Supporting Information

Optimization of Potent, Selective, and Orally Bioavailable Pyrrolodinopyrimidine-containing Inhibitors of Heat Shock Protein 90 (Hsp90). Identification of Development Candidate 2-amino-4-{4-chloro-2-[2-(4-fluoro-1H-pyrazol-1-yl)ethoxy]-6-methylphenyl}-N-(2,2-difluoropropyl)-5,7-dihydro-6H-pyrrolo[3,4-d]pyrimidine-6-carboxamide

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I. Chemistry

General experimental

Starting materials and solvents were purchased from commercial suppliers and were used without further purification. The reported yields are for purified material and are not optimized. $^1\text{H-NMR}$ spectra were recorded on a Bruker instrument operating either at 300 MHz, or 400 MHz. Chemical shifts (δ) are in part per million referenced to TMS.

Compound **2a**, **2b**, **2c**, and **3** were synthesized using the same procedure described in reference 9. All compounds which were tested in the biochemical assay were analyzed by both microanalysis (CHN) and LC/MS to confirm the purity $\geq 95\%$.

Synthesis of compounds **63** and **64**.

Reagents and conditions: (a) NaH, I₂, toluene, 81 °C, 12 h, 56%; (b) chloromethoxyethane, K₂CO₃, DMF, 23 °C, 12 h; (c) 4,4,5,5-tetramethyl-[1,3,2]dioxaborolane, Et₃N, Pd(OAc)₂, biphenyl-2-yl-dicyclohexyl-phosphane, dioxane, 80 °C, 2 h, 49%. (d) BBr₃, DCM, 0 °C, 20 min., 44%.

Reagents and conditions: (a) NCS, CH₃OH, 60 °C, 4 h, 98%; (b) NaNO₂, H₂SO₄, 23 °C, 0.5 h, 95%; (c) Fe, NH₄Cl, EtOH, 50 °C, 0.5 h, 83%; (d) NaNO₂, H₂SO₄, 23 °C, 0.5 h, quantitative yield; (e) CH₃I, K₂CO₃, CH₃CN, 23 °C, 12 h, 74%; (f) 4,4,5,5-tetramethyl-[1,3,2]dioxaborolane, Et₃N, PdCl₂(dppf)₂, CH₃CN, 90 °C, 3 h, 81%. (g) BBr₃, DCM, 0 °C, 10 minutes, 80%.

3,5-Dichloro-2-iodo-phenol

Sodium hydride (368 g, 9.2 mol, 60% dispersion in mineral oil) was added to a solution of **3,5-dichloro-phenol** (500 g, 3.067 mol) in dry toluene (5 L) at 0 °C under N₂ atmosphere. After the addition, the resulting mixture was allowed to warm up to room temperature and stirred for 20 minutes. The suspension was then cooled back to 0 °C, and iodine (779 g, 3.067 mol) was added slowly. Then the reaction mixture was stirred at room temperature overnight. TLC (petroleum ether/DCM 1:1) indicated complete consumption of 3,5-dichloro-phenol. The reaction mixture was quenched with 1N HCl (5 L) and diluted with ether (5.0 L). The separated organic layer was washed with brine (500 mL), dried over Na₂SO₄, and concentrated in vacuo to give crude compound 2, which was basified to pH = 10 with 3N NaOH (3 L) and extracted with DCM (1.5 L x 2), the separated aqueous layer was acidified to pH = 3 with HCl (2N, 3 L). The mixture was filtrated and the cake was dried in vacuo to give the title compound as a white solid (550 g, 56%).

1,5-Dichloro-3-ethoxymethoxy-2-iodo-benzene

Chloromethoxy-ethane (206 g, 2.2 mol) was added to a solution of **3,5-dichloro-2-iodo-phenol** (500 g, 1.7 mol) and K₂CO₃ (284 g, 2.1 mol) in DMF (5 L). The reaction mixture was stirred at room temperature for 12 h. TLC (petroleum ether/EtOAc 2:1) indicated complete consumption of 3,5-dichloro-2-iodo-phenol. Most of the DMF was removed in vacuum and H₂O (5.0 L) was added. The resulting mixture was extracted with Et₂O (2.5 L x 3) and washed brine (1 L), dried over Na₂SO₄, and concentrated in vacuo to give crude title compound (543 g, 90%) as a white solid, which was used for the next step directly.

2-(2,4-Dichloro-6-ethoxymethoxy-phenyl)-4,4,5,5-tetramethyl-[1,3,2]dioxaborolane

A solution of **1,5-dichloro-3-ethoxymethoxy-2-iodo-benzene** (250 g, 0.72 mol), 4,4,5,5-tetramethyl-[1,3,2]dioxaborolanein (223 mL, 1.5 mol), Et₃N (300 mL, 2.4 mol) in dioxane (1500 mL) was purged with N₂ for 30 minutes. Then Pd(OAc)₂ (8.7 g, 0.04 mol) and biphenyl-2-yl-dicyclohexyl-phosphane (27 g, 0.08 mol) were added to the resulting mixture. After the addition, the reaction mixture was stirred at 80 °C for 2 h. TLC (petroleum ether/EtOAc 50:1) indicated complete consumption of **1,5-dichloro-3-ethoxymethoxy-2-iodo-benzene**. The resulting mixture was filtered and the filtrate was concentrated in vacuo to give crude title compound. The title compound was crystallized from petroleum ether (2.0 L) as a white solid (120 g, yield: 48.5%).

