assays

Louis J. Lombardo,* Francis Y. Lee, Ping Chen, Derek Norris, Joel C. Barrish, Kamelia Behnia, Stephen Castaneda, Lyndon A. M. Cornelius, Jagabandhu Das, Arthur M. Doweyko, Craig Fairchild, John T. Hunt, Ivan Inigo, Kathy Johnston, Amrita Kamath, David Kan, Herbert Klei, Punit Marathe, Suhong Pang, Russell Peterson, Sidney Pitt, Gary L. Schieven, Robert J. Schmidt, John Tokarski, Mei-Li Wen, John Wityak, and Robert M. Borzilleri

Contents of Supporting Information:

I. Characterization data for compounds **1** through **12**.

General Experimental Details

Analytical high pressure liquid chromatography (HPLC) and liquid chromatography/mass spectrometry (LC/MS) analyses were conducted using Shimadzu LC-10AS pumps and a SPD-10AV UV-vis detector. MS detection was performed with a Micromass Platform LC spectrometer. HPLC and LC/MS methods are detailed below. Preparative Reverse Phase (RP) HPLC was performed using two Shimadzu LC-8A pumps and a SPD-10AV UV-vis detector set at 220 nm on C18 RP columns (YMC Pack ODSA S5 20 × 100 mm or 30 × 250 mm) using with methanol/water mixtures buffered with 0.1% trifluoroacetic acid. NMR (^{1}H and ^{13}C) spectra were recorded on one of the following instruments: JEOL ECL-500 MHz , JEOL ECL-400 MHz, Bruker AVANCE 400 MHz Bruker DRX-400 MHz spectrometer and calibrated using an internal reference. High-resolution mass spectra (HMRS) were recorded on a JEOL SX102 mass spectrometer. Elemental analyses were performed by Robertson Microlit Laboratories and the results obtained are within ±0.4% of the theoretical values, unless otherwise indicated.

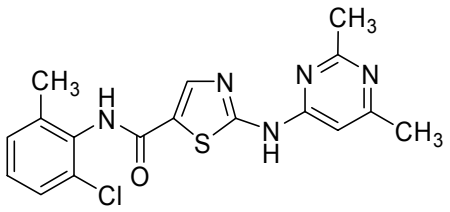
HPLC methods

Method A :A linear gradient program using 10% methanol, 90% water, 0.2% H_3PO_4 (solvent A) and 90% methanol, 10% water, 0.2% H_3PO_4 (solvent B); t = 0 min, 0% B, t = 4 min, 100% B was employed on a YMC S5 Combiscreen 4.6 × 50 mm column. Flow rate was 4 mL/min and UV detection was set to 220 nm. The LC column was maintained at ambient temperature.

Method B: A linear gradient program using 10% methanol, 90% water, 0.2% H_3PO_4 (solvent A) and 90% methanol, 10% water, 0.2% H_3PO_4 (solvent B); t = 0 min, 0% B, t = 8 min, 100% B was employed on a Zorbax SB C18 4.6 × 75 mm column. Flow rate was 2.5 mL/min and UV detection was set to 220 nm. The LC column was maintained at ambient temperature.

Method C: A linear gradient program using 10% methanol, 90% water, 0.1% trifluoroacetic acid (TFA) (solvent A) and 90% methanol, 10% water, 0.1% TFA (solvent B); t = 0 min, 0% B, t = 4 min, 100% B was employed on a Chromolith SpeedROD, 4.6 × 50 mm column. Flow rate was 4 mL/min and UV detection was set to 254 nm. The LC column was maintained at ambient temperature.

Method D: A linear gradient program using 10% methanol, 90% water, 0.1% TFA (solvent A) and 90% methanol, 10% water, 0.1% TFA (solvent B); t = 0 min, 0% B, t = 2 min, 100% B was employed on a Waters Xterra 5 m, 4.6 mm × 30 mm column. Flow rate was 4 mL/min and UV detection was set to 220 nm. The LC column was maintained at ambient temperature.



