Supporting Information

MLN8054 and Alisertib (MLN8237): Discovery of Selective Oral Aurora A Inhibitors

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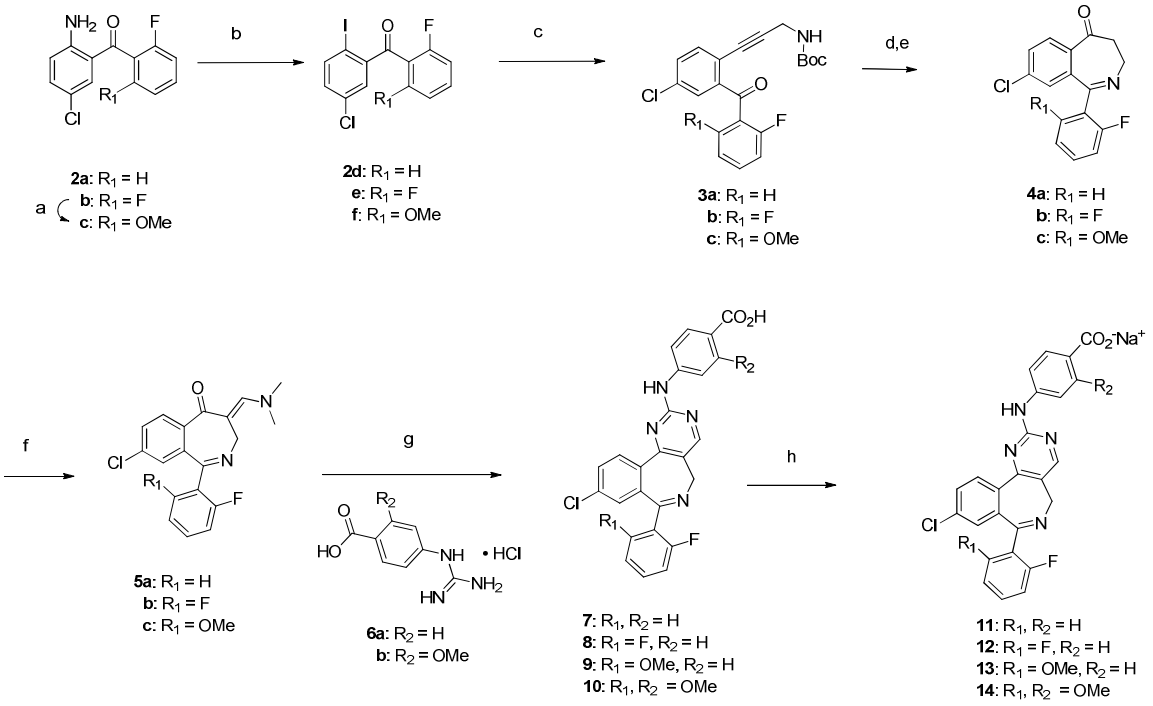
Contents

Eperimental Procedures

Preparation and Characterization of Compounds 7-10 and their sodium salts

Scheme 1



Reagents and conditions: a) Sodium methoxide, methanol, heat; b) HOAc, conc. HCl, $NaNO_2$, KI, EtOAc, water, 10°C; c) Prop-2-ynyl-carbamic acid tert-butyl ester, triethylamine, $PdCl_2(PPh_3)_2$, CuI, DCM, RT, overnight ; d) $HgSO_4$/formic acid or conc. HCl/DCM or TFA/water; e) potassium carbonate; f) DMF-DMA, DCM, 35° C; g) **6**, potassium carbonate, methanol, 55° C; h) sodium hydroxide, ethanol, water.

Experimental Methods

All solvents and reagents, unless otherwise stated, were commercially available and were used without further purification. All experiments were conducted under dry nitrogen or argon. Flash column chromatography was performed on prepacked silica gel cartridges. NMR spectra were recorded in the solvent reported on a Bruker 300 MHz Avance 1 or 400 MHz Avance 2 (5 mm QnProbe) with chemical shift in parts per million (ppm) downfield from TMS as a standard. NMR data are reported as follows: chemical shift (δ), multiplicity, coupling constants (Hz), and number of protons. Compound purity was determined by analysis of the diode array UV trace of an LC-MS spectrum using the following procedure: compounds were dissolved in DMSO, methanol, or acetonitrile, and

the solutions were analyzed using an Agilent 1100 LC interfaced to a micromass Waters Micromass Zspray mass detector (ZMD). One of two gradients was used to elute the compounds; either a formic acid (FA) gradient (acetonitrile containing zero to 100% 0.1% formic acid in water) or an ammonium acetate (AA) gradient (acetonitrile containing zero to 100% 10 mM ammonium acetate in water). High-resolution mass spectra (HRMS) were measured were performed using a QSTAR XL quadruple-time-of-flight mass spectrometer (Applied Biosystems/MDS Sciex) coupled with an Agilent 1100 series HPLC system (binary pump, autosampler and degasser). Unless stated otherwise, the purity of tested compounds was > 95% as determined by HPLC analysis.

Compounds **2a**[1], **2b**[2], **2d**[3], **4a**[2], **5a**[3], and **6b**[4] were prepared as described in the literature. Compound **6a** was purchased from Sigma-Aldrich and used without further purification.