2-(2,4-Dichloro-6-hydroxy-phenyl)-boronic acid (61)

Boron tribromide (524 g, 2.1 mol) was added to a stirred solution of **2-(2,4-Dichloro-6-ethoxymethoxy-phenyl)-4,4,5,5-tetramethyl-[1,3,2]dioxaborolane** (150 g, 0.43 mol) in dry CH₂Cl₂ (1200 mL) was added dropwise at 0 °C under N₂ atmosphere. The resulting solution was stirred for 20 minutes. TLC (petroleum ether/EtOAc 50:1) indicated complete consumption of **2-(2,4-Dichloro-6-ethoxymethoxy-phenyl)-4,4,5,5-tetramethyl-[1,3,2]dioxaborolane**. The reaction mixture was poured into ice water and the aqueous solution was basified to pH ~ 10 by adding 3N NaOH (750 mL). The aqueous layer was collected and adjusted to pH ~ 3 with 1N HCl (2000 mL). The aqueous solution was extracted with EtOAc (3 x 2000 mL). The combined organic layers were washed with brine (1.0 L), dried over Na₂SO₄, and concentrated in vacuo to give compound **61** as a white solid (39.2 g, 43.7%). ¹H NMR (400 MHz, CD₃OD) δ ppm: 6.88 (d, *J* = 1.2, 1 H), 6.73 (d, *J* = 1.2, 1 H). MS: ([2M-2H₂O]⁺ *m/z* = 377).

4-Chloro-2-methyl-6-nitro-phenylamine

N-chlorosuccinimide (365g, 2.6 mol) was added to a solution of 2-Methyl-6-nitro-phenylamine (375 g, 2.4 mol) in MeOH (4000 mL) at 60 °C. The mixture was refluxed for 4 h and TLC (petroleum ether EtOAc = 10:1) showed the reaction was complete. The mixture was concentrated and the residue was dissolved in EtOAc (4L) and washed with 5% of aqueous NaOH solution for three times. The organic layer was dried and concentrated to give the title compound (450 g, 98%) as a brown solid.

5-Chloro-2-iodo-1-methyl-3-nitro-benzene

A solution of sodium nitrite (146 g, 2.9 mol) in H₂SO₄ (750 mL) was added dropwise into a solution of compound **4-chloro-2-methyl-6-nitro-phenylamine** (250 g, 1.4 mol) in acetic acid (2000 mL). The mixture was allowed to stir at room temperature for 0.5 h and TLC (petroleum ether EtOAc = 10:1) showed the reaction was complete. Water (4 L) and urea (146g) were added and the mixture was stirred at room temperature for 10 min, A solution of KI (320 g) in H₂O (2 L) was added dropwise. The mixture was stirred at room temperature for 30 minutes and was filtered. The filter cake was washed with more water and dried in vacuum to give the title compound (400 g, 95%) as a brown solid.

5-Chloro-2-iodo-3-methyl-phenylamine

A solution of NH₄Cl (356 g, 2.9 mol) in H₂O (4 L) was added into a solution of **5-chloro-2-iodo-1-methyl-3-nitro-benzene** (400 g, 1.34 mol) in EtOH (4 L), The mixture was heated to 50 °C and Fe (350 g, 6.25mol) was added at one portion. The mixture was allowed to stir at reflux for 30 min and TLC (petroleum ether EtOAc = 10:1) showed the

reaction was complete. The mixture was filtered and EtOH was removed by evaporation. The aqueous solution was extracted with EtOAc (5 L) and the organic layer was dried, filtered, and concentrated to give the title compound (300 g, 83%) as a brown solid.

5-Chloro-2-iodo-3-methyl-phenol

A solution of sodium nitrite (146 g, 2.9 mol) in H₂SO₄ (750 mL) was added dropwise into a solution of **5-chloro-2-iodo-3-methyl-phenylamine** (250 g, 0.94 mol) in acetic acid (2000 mL). The mixture was allowed to stir at room temperature for 0.5 h and TLC (petroleum ether EtOAc = 20:1) showed the reaction was complete. The mixture was added dropwise into boiling water (5 L) after stirred at reflux for 10 min. The mixture was extracted with EtOAc (5 L) and the organic layer was dried, filtered, and concentrated to give crude title compound (300 g, 120%) as a brown solid. The crude product was used direct to the next step.

5-Chloro-2-iodo-1-methoxy-3-methyl-benzene

Iodomethane (238 g, 1.6 mol) was added to a solution of **5-chloro-2-iodo-3-methyl-phenol** (300g, 1.12 mol) and K₂CO₃ (185 g, 1.33 mol) in CH₃CN (6 L). The mixture was stirred at room temperature for 12 h and TLC (petroleum ether EtOAc = 20:1) showed the reaction was complete. The mixture was concentrated and the residue was dissolved in EtOAc (4 L) and washed with H₂O (2 L). The organic layer was dried, filtered, and concentrated to give the crude product which was purified through a silica gel to give the title compound (233 g, 74%) as an orange solid.