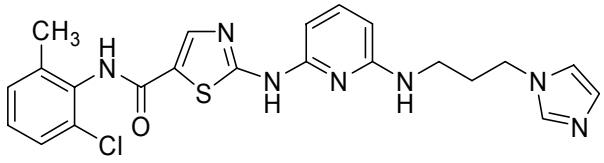
N-(2-Chloro-6-methylphenyl)-2-(2,6-dimethylpyrimidin-4-ylamino)thiazole-5-carboxamide (1)

^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.96 (s, 1H), 9.96 (s, 1H), 8.29 (s, 1H), 7.40-7.42 (m, 1H), 7.25-7.31 (m, 2H), 6.74 (s, 1H), 2.56 (s, 3H), 2.36 (s, 3H), 2.25 (s, 3H).

HPLC Method / t_R / purity: C / 1.91 min / 99%.

LC/MS (ESI): *m/z* 374 (MH^+)

HRMS (calc'd for $C_{17}H_{17}ClN_5OS$): 374.0842 amu; found: 374.0840 amu (Δ = 0.1 mmu).



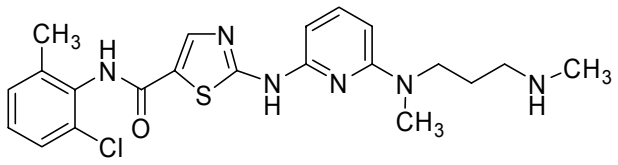
2-(6-(3-(1H-Imidazol-1-yl)propylamino)pyridin-2-ylamino)-N-(2-chloro-6-methylphenyl)thiazole-5-carboxamide (2)

^{1}H-NMR (500 MHz, CD_3OD): δ 8.99 (s, 1H), 8.36 (s, 1H), 7.70 (s, 1H), 7.65 (bs, 1H), 7.54 (s, 1H), 7.36-7.38 (dd, *J* = 2.2, 7.2 Hz, 1H), 7.25-7.28 (m, 2H), 6.39-6.44 (m, 2H), 4.46 (t, *J* = 7.4 Hz, 2H), 3.55 (t, *J* = 7.2 Hz, 2H), 2.32-2.36 (m, 5H).

HPLC Method / t_R / purity: A / 2.33 min / 98%.

LC/MS (ESI): *m/z* 468 (MH^+).

HRMS (calc'd for $C_{22}H_{23}ClN_7OS$): 468.1373 amu; found: 468.1394 amu (Δ = 2.1 mmu).



N-(2-Chloro-6-methylphenyl)-2-(6-(methyl(3-(methylamino)propyl)amino)pyridin-2-ylamino)thiazole-5-carboxamide (3)

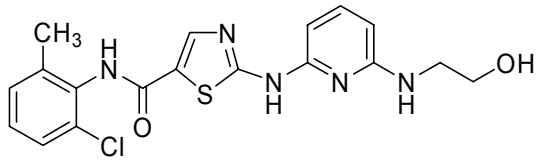
^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.43 (s, 1H), 9.94 (s, 1H), 8.31 (bs, 3H), 8.27 (s, 1H), 7.48-7.44 (m, 1H), 7.29-7.23 (m, 3H), 6.29 (d, J = 8.14 Hz, 1H), 6.14 (d, J = 8.14 Hz, 1H), 3.72-3.68 (m, 2H), 3.07-2.99 (m, 2H), 3.01 (s, 6H), 2,24 (s, 3H), 1.95-1.87 (m, 2H).

HPLC Method / t_R / purity: D / 1.45 min / 99%

LC/MS (ESI): *m/z* 445 (MH^+)

HRMS (calc'd for $C_{21}H_{26}ClN_6OS$): 445.1577 amu; found: 445.1558 amu (Δ = 1.9 mmu).

Anal. (calc'd for $C_{21}H_{25}ClN_6OS \bullet 1.0$ HCl $\bullet$ 0.2 H_2O): theoretical %C 51.99, %H 5.49, %N 17.33; found %C 51.72, %H 5.41, %N 17.32.



N-(2-Chloro-6-methylphenyl)-2-(6-(2-hydroxyethylamino)pyridin-2-ylamino)thiazole-5-carboxamide (4)

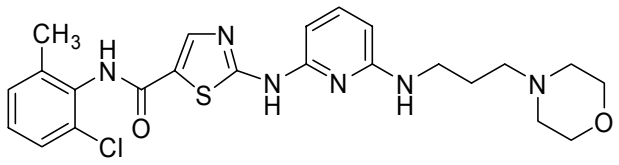
^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.27 (s, 1H), 9.77 (s, 1H), 8.19 (s, 1H), 7.38 (dd, J = 2.17, 7.23 Hz, 1H), 7.32-7.22 (m, 3H), 6.16 (d, J = 7.63 Hz, 1H), 6.09 (d, J = 8.13 Hz, 1H), 3.60-3.57 (m, 2H), 3.50-3.45 (m, 2H), 2.23 (s, 3H).