(2-amino-5-chlorophenyl)(2-fluoro-6-methoxyphenyl)methanone (2c). (2-Amino-5-chlorophenyl)-(2,6-difluorophenyl)methanone **2b** (447 g, 1.67 mol) was dissolved in methanol (7 L) in a round bottomed flask. The solution was stirred while sodium methoxide (451 g, 8.35 mol) was added. A reflux condenser was attached and the reaction was heated to reflux. After 4 days, additional sodium methoxide (180 g, 3.34 mol) was added and refluxing was continued overnight. The reaction was then allowed to cool to room temperature during which time a yellow precipitate formed. The reaction was then diluted with water (21 L) and the precipitate was filtered, washed with water, and dried at 50 °C overnight to provide 430.5 g (92% crude yield) product as a yellow solid. HPLC analysis indicated a purity of ~90%. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.60 (br s, 2H), 7.51 (dd, *J* = 8.5, 7 Hz, 1H), 7.31 (dd, *J* = 2.5, 9 Hz, 1H), 7.02 (d, *J* = 8.5 Hz, 1H), 6.91-6.97 (m, 2H), 6.89 (d, *J* = 9 Hz, 1H), 3.75 (s, 3H); LCMS: *m/z* 280.0 (M+H).

(5-chloro-2-iodophenyl)(2-fluoro-6-methoxyphenyl)methanone (2f). (2-amino-5-chlorophenyl)(2-fluoro-6-methoxyphenyl)methanone **2c** (80.0 g, 0.286 mol) was combined with acetic acid (211 mL, 3.72 mol) and conc. hydrochloric acid (97.7 mL, 1.17 mol) in a round bottomed flask equipped with a stir bar. The mixture was stirred and cooled in an ice bath then a solution of sodium nitrite (23.7 g, 0.343 mol) in water (98 mL) was slowly added over 1 hour with good stirring, keeping the reaction temperature below 9°C. The resulting mixture was stirred in the ice bath for 30 minutes, then cold ethyl acetate (279 mL) was added in one portion. The reaction was stirred for 5 minutes then a solution of potassium iodide (57.0 g, 0.343 mol) in water (98 mL) was added over 1.25 hours, keeping the reaction temperature below 9 °C. After the addition was complete, the reaction was allowed to warm to room temperature over 1.5 hours. A solution of sodium sulfite (72.1 g, 0.572 mol) in water (50 mL) and ethyl acetate (200 mL) were added simultaneously with good stirring during which time the brown reaction color dissipated to become light orange. The reaction mixture was transferred to a separatory funnel, the organic layer was separated, and aqueous layer was extracted with ethyl acetate. The organic extracts were combined and washed with saturated sodium bicarbonate solution until the aqueous layer remained basic, then was washed with brine. The organic layer was dried over sodium sulfate, filtered, and evaporated to leave a thick brown oil. 2-Propanol (250 mL) was added and heating was applied to dissolve the oil.

The solution was allowed to cool to room temperature during which time a yellow precipitate formed. The solids were collected on a Buchner funnel, washed with additional 2-propanol, and dried in vacuo at 40°C overnight to yield 88 g (79%) product as a yellow powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, *J* = 8.5 Hz, 1H), 7.39-7.45 (m, 2H), 7.12 (dd, *J* = 2.8, 8.5 Hz, 1H), 6.73-6.79 (m, 2H), 3.73 (s, 3H); LCMS: *m/z* 391.0 (M+H).

Compound **2e**. By employment of the above procedure, starting from **2b**, compound **2e** was prepared.

(5-chloro-2-iodophenyl)(2,6-difluorophenyl)methanone (2e). Yield 90%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, *J* = 8.2 Hz, 1H), 7.45-7.56 (m, 1H), 7.43 (d, *J* = 2.4 Hz, 1H), 7.18 (dd, *J* = 2.4, 8.2 Hz, 1H), 6.95-7.03 (m, 2H); LCMS: *m/z* 379.0 (M+H).

tert-butyl {3-[4-chloro-2-(2-fluoro-6-methoxybenzoyl)phenyl]prop-2-yn-1-yl}carbamate (3c). Methylene chloride (750 mL) was added to a mixture of (5-chloro-2-iodophenyl)(2-fluoro-6-methoxyphenyl)methanone **(2f)** (114 g, 0.292 mol), N-BOC propargylamine (49.8 g, 0.321 mol), bis(triphenylphosphine)palladium(II) chloride (10 g, 0.02 mol), and copper (I) iodide (3 g, 0.02 mol) in a round bottomed flask equipped with a stir bar. The resulting solution was cooled to 0 °C and sparged with nitrogen for 30 minutes. Degassed triethylamine (193.2 mL, 1.386 mol), was then added dropwise and the reaction was allowed to gradually warm to room temperature with stirring. After 8 hrs, a TLC (25% EtOAc/Hexanes) showed no starting material remaining with product as the major spot. The volatiles were removed in vacuo, leaving crude product as an orange solid which was purified by column chromatography (silica gel, 25% ethyl acetate/hexanes) to yield 113 g (93% yield) product as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, *J* = 1.6 Hz, 1H), 7.47 – 7.35 (m, 3H), 6.83 – 6.72 (m, 2H), 4.44 (br s, 1H), 3.87 (d, *J* = 4.4 Hz, 2H), 3.73 (s, 3H), 1.45 (s, 9H); LCMS: *m/z* 418.1 (M+H).