2-(4-Chloro-2-methoxy-6-methyl-phenyl)-4,4,5,5-tetramethyl-[1,3,2]dioxaborolane

$\text{PdCl}_2(\text{dppf})_2$ (13 g, 0.02 mol) and Et_3N (470 mL, 3.4 mol) were added to a solution of **5-chloro-2-iodo-1-methoxy-3-methyl-benzene** (150 g, 0.53 mol) in CH_3CN (1600 mL). 4,4,5,5-tetramethyl-[1,3,2]dioxaborolane (240 mL, 1.6 mol) was added dropwise at 23 °C. The mixture was heated to reflux for 3 h and TLC (petroleum ether EtOAc = 20:1) showed the reaction was complete. The mixture was concentrated and the residue was dissolved in EtOAc (1 L) and washed with H_2O (1 L). The organic layer was dried, filtered, and concentrated to give the crude product which was purified through a silica gel chromatography to give the title compound (140 g, 81%) as an off yellow solid.

2-(4-Chloro-2-hydroxy-6-methyl-phenyl)-boronic acid (**63**)

Boron tribromide (90 mL, 0.94 mol) was added dropwise to a solution of **2-(4-chloro-2-methoxy-6-methyl-phenyl)-4,4,5,5-tetramethyl-[1,3,2]dioxaborolane** (100 g, 0.35 mol) in DCM (1.2 L) at 0 °C. The mixture was allowed to stir at 0 °C for 10 minutes and TLC (petroleum ether EtOAc = 20:1) showed the reaction was complete. The mixture was poured into ice water and extracted with EtOAc (3 x 1 L). The organic layer was dried, filtered, and concentrated to give a solid residue. The residue was washed with petroleum ether to give compound **63** (50 g, 80%) as an off white solid. ^1H NMR (400 MHz, CD_3OD) δ 6.67 (s, 1H), 6.58 (d, J = 1.0, 1H), 2.19 (s, 3H). MS: ($[\text{2M}-2\text{H}_2\text{O}]^-$ m/z = 335).

Synthesis of compounds 9, 10, and 11.

Reagents and conditions: (a) BBr_3 , DCM, 0 °C, 24 h, 68%; (b) 1-iodo-4,4,4-trifluorobutane, K_2CO_3 , DMF, 60 °C, 12 h, 69%.

Reagents and conditions: (a) **61**, $\text{Pd}(\text{PPh}_3)_4$, Na_2CO_3 , 85 °C, 2 h, 76%; (b) 1-iodo-4,4,4-trifluorobutane, K_2CO_3 , DMF, 60 °C, 12 h, 50% (two steps).

2-(2-amino-6-methylpyrimidin-4-yl)-3-chlorophenol (**9**)

Boron tribromide (1 M in heptane; 10 mL, 10 mmol) was added to a suspension of compound **8** (2.46 g, 9.9 mmol) in DCM (35 mL) at 0 °C. The resulting mixture was warmed to ambient temperature and stirred for 24 h. An additional boron tribromide (20 mL) and DCM (65 mL) were carefully added at 0 °C and the resulting mixture was warmed to ambient temperature and stirred for 24 h. After stirring at 23 °C for 72 h, LCMS indicated less than 5% starting material remaining. The reaction mixture was cooled at 0 °C prior to adding water (0.5 mL every 2 minutes for a total of 5 mL) carefully. The mixture was then poured into ice water/diethylether (1:1, 200 mL total). Sodium bicarbonate was added portion wise until the mixture was neutralized to pH = 7. EtOAc (200 mL) was added. The mixture was stirred for 30 minutes, the two phases were

separated and the organic phase was washed with brine (50 mL). The aqueous phase was extracted with more EtOAc (100 mL). The combined organic phase was dried over MgSO_4 , filtered, and concentrated until ~ 10 mL of solvent left. The solid was filtered and washed with Et_2O (10 mL) and dried to afford the title compound as a white solid (1.57 g, 68%). ^1H NMR(400 MHz, CDCl_3) δ 10.24 (s, 1H), 7.20 (dd, $J = 8.1$, 1H), 6.94 (d, $J = 7.8$, 1H), 6.87 (d, $J = 8.3$, 1H), 6.60 (s, 2H), 6.49 (s, 1H), 2.26 (s, 3H). MS: (M^+H m/z = 236.0, 238.0). Anal. Calcd for $\text{C}_{11}\text{H}_{10}\text{N}_3\text{O}$: C, 56.06%; H, 4.28%; N, 17.83%. Found: C, 55.91%; H, 4.25%; N, 17.56%.

4-[2-Chloro-6-(4,4,4-trifluoro-butoxy)-phenyl]-6-methyl-pyrimidin-2-ylamine (10).