HPLC Method / t_R / purity: D / 1.31 min / 97.5%

LC/MS (ESI): *m/z* 404 (MH^+)

HRMS (calc'd for $C_{18}H_{19}ClN_5O_2S$): 404.0948 amu; found: 404.0964 amu (Δ = 1.6 mmu).

Anal. (calc'd for $C_{18}H_{18}ClN_5O_2S \bullet 1.0$ HCl $\bullet$ 0.2 H_2O): theoretical %C 48.69, %H 4.40, %N 15.77; found %C 48.65, %H 4.18, %N 15.59.



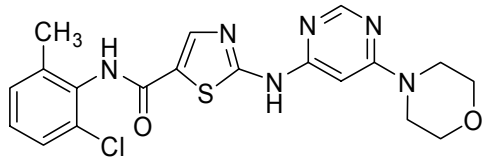
N-(2-Chloro-6-methylphenyl)-2-(6-(3-morpholinopropylamino)pyridin-2-ylamino)thiazole-5-carboxamide (5)

^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.39 (s, 1H), 9.55 (s, 1H), 9.57 (bs, 1H), 8.27 (s, 1H), 7.39 (d, J = 7.63 Hz, 1H), 7.33 (dd, J = 7.63 Hz, 7.63 Hz, 1H), 7.29-7.22 (m, 2H), 6.97 (bs, 1H), 6.19 (d, J = 7.63 Hz, 1H), 6.05 (d, J = 8.13 Hz, 1H), 3.88 (d, J = 13.74 Hz, 3H), 3.55 (t, J = 11.69 Hz, 2H), 3.46-3.37 (m, 3H), 3.29-3.23 (m, 2H), 3.03-2.93 (m, 2H), 2.23 (s, 3H), 2.03-1.97 (m, 2H).

HPLC Method / t_R / purity: D / 1.32 min / 98.5%

LC/MS (ESI): *m/z* 487 (MH^+)

HRMS (calc'd for $C_{23}H_{28}ClN_6O_2S$): 487.1683 amu; found: 487.1696 amu (Δ = 1.3 mmu).



N-(2-Chloro-6-methylphenyl)-2-(6-morpholinopyrimidin-4-ylamino)thiazole-5-carboxamide (6)

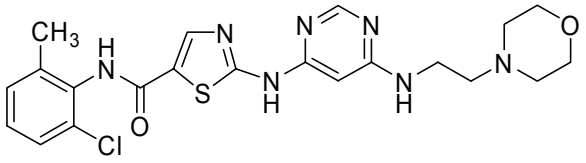
^{1}H-NMR (500 MHz, DMSO-d_6): δ 11.75 (bs, 1H), 9.98 (s, 1H), 8.45 (s, 1H), 8.26 (s, 1H), 7.39 (dd, J = 1.65, 7.38 Hz, 1H), 7.29-7.24 (m, 2H), 6.29 (s, 1H), 3.70-3.68 (m, 4H), 3.53-3.51 (m, 4H), 2.23 (s, 3H).

HPLC Method / t_R / purity: B / 7.08 min / 98%.

LC/MS (ESI): *m/z* 431 (MH^+)

HRMS (calc'd for $C_{19}H_{20}ClN_6O_2S$): 431.1057 amu; found: 431.1055 amu (Δ = 0.2 mmu).

Anal. (calc'd for $C_{19}H_{19}ClN_6O_2S$.1.0 HCl): theoretical %C 48.83, %H 4.31, %N 17.98; found %C 48.76, %H 4.21, %N 17.80.