Compounds **3a** and **3b**. By employment of the above procedure, starting from **2d or 2e**, compounds **3a** and **3b** were prepared.

tert-butyl {3-[4-chloro-2-(2-fluorobenzoyl)phenyl]prop-2-yn-1-yl}carbamate (3a). Yield 90%. 1H NMR (300 MHz, $CDCl_3$) δ 7.71 (td, J = 7.4, 1.8 Hz, 1H), 7.61 – 7.51 (m, 2H), 7.45 – 7.39 (m, 2H), 7.29 (dd, *J* = 7.6, 6.6 Hz, 1H), 7.11 (ddd, *J* = 10.2, 8.3, 0.9 Hz, 1H), 4.13 (br s, 1H), 3.80 (d, *J* = 5.3 Hz, 2H), 1.43 (s, 9H). LCMS: *m/z* 388.1 (M+H).

tert-butyl {3-[4-chloro-2-(2,6-difluorobenzoyl)phenyl]prop-2-yn-1-yl}carbamate (3b). Yield 61%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, *J* = 2.0 Hz, 1H), 7.55 – 7.35 (m, 3H), 7.07 – 6.88 (m, 2H), 4.52 (br s, 1H), 3.84 (d, *J* = 5.4 Hz, 2H), 1.56 – 1.34 (s, 9H); LCMS: *m/z* 406 (M+H).

8-Chloro-1-(2,6-difluorophenyl)-3,4-dihydrobenzo[c]azepin-5-one (4b). tert-Butyl {3-[4-chloro-2-(2,6-difluorobenzoyl)phenyl]prop-2-yn-1-yl}carbamate **(3b)** (9.2 g, 23

mmol) was dissolved in dioxane (200 mL) in a round bottomed flask. Hydrochloric acid (5N, 200 mL) was added and the solution was stirred at room temperature for 14 hours, then at 60 °C for 2 hours. The solution was diluted with methylene chloride (200 mL) and solid sodium bicarbonate was added portionwise until the solution pH was basic to litmus paper. The mixture was allowed to stir for 2 hours then the organic phase was separated and the aqueous phase was extracted with methylene chloride (2x100 mL). The combined organic phases were washed with water (3x50 mL), dried over sodium sulfate, and evaporated to provide 4.2 g (100% yield) product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, *J* = 8.2 Hz, 1H), 7.47 (dd, *J* = 1.8, 8.2 Hz, 1H), 7.28-7.39 (m, 1H), 7.10 (d, *J* = 1.8 Hz, 1H), 6.87-6.97 (m, 2H), 3.97 (m, 2H), 3.03 (m, 2H) ; LCMS: *m/z* 306 (M+H).

8-Chloro-1-(2-fluoro-6-methoxyphenyl)-3,4-dihydrobenzo[c]azepin-5-one (4c). Solid tert-butyl {3-[4-chloro-2-(2-fluoro-6-methoxybenzoyl)phenyl]prop-2-yn-1-yl}carbamate **(3c)** (110 g, 0.26 mol) was added portionwise to a stirring solution of trifluoroacetic acid (450 mL, 5.8 mol) and water (14.2 mL, 0.790 mol). After the addition was complete the solution was warmed to 38 °C and held with stirring for 18 hours. The mixture was concentrated on the rotary evaporator to leave a brown residue which was dissolved in methylene chloride (1000 mL). The solution was stirred and potassium carbonate (100 g, 0.7 mol) was added portionwise. The heterogeneous solution was allowed to stir at room temperature for 6 hrs. HPLC indicated that ring closure was complete. The reaction mixture was filtered through a Celite bed to remove solids and volatiles were removed in vacuo to provide a brown foam (83 g) which was used as is in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.4 Hz, 1H), 7.47 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.33 (td, *J* = 8.4, 6.6 Hz, 1H), 7.15 (d, *J* = 2.0 Hz, 1H), 6.79 (td, *J* = 8.7, 0.7 Hz, 1H), 6.70 (d, *J* = 8.4 Hz, 1H), 3.97 (br s, 2H), 3.65 (s, 3H), 3.07 (t, *J* = 5.5 Hz, 2H). LCMS: *m/z* 318 (M+H).