1-Iodo-4,4,4-trifluorobutane (121 mg, 0.51 mmol) was added to a solution of **9** (100 mg, 0.42 mmol) and K_2CO_3 (117 mg, 0.85 mmol) in DMF (5 mL). The resulting mixture was stirred at 60 °C for 12 h. After cooling down to rt, the reaction mixture was partitioned between ethyl acetate (20 mL) and water (20 mL). The organic phase was dried, filtered, and concentrated to dryness. The desired product was purified by silica gel column and eluted at 60-70% EtOAc in hexanes to afford the title compound as a solid (101 mg, 69% yield). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm: 7.38 (t, $J = 8.3$, 1 H), 7.10 (dd, $J = 13$, 8.0, 2 H), 6.55 (s, 2 H), 6.41 (s, 1 H), 4.01 (t, $J = 5.8$, 2 H), 2.24 (s, 3 H), 2.10 - 2.22 (m, 2 H), 1.70 - 1.82 (m, 2 H); MS: ($\text{M}+\text{H}$ m/z) = 346.20/348.20; HRMS: Calcd. For $\text{C}_{15}\text{H}_{16}\text{ClF}_3\text{N}_3\text{O}$: 347.095843; Found: 347.094902; Error = -2.71 ppm; Anal. calcd. for $\text{C}_{15}\text{H}_{15}\text{ClF}_3\text{N}_3\text{O} \bullet 0.03\text{HOAc} \bullet 0.1\text{H}_2\text{O}$: C: 51.78; H: 4.42; N: 12.03; Found: C: 51.58; H: 4.31; N: 12.00. HPLC Purity (254nm): 98.0%, RT = 9.825 min.

4-[2,4-Dichloro-6-(4,4,4-trifluoro-butoxy)-phenyl]-6-methyl-pyrimidin-2-ylamine (11)

Sodium carbonate (0.85 mL, 1.71 mmol, 2 N) was added to a solution of **61** (147 mg, 0.73 mmol), 2-amino-4-chloro-6-methyl pyridine (112 mg, 0.8 mmol) in dioxane (12 mL). The mixture was bubbled with N₂ for 15 min. Pd(PPh₃)₄ (82 mg, 0.071 mmol) was then added to this solution. The resulting reaction mixture was heated at 85 °C for 2 h. After cooling down to rt, the reaction mixture was concentrated to dryness, and the resulting residue was partitioned between water (20 mL) and EtOAc (2 x 30 mL). The organic phase was separated and collected. The aqueous phase was acidified with 1 M HCl to pH = 3, and extracted with more ethyl acetate (2 x 20 mL). The combined organic layer was dried, filtered, and concentrated to dryness. This residue was used for the next step reaction without further purification.

1-Iodo-4,4,4-trifluorobutane (338 mg, 1.42 mmol) was added to a solution of K₂CO₃ (196 mg, 1.42 mmol) and the above residue in DMF (6 mL). The resulting reaction mixture was heated at 60 °C for 12 h. After cooling down to rt, the reaction mixture was diluted with 10 mL water, and then extracted with ethyl acetate (2 x 50 mL). The combined organic layer was washed with brine (10 mL), dried, filtered, and concentrated to dryness. The desired product was purified by silica gel column and eluted at 40-50% EtOAc in hexanes to afford the title compound as a solid (136 mg, 50% over two steps). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm: 7.36 (t, *J* = 8.2, 1 H), 7.08 (dd, *J* = 8.2, 3.2, 2 H), 6.55 (s, 2 H), 6.39 (s, 1 H), 3.96 - 4.06 (m, 6 H), 2.24 (s, 3 H), 1.82 (s, 3 H), 1.66 - 1.76 (m, 2 H), 1.03 (s, 9 H); MS: (M+H m/z) = 380.05/382.00; HRMS: Calcd. for C₁₅H₁₅Cl₂F₃N₃O (M+H): 381.056869; Found: 381.056361; Error = -1.33 ppm; Anal. calcd for C₁₈H₂₅ClN₄O•1.14HOAc•0.97H₂O: C: 56.02; H: 7.30; N: 12.89. Found: C: 56.07; H: 7.04; N: 12.89; HPLC Purity (254nm): 99.0%, RT = 10.525 min.

Synthesis of compounds 24 and 25.

Reagents and conditions: (a) BrCH₂CH₂Cl, K₂CO₃, DMF, 70 °C, 3 h, 67%; (b) azetidine-HCl, KI/K₂CO₃, 120 °C, microwave, 30 min; (c) TFA, 23 °C, 3 h; (d) CDI, cyclobutylamine, TEA, THF, 60 °C, 2 h, 48% over three steps.

2-Amino-4-[2,4-dichloro-6-(2-chloro-ethoxy)-phenyl]-5,7-dihydro-pyrrolo[3,4-d]pyrimidine-6-carboxylic acid tert-butyl ester

A mixture of tert-butyl 2-amino-4-(2,4-dichloro-6-hydroxyphenyl)-5,7-dihydro-6H-pyrrolo[3,4-d]pyrimidine-6-carboxylate (200 mg, 0.5 mmol), 1-bromo-2-chloroethane (144 mg, 1 mmol), cesium carbonate (492 mg, 1.5 mmol), and 4 mL anhydrous DMSO was stirred at 70 °C for 2 h. After cooling down to rt, the reaction mixture was partitioned between ethyl acetate (30 mL) and water (30 mL). The organic phase was separated, washed with water (1 x 30mL) and brine (1 x 30mL), dried over sodium sulfate, and concentrated to dryness, and purified by column with 40% EtOAc/heptane to afford the title compound as a solid (156 mg, 67.4% yield). MS: (M+H m/z) = 459.10/461.05.