N-(2-Chloro-6-methylphenyl)-2-(6-(2-morpholinoethylamino)pyrimidin-4-ylamino)thiazole-5-carboxamide (7)

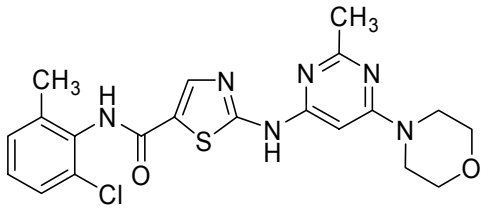
^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.60 (s, 1H), 9.91 (s, 1H), 8.40 (s, 1H), 8.21 (s, 1H), 7.59 (bs, 1H), 7.39 (dd, J = 1.74, 7.83 Hz, 1H), 7.29-7.23 (m, 2H), 6.15 (s, 1H), 3.94-3.69 (m, 4H), 3.67-3.62 (m, 2H), 3.47-3.14 (m, 4H), 3.30-3.27 (m, 2H), 2.22 (s, 3H).

HPLC Method / t_R / purity: D / 1.20 min / 100%.

LC/MS (ESI): *m/z* 474 (MH^+)

HRMS: (calc'd for $C_{21}H_{25}ClN_7O_2S$): 474.1479 amu; found: 474.1473 amu (Δ = 0.6 mmu).

Anal. (calc'd for $C_{21}H_{24}ClN_7O_2S \bullet 1.0$ HCl): theoretical %C 49.41, %H 4.93, %N 19.20; found %C 49.40, %H 4.94, %N 18.91.



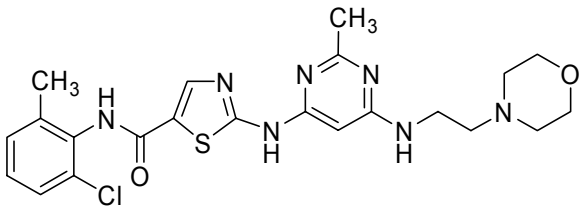
N-(2-Chloro-6-methylphenyl)-2-(2-methyl-6-morpholinopyrimidin-4-ylamino)thiazole-5-carboxamide (8)

^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.52 (s, 1H), 9.88 (s, 1H), 8.21 (s, 1H), 7.39 (d, J = 6.10 Hz, 1H), 7.29-7.23 (m, 3H), 6.04 (s, 1H), 3.67-3.65 (m, 4H), 3.49-3.46 (m, 4H), 2.41 (s, 3H), 2.23 (s, 3H).

HPLC Method / t_R / purity: D / 1.41 min / 99%.

LC/MS (ESI): *m/z* 445 (MH^+).

HRMS (calc'd for $C_{20}H_{22}ClN_6O_2S$): 445.1213 amu; found: 445.1234 amu (Δ = 2.1 mmu).



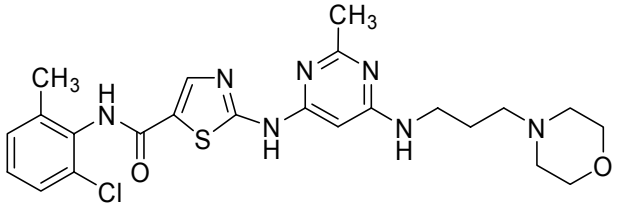
N-(2-Chloro-6-methylphenyl)-2-(2-methyl-6-(2-morpholinoethylamino)pyrimidin-4-ylamino)thiazole-5-carboxamide (9)

^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.39 (bs, 1H), 9.85 (s, 1H), 8.19 (s, 1H), 7.40 (m, 1H), 7.30-7.20 (m, 2H), 3.56 (t, J = 4.4 Hz, 4H), 2.53 (s, 1H), 2.49 (m, 8H), 2.35 (s, 3H), 2.22 (s, 3H).

HPLC Method / t_R / purity: A / 2.39 min / 95%.

LC/MS (ESI): *m/z* 488 (MH^+).

HRMS (calc'd for $C_{22}H_{27}ClN_7O_2S$): 488.1635 amu; found: 488.1617 amu (Δ = 1.8 mmu).



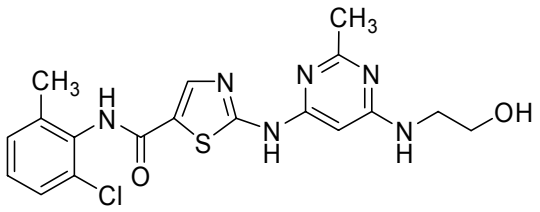
N-(2-Chloro-6-methylphenyl)-2-(2-methyl-6-(3-morpholinopropylamino)pyrimidin-4-ylamino)thiazole-5-carboxamide (10)

^{1}H-NMR (500 MHz, DMSO-d_6): δ 11.16 (bs, 1H), 10.08 (s, 1H), 8.33 (s, 1H), 7.40 (dd, J = 1.65, 7.38 Hz, 1H), 7.30-7.25 (m, 3H), 6.28 (bs, 1H), 4.01-3.90 (m, 2H), 3.89-3.80 (m, 2H), 3.53-3.43 (m, 2H), 3.19-3.13 (m, 2H), 3.09-3.03 (m, 2H), 2.60-2.53 (m, 2H), 2.45 (s, 3H), 2.24 (s, 3H), 2.07-1.99 (m, 2H).