8-Chloro-4-[(dimethylamino)methylene]-1-(2-fluoro-6-methoxyphenyl)-3,4-dihydro-5*H*-2-benzazepin-5-one (5c). 1,1-Dimethoxy-N,N-dimethylmethanamine (334.47 mL, 2.5178 mol) was added to 8-chloro-1-(2-fluoro-6-methoxyphenyl)-3,4-dihydro-5H-2-benzazepin-5-one **(4c)** (80.0 g, 0.252 mol) and the homogeneous solution was stirred at 40 °C overnight after which an LCMS of the reaction showed ~3% of starting material remaining. 2-Methoxy-2-methylpropane (335 mL) was added to the reaction. It was then cooled to 0 °C with stirring. The precipitated solid was collected by filtration and washed with additional 2-methoxy-2-methylpropane (335 mL). The collected product was dried in vacuo for 6 hrs to provide 60 g (64% yield) product as a tan solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99 (d, *J* = 8.4 Hz, 1H), 7.80 (s, 1H), 7.45 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.36 – 7.24 (m, 1H), 7.09 (d, *J* = 1.7 Hz, 1H), 6.93 – 6.54 (m, 2H), 4.92 (d, *J* = 12.6 Hz, 1H), 3.80 (br s, 3H), 3.72 (d, *J* = 12.6 Hz, 1H), 3.27 (s, 6H). LCMS: *m/z* 373 (M+H).

Compound **5b**. By employment of the above procedure, starting from **4b**, compound **5b** was prepared.

8-Chloro-4-[(dimethylamino)methylene]-1-(2,6-difluorophenyl)-3,4-dihydro-5*H*-2-benzazepin-5-one (5b). Yield 45%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (dd, *J* = 8.4, 2.8 Hz, 1H), 7.60 (s, 1H), 7.35 – 7.25 (m, 1H), 7.24 – 7.08 (m, 1H), 6.88 (d, *J* = 4.9 Hz, 1H), 6.74 (t, *J* = 8.3 Hz, 2H), 4.76 (d, *J* = 12.4 Hz, 1H), 3.52 (d, *J* = 12.4 Hz, 1H), 3.14 – 3.00 (m, 6H); LCMS: *m/z* 361.1 (M+H).

4-{[9-chloro-7-(2-fluoro-6-methoxyphenyl)-5*H*-pyrimido[5,4d][2]benzazepin-2-yl]amino}-2-methoxybenzoic acid (10). Methanol (50 mL) was added to a mixture of 8-chloro-4-[(dimethylamino)methylene]-1-(2-fluoro-6-methoxyphenyl)-3,4-dihydro-5H-2-benzazepin-5-one **(5c)** (2.39 g, 0.00642 mol), 4-{[amino(imino)methyl]amino}-2-methoxybenzoic acid•HCl **(6b)** (1.77 g, 0.00720 mo1) , and potassium carbonate•1.5 H_2O (2.65 g, 0.0160 mol) in a 100-mL round bottomed flask equipped with a stir bar and reflux condenser. The reaction mixture was stirred at reflux for 16 hours then was cooled to room temperature, diluted with water (450 mL) and acidified to pH 1 with 1N HCl. Diethyl ether (200 mL) was added and the mixture was stirred for 15 minutes. The resultant precipitate was collected by filtration and purified by flash silica gel chromatography (NH_4OH:MeOH:DCM 0.5:5:94.5 to 2:20:78) to yield the ammonium salt of the product as a tan solid. The solid was suspended in water (100 mL) and, with rapid stirring, 1N HCl was added to bring the mixture to pH 1. The mixture was stirred for approximately 30 minutes, then diethyl ether (50 mL) and ethyl acetate (5 mL) were added and the mixture was stirred at room temperature for approximately 1 hour. The product was collected on a fritted funnel, washed with water (50 mL), then diethyl ether (50 mL), and dried in vacuo at 40 °C overnight to provide 1.65 g (50% yield) product. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.08 (s, 1H), 10.23 (s, 1H), 8.72 (s, 1H), 8.29 (d, *J* = 8.5 Hz, 1H), 7.95 (s, 1H), 7.80 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.70 (d, *J* = 8.6 Hz, 1H), 7.4 – 7.35 (m, 2H), 7.21 (br s, 1H), 6.9 (br s, 2H), 4.9 (br s, 1H), 3.9 (br s, 1H), 3.85 (s, 3H), 3.3 (s, 3H); LCMS: *m/z* 519.0 (M+H).

4-{[9-chloro-7-(2-fluorophenyl)-5*H*-pyrimido[5,4-d][2]benzazepin-2-yl]amino}benzoic acid (7). By employment of the above procedure, starting from **5a** and **6a**, compound **7** was prepared. Yield 51%. ^{1}H NMR (300 MHz, DMSO-*d*6) δ 12.58 (s, 1H), 10.30 (s, 1H), 8.75 (s, 1H), 8.32 (d, *J* = 8.5 Hz, 1H), 8.04 – 7.83 (m, 5H), 7.65 (td, *J* = 7.6, 1.6 Hz, 1H), 7.60 – 7.50 (m, 1H), 7.40 – 7.29 (m, 2H), 7.20 (dd, *J* = 10.6, 8.4 Hz, 1H), 4.96 (br s, 1H), 3.68 (br s, 1H). LCMS: *m/z* 459.2 (M+H).