2-Amino-4-[2,4-dichloro-6-[2-(3,3-difluoro-azetidin-1-yl)-ethoxy]-phenyl]-5,7-dihydro-pyrrolo[3,4-d]pyrimidine-6-carboxylic acid tert-butyl ester

3,3-Difluoroazetidine hydrochloride (79 mg, 0.61 mmol) was added to a solution of tert-butyl 2-amino-4-[2,4-dichloro-6-(2-chloroethoxy)phenyl]-5,7-dihydro-6H-pyrrolo[3,4-d]pyrimidine-6-carboxylate (140 mg, 0.31 mmol), K_2CO_3 (126 mg, 0.92 mmol), and KI (76 mg, 0.46 mmol) in DMF (4 mL). The mixture was heated at 120 °C in a microwave for 30 minutes. After cooling down to 23 °C, the reaction mixture was partitioned between EtOAc (20 mL) and water (20 mL). The organic phase was separated, washed with water (1 x 20 mL) and brine (1 x 20 mL), dried, and purified by silica gel chromatography. The desired product was eluted with 60% EtOAc/Heptanes to afford the title compound as a solid (84 mg, 53% yield). MS: (M+H m/z) = 516.10/518.0.

4-[2,4-Dichloro-6-[2-(3,3-difluoro-azetidin-1-yl)-ethoxy]-phenyl]-6,7-dihydro-5H-pyrrolo[3,4-d]pyrimidin-2-ylamine

Trifluoroacetic acid (2 mL) was added to a mixture of tert-butyl 2-amino-4-[2,4-dichloro-6-[2-(3,3-difluoroazetidin-1-yl)ethoxy]phenyl]-5,7-dihydro-6H-pyrrolo[3,4-d]pyrimidine-6-carboxylate (84 mg, 0.163 mmol). The mixture was stirred at 23 °C for 3 h. After removing excess TFA, the resulting residue was dissolved in a small amount of CH_3CN , neutralized with $NaHCO_3$, and concentrated to afford a solid. This solid residue was then suspended in ethyl acetate, and filtered. The resulting filtrate was concentrated to afford the title compound. MS: (M+H m/z) = 416.05/418.00.

2-Amino-4-[2,4-dichloro-6-[2-(3,3-difluoro-azetidin-1-yl)-ethoxy]-phenyl]-5,7-dihydro-pyrrolo[3,4-d]pyrimidine-6-carboxylic acid cyclobutylamide (24).

Cyclobutylamine (23 mg, 0.326 mmol, in 1 mL of THF) was added to a solution of CDI (53 mg, 0.326 mmol) in THF (2 mL) at 0 °C. After stirring at 23 °C for 3 h, a solution of 4-{2,4-dichloro-6-[2-(3,3-difluoroazetidin-1-yl)ethoxy]phenyl}-6,7-dihydro-5H-pyrrolo[3,4-d]pyrimidin-2-amine (68 mg, 0.163 mmol) in THF (2 mL) and TEA (0.07 mL, 0.502 mmol, neat) was added to the cyclobutylamine/CDI mixture, and the resulting reaction mixture was stirred at 60 °C for 2 h. After concentrating to dryness, the resulting residue was concentrated to dryness, and purified prep HPLC to afford the title compound as a solid (40 mg, 48% yield over three steps). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm: 7.34 (d, *J* = 1.5, 1 H), 7.25 (d, *J* = 1.8, 1 H), 6.85 (s, 2 H), 6.47 - 6.57 (m, 2 H), 4.26 - 4.48 (m, 2 H), 4.09 - 4.17 (m, 3 H), 3.98 - 4.08 (m, 2 H), 3.36 - 3.47 (m, 2 H), 2.74 (br. s., 2 H), 2.08 (dd, *J* = 6.8, 3.5, 2 H), 1.85 - 1.97 (m, 2 H), 1.54 (dd, *J* = 9.9, 7.3, 2 H). MS: (M+H *m/z*) = 513.10/515.05; HRMS: Calcd for C₂₂H₂₅Cl₂F₂N₆O₂ (M+H): 513.1379; Found: 513.1378; HPLC Purity (254nm): 98.4%, RT = 11.425 min.

2-Amino-4-[2,4-dichloro-6-(2-ethylamino-ethoxy)-phenyl]-5,7-dihydro-pyrrolo[3,4-d]pyrimidine-6-carboxylic acid cyclobutylamide (25)

Following the procedure for the preparation of **24** but substituting **55** with **48e** and substituting azetidine hydrochloride with ethyl amine provided the title compound with 14% yield.

¹H NMR (400 MHz, DMSO-*d*₆) δ ppm: 7.34 (s, 1 H), 7.31 (s, 1 H), 6.82 (s, 2 H), 6.51 (d, *J* = 7.8, 1 H), 4.40 (br. s., 2 H), 4.01 - 4.19 (m, 4 H), 2.73 (t, *J* = 4.9, 2 H), 2.38 - 2.45 (m, 2 H), 2.02 - 2.14 (m, 2 H), 1.83 - 1.96 (m, 4 H), 1.44 - 1.61 (m, 2 H), 0.87 (t, *J* =

7.1, 3 H). MS: (M+H m/z) = 465.10/467.05; HRMS: Calcd. for $C_{21}H_{27}Cl_2N_6O_2$ (M+H): 465.1567; Found: 465.1568; HPLC Purity (254nm): 96.9%, RT = 8.201 min.

Synthesis of compounds 36 and 37.