HPLC Method / t_R / purity: B / 4.76 min / 98%.

LC/MS (ESI): *m/z* 502 (MH^+).

HRMS (calc'd for $C_{23}H_{29}ClN_7O_2S$): 502.1792 amu; found: 502.1787 amu (Δ = 0.5 mmu).



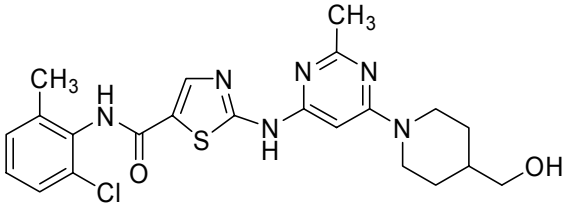
N-(2-Chloro-6-methylphenyl)-2-(6-(2-hydroxyethylamino)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide (11)

^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.27 (s, 1H), 9.77 (s, 1H), 8.19 (s, 1H), 7.38 (dd, J = 2.17, 7.23 Hz, 1H), 7.32-7.22 (m, 3H), 6.16 (d, J = 7.63 Hz, 1H), 6.09 (d, J = 8.13 Hz, 1H), 3.60-3.57 (m, 2H), 3.50-3.45 (m, 2H), 2.23 (s, 3H).

HPLC Method / t_R / purity: D / 1.26 min / 99%.

LC/MS (ESI): *m/z* 419 (MH^+).

HRMS (calc'd for $C_{18}H_{20}ClN_6O_2S$): 419.1057 amu; found: 419.1047 amu (Δ = 1.0 mmu).



N-(2-Chloro-6-methylphenyl)-2-(6-(4-(hydroxymethyl)piperidin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide (12)

^{1}H-NMR (500 MHz, CD_3OD): δ 8.22 (s, 1H), 7.35 (dd, J = 7.1, 1.6 Hz, 1H), 7.26-7.23 (m, 2H), 6.39 (bs, 1H), 3.45 (d, J = 6.0 Hz, 2H), 3.31-3.29 (m, 4H), 2.65 (s, 3H), 2.31 (s, 3H), 2.00-1.80 (m, 3H), 1.35-1.32 (m, 2H).

HPLC Method / t_R / purity: A / 2.54 min/ 96%.

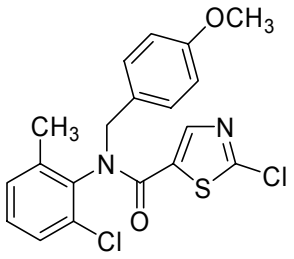
LC/MS (ESI): *m/z* 473 (MH^+).

II. Experimental procedures for the preparation of compound **13**.

General Experimental Details

All non-aqueous reactions were carried out under an atmosphere of nitrogen or argon at ambient temperature unless otherwise noted. Commercial reagents and solvents were used without further purification. Compounds were characterized using the methods detailed in Section I.

The preparation of 2-chlorothiazole (**14**) from commercially available 2-aminothiazole is described by Begtrup et al. (*Acta Chem. Scandinavica* **1992**, *46*, 372-383). 4-Amino-6-chloro-2-methylpyrimidine was prepared from the commercially available 4,6-dihydroxy-2-methylpyrimidine in 2 steps using the method of Henze et al. (*J. Org. Chem.* **1952**, *17*, 1320-1327).



N-(4-Methoxybenzyl)-2-chloro-N-(2-chloro-6-methylphenyl)thiazole-5-carboxamide (15)

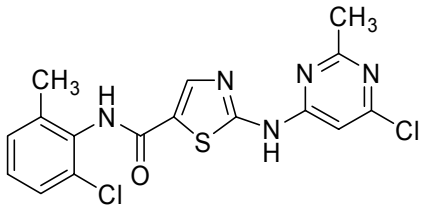
Step A. 2-Chloro-N-(2-chloro-6-methylphenyl)thiazole-5-carboxamide.