4-{[9-chloro-7-(2,6-difluorophenyl)-5*H*-pyrimido[5,4-d][2]benzazepin-2-yl]amino}benzoic acid (8). By employment of the above procedure, starting from **5b** and **6a**, compound **8** was prepared. Yield 45%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.11 (br s, 1H), 9.87 (s, 1H), 8.33 (s, 1H), 7.89 (d, *J* = 8.5 Hz, 1H), 7.66 – 7.34 (m, 5H), 7.12 (dd, *J* = 7.5, 6.8 Hz, 1H), 6.92 (d, *J* = 1.8 Hz, 1H), 6.73 (t, *J* = 8.4 Hz, 2H), 4.52 (br s, 1H), 3.55 (br s, 1H); LCMS: *m/z* 477.1 (M+H).

4-{[9-chloro-7-(2-fluoro-6-methoxyphenyl)-5*H*-pyrimido[5,4-d][2]benzazepin-2-yl]amino}benzoic acid (9). By employment of the above procedure, starting from **5c** and **6a**, compound **9** was prepared. Yield 69%. ^{1}H NMR (300 MHz, DMSO-*d*6) δ

12.53 (br s, 1H), 10.29 (s, 1H), 8.71 (s, 1H), 8.28 (d, *J* = 8.5 Hz, 1H), 7.95 (q, *J* = 8.9 Hz, 4H), 7.81 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.42 (q, *J* = 8.3 Hz, 1H), 7.21 (br s, 1H), 6.88 (br s, 2H), 4.85 (br s, 1H), 3.96 (br s, 1H), 3.39 (br s, 3H). LCMS: *m/z* 489.0 (M+H).

Sodium 4-{[9-chloro-7-(2-fluoro-6-methoxyphenyl)-5*H*-pyrimido[5,4-d][2]benzazepin-2-yl]amino}-2-methoxybenzoate (14). To a stirred suspension of 4-{[9-chloro-7-(2-fluoro-6-methoxyphenyl)-5H-pyrimido[5,4-d][2]benzazepin-2-yl]amino}-2-methoxybenzoic acid **(10)** (98.0 g, 190 mmol) in ethanol (2.0 L) was added a solution of sodium hydroxide in water (1.044M, 199 mL, 208 mmol). The resultant homogeneous solution was stirred for 1 hour, during which time a thick precipitate formed. The product was collected by filtration, and washed with ethanol (0.5 L) then diethyl ether (1.0 L). The resultant solid was dried in vacuo at 60-70 °C for 4 days to provide 88.6 g (86.8%) of sodium 4-{[9-chloro7-(2-fluoro-6-methoxyphenyl)-5H-pyrimido[5,4-d][2]benzazepin-2-yl]amino}-2-methoxybenzoate as a light tan solid, mp 225° C (decomp). ^{1}H NMR (400 MHz, DMSO-*d*6) δ 9.86 (s, 1H), 8.60 (s, 1H), 8.29 (d, *J* = 8.53 Hz, 1H), 7.79 (dd, *J* = 8.53, 2.01 Hz, 1H), 7.60 (br s, 1H), 7.40 (m, 1H), 7.29 (d, *J* = 8.28 Hz, 1H), 7.25-7.15 (m, 2H), 6.9 (br s, 2H), 4.9 (br s, 1H), 3.8 (br s, 1H), 3.70 (s, 3H), 3.35 (br s, 3H); Calculated *m/z* value of $[M+H]^+$ 519.1230; Experimental *m/z* value of $[M+H]^+$ 519.1214.

Compounds **11, 12,** and **13**. By employment of the above procedure, starting from compounds **7**, **8**, or **9**, compounds **11, 12, and 13** were prepared.

Sodium 4-{[9-chloro-7-(2-fluorophenyl)-5*H*-pyrimido[5,4-d][2]benzazepin-2-yl]amino}benzoate (11). Yield 93%. ^{1}H NMR (300 MHz, DMSO-*d*6) δ 9.81 (s, 1H), 8.64 (s, 1H), 8.27 (d, *J* = 8.5 Hz, 1H), 7.85 (m, 3H), 7.71 (d, *J* = 8.6 Hz, 2H), 7.65 – 7.56 (m, 1H), 7.50 (dd, *J* = 13.5, 5.8 Hz, 1H), 7.37 – 7.23 (m, 2H), 7.16 (dd, *J* = 10.6, 8.4 Hz, 1H), 4.86 (s, 1H), 3.76 (s, 1H). Calculated *m/z* value of $[M+H]^+$ 459.1019; Experimental *m/z* value of $[M+H]^+$ 459.1015.

Sodium 4-{[9-chloro-7-(2,6-difluorophenyl)-5*H*-pyrimido[5,4-d][2]benzazepin-2-yl]amino}benzoate (12). Yield 88%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 9.86 (s, 1H), 8.65 (s, 1H), 8.28 (d, *J* = 8.5 Hz, 1H), 7.89 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.80 (d, *J* = 8.6 Hz, 2H), 7.69 (d, *J* = 8.6 Hz, 2H), 7.53 (dt, *J* = 15.1, 8.3 Hz, 1H), 7.32 (d, *J* = 2.1 Hz, 1H), 7.215 (t, *J* = 8.5 Hz, 2H), 4.89 (br s, 1H), 3.88 (br s, 1H). Calculated *m/z* value of $[M+H]^+$ 477.0924; Experimental *m/z* value of $[M+H]^+$ 477.0931.