Reagents and conditions: (a) amine, CDI, TEA, DMF, 0 °C to rt; add **46**, rt to 45 °C, 2 h, 43%; (b) **61**, Na_2CO_3 , $Pd(PPh_3)_4$, dioxane, 85 °C, 24 h, 51%; (c) 1-(2-Chloro-ethyl)-4-methyl-1H-pyrazole, Cs_2CO_3 , KI, DMSO, 100 °C, 6 h, 35%.

2-Amino-4-iodo-5,7-dihydro-pyrrolo[3,4-d]pyrimidine-6-carboxylic acid bicyclo[1.1.1]pent-1-ylamide

Following the procedure for the preparation of **47e** but substituting cyclobutylamine with bicyclo[1.1.1]pent-1-ylamine provided the title compound in 43% yield. 1H NMR (300 MHz, DMSO- d_6) δ 7.09 (br. s., 1H), 7.02 (br. s., 2H), 4.38 (s, 2H), 4.23 (s, 2H), 2.37 (s, 1H), 1.96 (s, 6H). MS: (M+H m/z = 372.2).

2-Amino-4-(2,4-dichloro-6-hydroxy-phenyl)-5,7-dihydro-pyrrolo[3,4-d]pyrimidine-6-carboxylic acid bicyclo[1.1.1]pent-1-ylamide

Following the procedure for the preparation of **48e** but substituting **47e** with **2-Amino-4-iodo-5,7-dihydro-pyrrolo[3,4-d]pyrimidine-6-carboxylic acid bicyclo[1.1.1]pent-1-ylamide** provided the title compound in 51% yield. MS: (M+H m/z = 406.2).

1-(2-Chloro-ethyl)-4-methyl-1H-pyrazole

A solution of 4-methyl pyrazole (0.177 g, 2.16 mmol) and 1-chloro-2-iodoethane (1.20 g, 6.30 mmol) in 1,4-dioxane (5.0 mL) was treated with cesium carbonate (1.10 g, 3.38 mmol) and heated to 95 °C. After 24 h, the rxn mixture was cooled to rt, diluted with DCM (10 mL), filtered, and concentrated. The resulting residue was purified on silica gel eluting with 0-70% EtOAc in hexanes to provide the title compound as a clear oil (49 mg, 16%). ^1H NMR (300 MHz, CDCl_3) δ 7.46 (s, 1H), 7.33 (s, 1H), 4.49 (t, J = 5.93 Hz, 2H), 3.92 (t, J = 5.84 Hz, 2H), 2.12 (s, 3H).

2-Amino-4-{2,4-dichloro-6-[2-(4-methyl-pyrazol-1-yl)-ethoxy]-phenyl}-5,7-dihydro-pyrrolo[3,4-d]pyrimidine-6-carboxylic acid bicyclo[1.1.1]pent-1-ylamide (**36**)

A solution of **2-Amino-4-(2,4-dichloro-6-hydroxy-phenyl)-5,7-dihydro-pyrrolo[3,4-d]pyrimidine-6-carboxylic acid bicyclo[1.1.1]pent-1-ylamide** (59 mg, 0.15 mmol) in DMSO (1.5 mL) was treated with cesium carbonate (177 mg, 0.543 mmol), potassium iodide (7 mg), and **1-(2-Chloro-ethyl)-4-methyl-1H-pyrazole** (49 mg, 0.34 mmol) and heated to 100°C. After 6h, the reaction mixture was cooled to rt and filtered. Preparative

HPLC provided the title compound as a white powder (26 mg, 35%). ¹H NMR (300 MHz, DMSO-d₆) δ 7.33 (d, *J* = 1.13 Hz, 1H), 7.25 (d, *J* = 1.51 Hz, 1H), 7.10 (s, 1H), 6.97 (s, 1H), 6.91 (br. s., 1H), 6.80 (br. s., 2H), 4.19 - 4.40 (m, 6H), 3.95 (d, *J* = 12.81 Hz, 1H), 3.68 (d, *J* = 13.75 Hz, 1H), 2.35 (s, 1H), 1.94 (s, 6H), 1.90 (s, 3H). MS: (M+H *m/z* = 514.2). Anal. Calcd. for C₂₄H₂₅N₇O₂Cl₂•2.0 H₂O: C, 52.37%; H, 5.31%; N, 17.81%. Found: C, 52.48%; H, 4.99%; N, 17.63%.

Reagents and conditions: (a) **Methanesulfonic acid 2-(4-chloro-pyrazol-1-yl)-ethyl ester**, K₂CO₃, KI, DMF, 120 °C, 40 minutes, 48%; (b) HCl, DCM, 23 °C, 12 h, quant.; (c) bicyclo[1.1.1]pent-1-ylamine, CDI, TEA, DMF, 0 to 60 °C, 3 h, 39%.

Methanesulfonic acid 2-(4-chloro-pyrazol-1-yl)-ethyl ester

Sodium hydride (136 mg, 60% dispersion in mineral oil) was added to a solution of 4-chloro pyrazole (0.34 g, 3.3 mmol) in DMF (3 mL) under nitrogen atmosphere. The reaction was stirred at 23 °C for 1 h and was canulated into a solution of Methanesulfonic acid 2-bromo-ethyl ester (810 mg, 4 mmol) in DMF (5 mL). The solution was heated at 70 °C for 3 h. The reaction was quenched with H₂O (20 mL) and extracted with EtOAc (2 x 40 mL). The combined organic layer was dried, filtered, and concentrated to get a brown oil. This curde mixture was purified by column chromatography to provide the title compounds as a brown oil (59 mg, 8%).