A solution of 2-chlorothiazole (**14**, 480 mg, 40.0 mmol) in THF (10 mL) was cooled to -78 ^{o}C and treated dropwise with 2.5 M *n*-butyllithium in hexanes (1.68 mL, 4.2 mmol) over a period of 20 min, keeping the temperature below -75 ^{o}C. After the addition was complete, the mixture was stirred at -78 ^{o}C for 15 min and then treated with a solution of 2-chloro-6-methylphenylisocyanate (600 μL, 4.4 mmol) in THF (5 mL). The mixture was stirred at -78 ^{o}C for 2 h, quenched with saturated aqueous NH_4Cl, warmed to room temperature and partitioned between EtOAc and H_2O. The EtOAc phase was separated, washed with brine, dried ($MgSO_4$) and concentrated *in vacuo* to afford a semi-solid. The crude product was purified by recrystallization from EtOAc-hexanes to give the intermediate as a pale yellow solid (988 mg, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.97 (s, 1H), 7.29-7.25 (m, 1H), 7.15- 7.18 (m, 2H), 2.25 (s, 3H); MS-ESI *m/z* 286 (MH^+).

Step B. N-(4-Methoxybenzyl)-2-chloro-N-(2-chloro-6-methylphenyl)thiazole-5-carboxamide.

A solution of 2-chloro-N-(2-chloro-6-methylphenyl)thiazole-5-carboxamide (574 mg, 2.0 mmol) in DMF (5 mL) was treated with 95 % NaH (60 mg, 2.4 mmol) and stirred at room temperature for 30 min. The mixture was treated with 4-methoxybenzyl chloride (325 μL, 2.4 mmol) and tetrabutylammonium iodide (148 mg, 0.40 mmol), and then stirred at room temperature for 16 h. The mixture was partitioned between H_2O and EtOAc and the EtOAc phase was separated, washed with brine, dried ($MgSO_4$) and concentrated under vacuum. The crude product was purified by flash chromatography on SiO_2 using 9:1 EtOAc-hexanes to give compound **15** as a colorless viscous oil (676 mg, 95%). ^{1}H NMR (500

MHz, DMSO-d_6) δ 7.48 (d, 1H, J = 8.3 Hz), 7.42 (s, 1H), 7.41 (dd, 1H, J = 7.7, 8.3 Hz), 7.28 (d, 1H, J = 7.7 Hz), 7.11 (d, 2H, J = 8.3 Hz), 6.78 (d, 2H, J = 8.8 Hz), 5.85 (d, 1H, J = 14.0 Hz), 4.50 (d, 1H, J = 14.0 Hz), 3.66 (s, 3H), 1.72 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.3, 155.3, 145.8, 140.5, 136.1, 134.0, 133.7, 131.6, 131.5, 130.9, 128.9, 127.6, 113.9, 55.2, 51.6, 17.9; MS-ESI m/z 408 (MH^+).



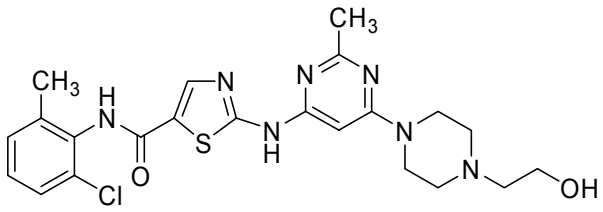
2-(6-Chloro-2-methylpyrimidin-4-ylamino)-N-(2-chloro-6-methylphenyl)thiazole-5-carboxamide (16)

Step A. N-(4-Methoxybenzyl)-2-(6-chloro-2-methylpyrimidin-4-ylamino)-N-(2-chloro-6-methylphenyl)thiazole-5-carboxamide.