Sodium 4-{[9-chloro-7-(2fluoro-6-methoxyphenyl)-5*H*-pyrimido[5,4-d][2]benzazepin-2-yl]amino}benzoate (13). Yield 98%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 9.84 (s, 1H), 8.63 (s, 1H), 8.25 (d, *J* = 8.5 Hz, 1H), 7.88 – 7.75 (m, 3H), 7.69 (d, *J* = 8.6 Hz, 2H), 7.49 – 7.35 (m, 1H), 7.18 (s, 1H), 6.86 (br s, 2H), 4.81 (br s, 1H), 3.82 (br s, 1H), 3.32 (br s, 3H). Calculated *m/z* value of $[M+H]^+$ 489.1124; Experimental *m/z* value of $[M+H]^+$ 489.1104.

Table 1. Invitrogen Kinase Panel Percent Activity with 1 uM MLN8054 (**8**)

Kinase	% Act	Kinase	% Act	Kinase	% Act	Kinase	% Act
Abl(h)	24	eEF-2K(h)	123	LKB1(h)	101	PKCßII(h)	100
Abl(m)	31	EGFR(h)	74	LOK(h)	95	PKCγ(h)	116
Abl(T315I)(h)	74	EGFR(L858R)(h)	81	Lyn(h)	46	PKCδ(h)	109
ALK(h)	95	EGFR(L861Q)(h)	92	Lyn(m)	52	PKCε(h)	123
ALK4(h)	115	EGFR(T790M)(h)	86	MAPK1(h)	91	PKCη(h)	120
Arg(h)	40	EGFR(T790M,L858R)(h)	51	MAPK2(h)	96	PKCι(h)	109
AMPK(r)	83	EphA1(h)	29	MAPK2(m)	105	PKCμ(h)	112
Arg(m)	63	EphA2(h)	31	MAPKAP-K2(h)	101	PKCθ(h)	112
ARK5(h)	98	EphA3(h)	88	MAPKAP-K3(h)	104	PKCζ(h)	99
ASK1(h)	120	EphA4(h)	90	MARK1(h)	106	PKD2(h)	90
Aurora-A(h)	0	EphA5(h)	80	MEK1(h)	110	PKG1α(h)	103
Axl(h)	41	EphA7(h)	67	MELK(h)	79	PKG1ß(h)	109
Blk(m)	53	EphA8(h)	87	Met(h)	140	Plk3(h)	105
Bmx(h)	64	EphB1(h)	35	MINK(h)	104	PRAK(h)	110
BRK(h)	104	EphB2(h)	69	MKK4(m)	120	PRK2(h)	90
BrSK1(h)	92	EphB3(h)	95	MKK6(h)	94	PrKX(h)	99
BrSK2(h)	99	EphB4(h)	84	MKK7ß(h)	95	PTK5(h)	90
BTK(h)	90	ErbB4(h)	74	MLCK(h)	95	Pyk2(h)	90
CaMKI(h)	98	FAK(h)	110	MLK1(h)	81	Ret(h)	80
CaMKII(r)	102	Fer(h)	57	Mnk2(h)	94	RIPK2(h)	88
CaMKIIß(h)	100	Fes(h)	133	MRCKα(h)	101	ROCK-I(h)	120
CaMKIIγ(h)	112	FGFR1(h)	43	MRCKß(h)	107	ROCK-II(h)	100
CaMKIIδ(h)	123	FGFR2(h)	52	MSK1(h)	93	ROCK-II(r)	92
CaMKIV(h)	104	FGFR3(h)	86	MSK2(h)	106	Ron(h)	94
CDK1/cyclinB(h)	110	FGFR4(h)	115	MSSK1(h)	100	Ros(h)	88
CDK2/cyclinA(h)	96	Fgr(h)	36	MST1(h)	107	Rse(h)	56
CDK2/cyclinE(h)	97	Flt1(h)	65	MST2(h)	100	Rsk1(h)	91
CDK3/cyclinE(h)	105	Flt3(D835Y)(h)	81	MST3(h)	107	Rsk1(r)	83
CDK5/p25(h)	94	Flt3(h)	91	MuSK(h)	97	Rsk2(h)	76
CDK5/p35(h)	100	Flt4(h)	6	NEK2(h)	110	Rsk3(h)	76
CDK6/cyclinD3(h)	116	Fms(h)	95	NEK3(h)	95	SAPK2a(h)	105
CDK7/cyclinH/MAT1(h)	105	Fyn(h)	31	NEK6(h)	92	SAPK2a(T106M)(h)	130
CDK9/cyclin T1(h)	116	GRK5(h)	109	NEK7(h)	99	SAPK2b(h)	95
CHK1(h)	109	GRK6(h)	110	NEK11(h)	116	SAPK3(h)	121
CHK2(h)	83	GSK3α(h)	110	NLK (h)	104	SAPK4(h)	87
CK1γ1(h)	92	GSK3ß(h)	102	p70S6K(h)	94	SGK(h)	99
CK1γ2(h)	116	Hck(h)	22	PAK2(h)	100	SGK2(h)	100
CK1γ3(h)	113	HIPK1(h)	117	PAK3(h)	94	SGK3(h)	116

