

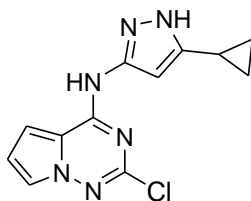
## Supporting Information

### **The Discovery of BMS-754807, a Small Molecule Inhibitor of Insulin-Like Growth Factor Receptor (IGF-1R) Kinase in Clinical Development.**

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Contents include synthetic procedure and analytical data for intermediates **5**, **7**, and **9d** and experimental details for data presented in Table 1 and 3 and the western blots used to generate Table 2. The purity of all compounds was <95% as confirmed by two orthogonal HPLC determinations.

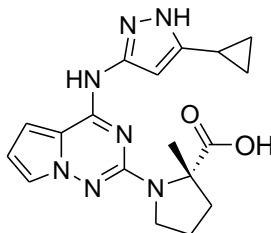
2-Chloro-N-(5-cyclopropyl-1H-pyrazol-3-yl)pyrrolo[1,2-f][1,2,4]triazin-4-amine (**5**)



To a two liter three necked round bottom flask equipped with a mechanical stirrer, a N<sub>2</sub> inlet and a thermometer were added, under N<sub>2</sub>, a solution of 5-cyclopropyl-1H-pyrazol-3-amine (36.0 g, 0.292 mol) in isopropyl alcohol (500 ml) (dissolved with heating) and diisopropylethylamine (93 ml, 0.534 mol). The solution was then cooled to 5 °C with an ice bath. 2,4-Dichloropyrrolo[1,2-f][1,2,4]triazine (BMS-738904) was added as one portion to the above solution. The ice bath was removed. The reaction mixture was allowed to warm to ambient temperature and stirred for 18 h. The reaction mixture was then diluted with isopropyl alcohol (300 mL), cooled to 5 °C and stirred for one hour at 0-5 °C. The white precipitates were then vacuum filtered and the resulting filter cake was washed with cold isopropyl alcohol (2 x 50 mL). The filter cake was house vacuum air dried for 18 h. The product **3** was collected as a white solid (69.9 g, 96%). HPLC purity, 98.0%; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 300 MHz) δ 12.33 (s, 1H), 10.95 (s, 1H), 7.73 (s, 1H), 7.32 (brs, 1H), 6.69 (dd, J = 4.3 and 2.6 Hz, 1H), 6.45 (s, 1H), 1.90-1.99 (m, 1H), 0.92-0.98 (m, 1H), 0.70-0.75 (m, 1H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 75 MHz) δ 150.87, 150.37, 118.96, 111.45, 110.54, 102.56, 94.25, 6.95, 6.104; HRMS calcd for C<sub>12</sub>H<sub>11</sub>ClN<sub>6</sub> 274.0734, found 274.0734.

HPLC conditions : Column: Xbridge RP18 2.5 uM, 4.6 mm x 75 mm; Solvent system: A: 90% water/10% MeCN 0.1% TFA all via volume; B: 90% MeCN/10% water 0.1% TFA all via volume. Step Gradient: Time 0: 10% B, Time 2 minute: 30% B, Time 10 minutes: 70% B, Time 12 minutes: 100% B, Time 14 minutes: 100% B, Stop time 15 minutes. Flow Rate: 1.5 mL/min. UV detection and 235 nm & 254 nm. Retention time: 6.3 minutes

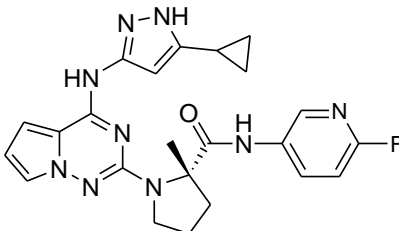
(S)-1-(4-(5-Cyclopropyl-1H-pyrazol-3-ylamino)pyrrolo[1,2-f][1,2,4]triazin-2-yl)pyrrolidine-2-carboxylic acid (**7**)