4-Amino-6-chloro-2-methylpyrimidine (14.4 g, 0.10 mol) was added in portions to a suspension of NaH (60 % dispersion, 12.0 g, 0.30 mol) in THF (300 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and then treated with compound **15** (27.2 g, 0.067 mol) in portions. The resulting mixture was heated at reflux for 4 h, cooled to room temperature and diluted with H_2O (10 mL). The mixture was acidified with 1 N HCl (500 mL) and extracted with 10 % MeOH / $CHCl_3$ (3 × 250 mL). The combined extracts were dried ($MgSO_4$) and concentrated *in vacuo* to give a solid. The crude product was purified by trituration with ether to give a solid (28.4 g, 83%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.54 (d, 1H, J = 8.5 Hz), 7.46 (s, 1H), 7.43 (dd, 1H, J = 8.3, 7.7 Hz), 7.30 (d, 1H, J = 7.7 Hz), 7.16 (d, 2H, J = 8.3 Hz), 6.74 (s, 1H), 6.83 (d, 2H, J = 8.8 Hz), 5.21 (d, 1H, J = 13.7 Hz), 4.44 (d, 1H, J = 13.7 Hz), 3.71 (s, 3H), 2.45 (s, 3H), 1.72 (s, 3H); MS-ESI m/z 515 (MH^+).

Step B. 2-(6-Chloro-2-methylpyrimidin-4-ylamino)-N-(2-chloro-6-methylphenyl)thiazole-5-carboxamide

A solution of N-(4-methoxybenzyl)-2-(6-chloro-2-methylpyrimidin-4-ylamino)-N-(2-chloro-6-methylphenyl)thiazole-5-carboxamide (6.6 g, 13 mmol) dissolved in 50 % TFA in CH_2Cl_2 (50 mL) was treated with triflic acid (6.8 g, 45 mmol) and stirred at room temperature for 3 h. The mixture was poured onto crushed ice (150 g) and extracted with $CHCl_3$ (3 × 100 mL). The desired product precipitated from solution upon standing at room temperature and was collected by filtration and dried under high vacuum to give compound **16** (5.0 g, 99%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.24 (br s, 1H), 10.02 (s, 1H), 8.32 (s, 1H), 7.41 (d, 1H, J = 7.7 Hz), 7.30 (dd, 1H, J = 6.1, 6.7 Hz), 7.26 (d, 1H, J = 7.7 Hz), 6.95 (s, 1H), 2.59 (s, 3H), 2.24 (s, 3H); MS-ESI m/z 395 (MH^+).



N-(2-Chloro-6-methylphenyl)-2-(6-(4-(2-hydroxyethyl)piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide, hydrochloride salt (13)

A mixture of compound **16** (34.7 g, 88.1 mmol), diisopropylethylamine (3.6 mL, 0.17 mol) and 1-(2-hydroxyethyl)piperazine (54 mL, 0.44 mol) in 1,4-dioxane (300 mL) was refluxed for 12 h. The mixture was concentrated under vacuum and the solid was triturated successively with H_2O, aqueous MeOH, ether (twice) and dried under high vacuum. The solid was resuspended in ether-methanol, treated with 2 N HCl (44 mL) and vigorously stirred for 30 min. The precipitate was collected by filtration, and triturated successively with ether, ether-methanol, and then ether. The solid was dried under high vacuum to give compound **13** (42.2 g, 91%) as the hydrochloride salt. mp = 279-280^{o}C (free base); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.67 (br s, 1H), 10.50 (br s, 1H), 9.96 (s, 1H), 8.27 (s, 1H), 7.40 (d, 1H, J = 7.7 Hz), 7.28 (dd, 1H, J = 6.6, 6.7 Hz), 7.25 (d, 1H, J = 7.7 Hz), 6.17 (s, 1H), 4.33 (d, 2H, J = 12.6 Hz), 3.79 (dd, 2H, J = 5.0, 5.5 Hz), 3.60 (d, 2H, J = 11.6 Hz), 3.38 (dd, 2H, J = 12.1, 12.6 Hz), 3.22 – 3.19 (m, 2H), 3.13 – 3.07 (m, 2H), 2.45 (s, 3H), 2.24 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.7, 162.8, 162.1, 160.4, 157.5, 141.2, 139.4, 133.8, 133.0, 129.6, 128.8, 127.6, 126.5, 84.0, 58.1, 55.2, 51.1 (2), 41.2 (2), 25.7, 18.8; MS-ESI *m/z* 488 (100, MH^+), 490 (40, $(MH+2)^+$); HRMS (calc'd for $C_{22}H_{27}ClN_7O_2S$): 488.1635 amu; found: 488.1636 amu (Δ = 0.1 mmu); Anal. (calc'd for $C_{22}H_{26}ClN_7O_2S$): theoretical %C 54.14, %H 5.37, %N 20.09; found %C 53.90, %H 5.30, %N 20.07.