CK1δ(h)	129	HIPK2(h)	112	PAK4(h)	96	SIK(h)	66
CK1(y)	115	HIPK3(h)	111	PAK5(h)	100	Snk(h)	110
CK2(h)	108	IGF-1R(h)	88	PAK6(h)	108	SRPK1(h)	105
CK2α2(h)	120	IKKα(h)	114	PAR-1Bα(h)	101	SRPK2(h)	87
CLK3(h)	81	IKKß(h)	99	PASK(h)	114	STK33(h)	96
cKit(D816V)(h)	101	IR(h)	88	PDGFRα(h)	110	Syk(h)	99
cKit(D816H)(h)	83	IRR(h)	95	PDGFRß(h)	98	TAK1(h)	111
cKit(h)	91	IRAK1(h)	110	PDK1(h)	81	TBK1(h)	100
c-RAF(h)	115	IRAK4(h)	97	PhKγ2(h)	98	Tie2(h)	51
CSK(h)	105	Itk(h)	118	Pim-1(h)	97	TrkA(h)	32
cSRC(h)	75	JAK2(h)	92	Pim-2(h)	101	TrkB(h)	112
DAPK1(h)	114	JAK3(h)	100	PKA(b)	86	TSSK1(h)	107
DAPK2(h)	103	JNK1α1(h)	122	PKA(h)	100	TSSK2(h)	101
DCAMKL2(h)	103	JNK2α2(h)	141	PKBα(h)	101	WNK2(h)	109
DDR2(h)	87	JNK3(h)	125	PKBß(h)	104	WNK3(h)	88
DMPK(h)	116	KDR(h)	61	PKBγ(h)	110	Yes(h)	20
DRAK1(h)	60	Lck(h)	59	PKCα(h)	108	ZAP-70(h)	96
DYRK2(h)	98	LIMK1(h)	110	PKCßI(h)	98	ZIPK(h)	97

Table 2. Invitrogen Kinase Panel Percent Activity with 1 uM alisertib (**10**).

Kinase	% Act	Kinase	% Act	Kinase	% Act	Kinase	% Act
ABL1	32	EGFR L861Q	33	MAP2K2 (MEK2)	102	PRKCB2 (PKC beta II)	104
ABL1 E255K	45	EPHA1	25	MAP2K6 (MKK6)	109	PRKCD (PKC delta)	83
ABL1 G250E	13	EPHA2	-11	MAP3K8 (COT)	94	PRKCE (PKC epsilon)	99
ABL1 T315I	65	EPHA3	78	MAP3K9 (MLK1)	55	PRKCG (PKC gamma)	107
ABL1 Y253F	36	EPHA4	6	MAP4K2 (GCK)	57	PRKCH (PKC eta)	108
ABL2 (Arg)	47	EPHA5	26	MAP4K4 (HGK)	100	PRKCI (PKC iota)	101
ACVR1B (ALK4)	101	EPHA8	102	MAP4K5 (KHS1)	110	PRKCN (PKD3)	102
ADRBK1 (GRK2)	102	EPHB1	17	MAPK1 (ERK2)	105	PRKCQ (PKC theta)	103
ADRBK2 (GRK3)	105	EPHB2	56	MAPK11 (p38 beta)	103	PRKCZ (PKC zeta)	106
AKT1 (PKB alpha)	106	EPHB3	92	MAPK12 (p38 gamma)	99	PRKD1 (PKC mu)	102
AKT2 (PKB beta)	99	EPHB4	29	MAPK13 (p38 delta)	103	PRKD2 (PKD2)	99
AKT3 (PKB gamma)	107	ERBB2 (HER2)	65	MAPK14 (p38 alpha)	109	PRKG1	101
ALK	83	ERBB4 (HER4)	60	MAPK3 (ERK1)	109	PRKG2 (PKG2)	104
AURKB (Aurora B)	0	FER	30	MAPKAPK2	111	PRKX	97
BLK	8	FES (FPS)	87	MAPKAPK3	6	PTK2 (FAK)	27
BMX	25	FGFR1	18	MAPKAPK5 (PRAK)	104	PTK6 (Brk)	93
BRAF	101	FGFR2	10	MATK (HYL)	106	RAF1 (cRAF)	105
BRAF V599E	92	FGFR3	46	MERTK (cMER)	85	RET	50
BTK	51	FGFR3 K650E	37	MET (cMet)	93	RET V804L	91
CAMK1D (CaMKI delta)	62	FGFR4	79	MET M1250T	104	RET Y791F	52
CAMK2A (CaMKII alpha)	5	FGR	3	MINK1	103	ROCK1	97
CAMK2B (CaMKII beta)	96	FLT1 (VEGFR1)	95	MST1R (RON)	108	ROCK2	102
CAMK2D (CaMKII delta)	103	FLT3	59	MST4	100	ROS1	20
CAMK4 (CaMKIV)	88	FLT3 D835Y	93	MUSK	48	RPS6KA1 (RSK1)	81
CDC42 BPA (MRCKA)	100	FLT4 (VEGFR3)	23	MYLK2 (skMLCK)	98	RPS6KA2 (RSK3)	69
CDC42 BPB (MRCKB)	97	FRK (PTK5)	94	NEK1	97	RPS6KA3 (RSK2)	54
CDK1/cyclin B	100	FYN	30	NEK2	96	RPS6KA4 (MSK2)	92
CDK2/cyclin A	93	GRK4	109	NEK4	101	RPS6KA5 (MSK1)	101
CDK5/p35	100	GRK5	99	NTRK1 (TRKA)	13	RPS6KB1 (p70S6K)	97
CHEK1 (CHK1)	83	GRK6	94	NTRK2 (TRKB)	22	SGK (SGK1)	103
CHEK2 (CHK2)	57	GRK7	98	NTRK3 (TRKC)	9	SGK2	107
CLK1	106	GSK3A (GSK3 alpha)	100	PAK3	98	SGKL (SGK3)	104
CLK2	71	GSK3B (GSK3 beta)	101	PAK4	98	SRC	26
CLK3	94	HCK	11	PAK6	100	SRC N1	18
CSF1R (FMS)	52	HIPK4	99	PASK	98	SRMS (Srm)	98
CSK	39	IGF1R	100	PDGFRA (PDGFR alpha)	94	SRPK1	93
CSNK1A1 (CK1 alpha 1)	103	IKBKB (IKK beta)	105	PDGFRA D842V	87	SRPK2	94
CSNK1D (CK1 delta)	101	INSR	101	PDGFRA T674I	95	STK22B (TSSK2)	111
CSNK1E (CK1 epsilon)	104	INSRR (IRR)	109	PDGFRB (PDGFR beta)	92	STK22D (TSSK1)	108
CSNK1G1 (CK1 gamma 1)	29	IRAK4	91	PDK1	72	STK23 (MSSK1)	97
CSNK1G2 (CK1 gamma 2)	115	ITK	101	PHKG1	100	STK24 (MST3)	104
CSNK1G3 (CK1 gamma 3)	108	JAK2	99	PHKG2	101	STK25 (YSK1)	96
CSNK2A1 (CK2 alpha 1)	102	JAK2 JH1 JH2	107	PIM1	100	STK3 (MST2)	92
CSNK2A2 (CK2 alpha 2)	93	JAK3	96	PIM2	98	STK4 (MST1)	96
DAPK3 (ZIPK)	97	KDR (VEGFR2)	65	PKN1 (PRK1)	94	STK6 (Aurora A)	0
DCAMKL2 (DCK2)	103	KIT	104	PLK1	101	SYK	101