2-Amino-4-{2,4-dichloro-6-[2-(4-chloro-pyrazol-1-yl)-ethoxy]-phenyl}-5,7-dihydro-pyrrolo[3,4-d]pyrimidine-6-carboxylic acid tert-butyl ester

Following the procedure for the preparation of **57** but substituting **65** with **methanesulfonic acid 2-(4-chloro-pyrazol-1-yl)-ethyl ester** provided the title compound in 48% yield. MS: (M+H m/z = 525.1).

^1H NMR (400 MHz, DMSO- d_6) δ 7.55 (s, 1H), 7.39 (s, 1H), 7.32 - 7.34 (m, 1H), 7.27 (s, 1H), 6.81 (br. s., 2H), 4.40 (d, J = 3.54 Hz, 4H), 4.28 - 4.34 (m, 2H), 3.91 (dd, J = 13.39, 24.00 Hz, 1H), 3.63 (t, J = 13.89 Hz, 1H), 1.38 - 1.48 (m, 9H).

4-{2,4-Dichloro-6-[2-(4-chloro-pyrazol-1-yl)-ethoxy]-phenyl}-6,7-dihydro-5H-pyrrolo[3,4-d]pyrimidin-2-ylamine

Following the procedure for the preparation of **60** but substituting **57** with **2-Amino-4-{2,4-dichloro-6-[2-(4-chloro-pyrazol-1-yl)-ethoxy]-phenyl}-5,7-dihydro-pyrrolo[3,4-d]pyrimidine-6-carboxylic acid tert-butyl ester** provided the title compound in quantitative yield. MS: (M+H m/z = 425.1).

2-Amino-4-{2,4-dichloro-6-[2-(4-chloro-pyrazol-1-yl)-ethoxy]-phenyl}-5,7-dihydro-pyrrolo[3,4-d]pyrimidine-6-carboxylic acid bicyclo[1.1.1]pent-1-ylamide (37)

Following the procedure for the preparation of **42** but substituting **60** with **4-{2,4-Dichloro-6-[2-(4-chloro-pyrazol-1-yl)-ethoxy]-phenyl}-6,7-dihydro-5H-pyrrolo[3,4-d]pyrimidin-2-ylamine** and substituting **2,2-difluoro-propylamine hydrochloride** with bicyclo[1.1.1]pent-1-ylamine provided the title compound as a pale yellow solid residue (23 mg, 39%). MS: (M+H m/z = 534.0). ^1H NMR (400 MHz, DMSO- d_6) δ 7.49 (s, 1H), 7.36 (s, 1H), 7.35 (d, J = 1.5, 1H), 7.28 (d, J = 1.8, 1H), 6.90 (s, 1H), 6.78 (s, 2H), 4.33 - 4.48 (m, 4H), 4.27 - 4.33 (m, 2H), 3.96 (d, J = 13.1, 1H), 3.72 (d, J = 13.1, 1H), 2.35 (s, 1H), 1.94 (s, 6H).

Anal. Calcd. for $\text{C}_{23}\text{H}_{22}\text{N}_7\text{O}_2\text{Cl}_3 \bullet 1.0 \text{ H}_2\text{O}$: C, 49.97%; H, 4.38%; N, 17.73%. Found: C, 50.36%; H, 4.23%; N, 17.40%.

II. Compound characterization table

Compound characterization table for novel compounds:

compd	LC/MS (M+1)	Purity (UV 254 nm)	^1H NMR	Elemental Analysis	HRMS
9	236.0	93%	√	√	
10	346.2	98%	√	√	√
11	380.1	99%	√	√	√
12	479.2	95%	√	√	
13	503.2	96%	√		√
14	492.2	98%	√		√
15	490.1	95%	√		√
16	504.2	95%	√	√	
17	436.2	95%	√		√
18	498.0	98%	√		√
19	499.2	96%	√		√
20	461.2	95%	√	√	
21	489.2	97%	√		√
22	452.2	95%	√		√
23	452.0	99%	√		√
24	513.1	98%	√		√
25	465.1	97%	√		√
26	488.1	99%	√	√	√
27	435.2	95%	√	√	
28	448.2	99%	√		√
29	463.1	99%	√	√	
30	474.1	98%	√	√	√

31	524.0	99%	√	√	√
32	500.2	99%	√	√	√
33	498.2	95%	√	√	√
34	517.0	99%	√	√	
35	514.0	99%	√	√	
36	514.2	95%	√	√	
37	534.0	99%	√	√	
38	518.0	95%	√	√	
39	534.0	99%	√	√	
40	514.2	99%	√	√	
41	531.0	95%	√	√	
42	510.0	99%	√	√	

All LC/MS data were gathered on an Agilent 1100 LC with MSD (Agilent model G1946B upgraded to D model) single-quadrupole mass spec detectors running with electrospray spray ionization source. The LC instrument includes a binary pump (Agilent model G1312A) with upper pressure limit of 400 bar attached to autosampler (Agilent model G1313A) which uses external try for sample submission. The column compartment (Agilent model G1316A) which is attached to diode array (Agilent model G1315A). The instrument acquisition and data handling was done with ChemStation rev. B.02.01.