III. Detailed description of pharmacokinetic assays.

Mouse 4 hour oral exposure assay

The *in vivo* exposure of compounds were assessed in male Balb-c mice after administration of a single oral dose of 50 mg/kg. The vehicle used was propylene glycol:water (1:1). There were three mice per compound. The mice were fasted overnight and throughout the study. Serum concentrations in mice were collected at 30 min, 1 and 4 h after oral dosing. Samples were analyzed for each compound by LC/MS/MS. Composite serum concentration-time profiles were constructed for pharmacokinetic analysis.

Rat pharmacokinetic study

The pharmacokinetics of BMS-354825 were investigated in male Sprague-Dawley rats which were fasted overnight, following a single dose of 10 mg/kg either intravenously (IV) as a 10 minute infusion or orally by gavage. There were three rats per group The dosing vehicle used was propylene glycol:water (1:1). The rats were fed 4 h post dose. Blood samples were collected at 15, 30, 45 min, 1, 2, 4, 6, 8 and 10 h after IV and oral dosing. An additional 10 min sample was collected after IV dosing. Approximately 0.3 ml of blood was collected from the jugular vein in tubes containing EDTA, and plasma was obtained by centrifugation. Plasma samples were stored at -20ºC until analysis. Samples were analyzed for BMS-354825 by LC/MS/MS.

Analytical method - LC/MS/MS

Samples from all the pharmacokinetic studies were analyzed by the following LC/MS/MS method. Plasma samples were treated with two volumes of acetonitrile containing 200 ng/mL of BMS-487418 as internal standard (IS). After centrifugation to remove precipitated proteins, a 10 µL portion of the clear supernatant was analyzed by LC/MS/MS.

The HPLC system consisted of two Shimadzu LC10AD pumps (Shimadzu, Columbia, MD), and a HTS PAL autosampler (Leap technologies, NC), and a Hewlett Packard Series 1100 (Hewlett Packard Palo Alto, CA) column compartment. The column used was a YMC C18-AQ, 2 mm × 50 mm, 3 µm particles (Waters), maintained at 60 ºC and a flow rate of 0.3 mL/min. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). Initial mobile phase composition was 85% solvent A and 15% solvent B. After sample injection, the mobile phase was changed to 5% solvent A and 95% solvent B over 1 min and held at that composition for an additional 1 min. The mobile phase was then returned to initial conditions and the column re-equilibrated for 1.0 min. Total analysis time was three minutes.

The HPLC was interfaced to either a Micromass Quattro Micro triple quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with an electrospray interface, or a Sciex API 3000 triple quadrupole mass spectrometer with a turbo ionspray interface. On the Micromass Quattro Micro, UHP nitrogen was used as the nebulizing and desolvation gas at flow rates of 100 L/hour for nebulization and 1000 L/hour for desolvation. The desolvation temperature was 300 ºC and the source temperature was

150 ºC. Data acquisition employed selected reaction monitoring (SRM). Positively charged ion representing the [MH^+] for BMS-354825 and the IS were selected in MS1 and collisionally dissociated with argon at a pressure of 2 × 10-3 Torr to form specific product ions which were subsequently monitored by MS2. All dwell times were 100 ms. The SRM transitions monitored were m/z 488 → 401 for BMS-354825, m/z 459 → 338 for the internal standard. Cone voltage was optimized at 45 V for BMS-354825 and 30 V for the internal standard, while the collision energy was 30 eV for BMS-354825 and 20 ev for the internal standard. The retention times for BMS-354825 and the internal standard, were around 1.0 and 1.2 min, respectively.

The Sciex API 3000 triple quadrupole mass spectrometer used the turbo ionspray interface with an ionspray voltage of 4500 V. Gas 1 and gas 2 were set at 8 and 800, respectively. The turbo ionspray temperature was set at 400 ºC. The declustering potential (DP) was 41 V for BMS-354825 and 61 V for the internal standard. The focusing potential (FP) was 170 V for BMS-354825 and 100 V for the internal standard. The collision energy was 41 eV for BMS-354825 and 29 eV for the internal standard. The nitrogen collision gas setting was six.