DYRK1A	104	KIT T670I	98	PLK2	103	TAOK2 (TAO1)	96
DYRK3	99	LCK	14	PLK3	111	TBK1	39
DYRK4	101	LYN A	12	PRKACA (PKA)	59	TEK (Tie2)	20
EGFR (ErbB1)	39	LYN B	14	PRKCA (PKC alpha)	115	TYRO3 (RSE)	49
EGFR L858R (ErbB1 L858R)	33	MAP2K1 (MEK1)	105	PRKCB1 (PKC beta I)	105	YES1	11
						ZAP70	105

Table 3. Functional Observational Battery in Sprague Dawley Rat with IV dosing of MLN8054 (**8**) and alisertib (**10**)[a]

	C_{max} (μg/mL)	Effect in FOB
Vehicle	N/A	No
MLN8054	0.6	No
	4.0	No
	17.6	yes
	38.2	yes
alisertib	54.5	No
	147.0	yes

a. Five minute IV infusion of the sodium salts **12** and **14** in 10% 2-HP-β-CD/0.25% PBS.

Table 4. Perkin Elmer General SEP panel in which no activity was observed with MLN8054 (**8**) and alisertib (**10**) at 1 and 10 μM

Adenosine, A1	Cholecystokinin, CCK1 (CCKA)	Glutamate, NMDA, Phencyclidine Site (Ionotropic)	Oxidase, MAO-A, Peripheral
Adenosine, A2	Dopamine Transporter	Histamine, H1	Oxidase, MAO-B, Peripheral
Adrenergic, Alpha 1, Non-selective	Dopamine, D1 (h)	Histamine, H2	Potassium Channel, ATP-Sensitive
Adrenergic, Alpha 2, Non-selective	Dopamine, D2s (h)	Histamine, H3	Serotonin Transporter
Adrenergic, Beta 1 (h)	Endothelin, ET-A (h)	Muscarinic, Non-selective, Central	Serotonin, 5HT1A (h)
Adrenergic, Beta 2 (h)	Esterase, Acetylcholine	Neurokinin, NK1	Serotonin, 5HT1B
Angiotensin II, AT1 (h)	GABA A, Agonist Site	Neuropeptide Y, Non-selective	Serotonin, 5HT2A (h)
Calcium Channel, Type L (Benzothiazepine Site)	GABA-B	Nicotinic, Neuronal (a-BnTx insensitive)	Sigma, Non-selective
Calcium Channel, Type L (Dihydropyridine Site)	Glucocorticoid (h)	Norepinephrine Transporter	Sodium, Site 2
Calcium Channel, Type N	Glutamate, NMDA Agonist Site (Ionotropic)	Opioid, Non-selective	Vasopressin 1

Supporting Information References

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