Elution Conditions:

Column: Waters Xbridge C18, 2.1x30 mm, 2.5 µm particle size; Column Temperature 80 °C

Solvent A: Water (0.1% formic acid and 0.05% ammonium formate)

Solvent B: Methanol (0.1% formic acid and 0.05% ammonium formate)

Gradient: 5-95 %B in 2.5 min, 95%B 2.5-3.0 min; Flow rate 1.2 mL/min

III. Biology

Hsp90 Biochemical Assay

Compounds were evaluated for potency against HSP-90 using a SPA (scintillation proximity assay) competition binding assay. Briefly, either full length or N-terminal HSP-90 that contains a polyhistine (6-His) tag on its C-terminus binds to copper on Yttrium-silicate scintillant beads via chelation to the His-tag. A tritiated proprietary small molecule ligand is used as the radiolabeled ligand in the competition assay. Binding of ³H-ligand to HSP-90 brings the isotope into proximity with the beads. Test

compounds which bind to HSP-90 can compete with ^3H -ligand at the ATP-binding site on the N-terminus of HSP-90.

The SPA assay for binding to HSP-90 was performed in 96-well flat bottom white plates (Corning #3604). 5 nM HSP-90 was incubated in binding buffer (100 mM Hepes, pH 7.5 and 150 mM KCl), 50 nM ^3H -ligand, various concentrations of inhibitors, and 100 μg /well SPA beads at room temperature for 30 minutes with shaking. Prior to adding the labeled ligand to the assay mix it was first diluted to 40% label with unlabeled ligand. For K_i determinations, inhibitors were diluted in 100% DMSO to 22 different concentrations prior to addition to the HSP-90/ ^3H -ligand solution. The final DMSO in the assay was 4% v/v. Copper-Ysi SPA beads (Perkin Elmer Health Sciences, #RPNQ0096) were diluted in binding buffer prior to addition. The beads were allowed to settle for 30 minutes after which the plates were counted using a TopCount NXT instrument (Perkin Elmer Inc.).

For K_i determinations, corrected CPM were transformed into % inhibition using the background wells (no enzyme) and DMSO only controls. The % inhibition values were plotted vs. log inhibitor concentration and fit to four parameter logistic equation:

$$Y = bottom + \left(\frac{top - bottom}{1 + 10^{(\log IC_{50} - X) \times slope}} \right)$$

Where Y is % Inhibition, top is the upper plateau, bottom is the lower plateau, X is the log of the competing compound concentration, and slope is the Hill Slope. The IC_{50} is then used to calculate the K_i by using the Cheng-Prusoff equation:

$$K_i = IC_{50} / \left(1 + \left(\frac{L}{K_d} \right) \right)$$

Where K_i is the inhibition constant, L is the total concentration of ^3H -ligand (50 nM), and K_d is the dissociation constant for the ligand (40 nM). The synthesis of the precursor of this ligand is described in Kung, P.-P. et al. *J. Med. Chem.* **2010**, 53, 499-503
tritium-labeled ligand

Cell based assay

Akt Luminex assay: non-small-cell lung carcinoma cell line NCI-H1299 (ATCC Number: CRL-5803) was cultured in DMEM+10%FBS, 10,000cells/well were cultured in 96-well plate. Compounds were added to each well starting at 10 uM with 3 fold serial dilution of 11 points of resulting concentrations. 24 hours post adding compounds, cell lysates were prepared and subjected to Luminex 100 system using the Beadlyte Akt/PKB kit – Upstate #46-605.

IV. X-ray crystallographic Studies of 10, 16 and 35 Crystallization conditions:

Compound stocks were made in 100 mM stock solutions using DMSO. For complex formation a ratio of 5:1 ligand: protein was used. Protein/ligand complexes were set up at 13 °C in the hanging drop method. These crystals were grown in 10-25% ethylene glycol, 0.1M phosphate citrate pH 4.2-4.6, and 0-20 mM TCEP pH 7. Crystals would appear between 1 and 3 days and harvested a week after set up.

Crystallographic data and refinement statistics.

Compound #	6	10	16	35
Data collection				
Space group	P2 ₁	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁
Unit cell (Å)	54.2, 44.1, 53.3, β = 115.7°	45.9, 79.4, 118.5	43.1, 79.5, 130.1	46.2, 79.6, 118.6
Resolution (Å)	1.7	2.0	2.65	1.7
Observations	74,217	125,213	57,618	162,391
Unique reflections	20,999	29,909	12,218	48,645
R_{sym}	0.047 (0.083)	0.059 (0.229)	0.074 (0.221)	0.052 (0.373)
I/σ	27.2 (13.4)	23.5 (7)	18.3 (6.5)	20.5 (3.2)
Completeness	0.827 (0.934)	0.994 (0.984)	0.898 (0.796)	0.994 (0.987)
Refinement				
R factor	0.197	0.186	0.211	0.187
R_{free}	0.228	0.220	0.285	0.212
RMSD				
Bond length (Å)	0.005	0.005	0.006	0.004
Bond angle (°)	1.2	0.9	0.9	1.0