To a one liter three necked round bottom flask equipped with a Dean-Stark apparatus, a condenser, a N<sub>2</sub> inlet, a magnetic stirrer and a thermometer were charged 2-chloro-N-(5-cyclopropyl-1H-pyrazol-3-yl)pyrrolo[1,2-f][1,2,4]triazin-4-amine (**5**) (45 g, 0.16 mol), (S)-2-methylpyrrolidine-2-carboxylic acid (90 g, 0.70 mol), potassium tert-butoxide (73.7 g, 0.65 mol) and 1-methyl-2-pyrrolidinone (250 mL). The suspension was heated under N<sub>2</sub> with stirring to 174 °C over a period of 3 h while tert-butanol was collected in the Dean-Stark trap. The reaction mixture was then stirred at 174 °C for 18 h. After cooling to rt, the reaction mixture was poured into a four liter beaker containing 1 N NaOH (1200 mL). This was stirred for 15 min and then extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 700 mL). The aqueous layer was vacuum filtered and the resulting filtrate was cooled to 5 °C with an ice bath. The cold filtrate was then acidified to PH = 3 with 12 N HCl (156 mL) and allowed to stir at ambient temperature for 30 min. The white suspension was vacuum filtered. The resulting filter cake was washed with H<sub>2</sub>O (6 x 150 mL) and then house vacuum air dried for 18 h. The filter cake was collected and dried for an additional 24 h at 60 °C in a house vacuum oven to afford the product **5** as an off white solid (53.4 g, 89%). HPLC purity, 91%; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 300 MHz) δ 12.16 (brs, 1H), 10.01 (s, 1H), 7.95 (s, 1H), 7.33 (s, 1H), 7.07 (d, J = 4.4 Hz, 1H), 6.43 (s, 1H), 6.38 (d, J = 4.4 Hz, 1H), 3.28-3.62 (m, 3H), 2.10-2.21 (m, 1H), 1.81-2.04 (m, 4H), 1.65 (s, 3H), 0.72-0.98 (m, 4H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 75 MHz) δ 176.07, 161.56, 149.43, 116.56, 110.74, 107.70, 100.19, 92.43, 69.81, 64.00, 48.05, 35.03, 30.04, 21.79, 7.23, 7.03, 6.31; HRMS calcd for C<sub>18</sub>H<sub>21</sub>ClN<sub>7</sub>O<sub>2</sub> 367.1757, found 367.1757.

HPLC conditions (same as above). Retention time: 5.1 minutes

(S)-1-(4-(5-Cyclopropyl-1H-pyrazol-3-ylamino)pyrrolo[1,2-f][1,2,4]triazin-2-yl)-N-(6-fluoropyridin-3-yl)pyrrolidine-2-carboxamide (**9d**)



To a flame dried, four necked, one liter round bottom flask equipped with a mechanic stirrer, a N<sub>2</sub> inlet and a thermometer, were charged (S)-1-(4-(5-cyclopropyl-1H-pyrazol-3-ylamino)pyrrolo[1,2-f][1,2,4]triazin-2-yl)pyrrolidine-2-carboxylic acid (**7**) (55.1 g, 91% purity, 0.15 mol) and dry DMF (500 mL). The solution was then cooled to 5 °C with an ice bath. Thionyl chloride (14.2 mL, 1.3 equiv.) was added dropwise while the temperature was kept below 20 °C. The reaction mixture was stirred at 5 to 20 °C for 1 h. 2-Fluoro-5-amino-pyridine (50.5 g, 3 equiv.) was added in one portion. The reaction mixture was allowed to warm to rt with stirring over a period of 22 h. The reaction mixture was then poured into 0.5 N NaHCO<sub>3</sub> solution (4 L). The resulting suspension was stirred at rt for 1 h before vacuum filtration. The filter cake was washed with H<sub>2</sub>O (100 mL) and then house vacuum air dried for 18 h. The filter solid was dissolved in EtOAc (200 mL). The solution was passed through a silica gel pad (250 g). The silica gel pad was rinsed with 20% hexane in EtOAc (2 L). The filtrate was concentrated in vacuo to give the crude solid product which was crystallized from a mixture of acetone and water (3 : 2, 500 mL) from 30 °C to -10 °C. The crystals were filtered and house vacuum oven dried at 60 °C for 24 h to give the pure product **8** as a white solid (38.2 g, 71% based on 91% pure compound **5**). HPLC purity, 98.7%.

#### Crystallization conditions

(S)-1-(4-(5-cyclopropyl-1H-pyrazol-3-ylamino)pyrrolo[1,2-f][1,2,4]triazin-2-yl)-N-(6-fluoropyridin-3-yl)pyrrolidine-2-carboxamide (**9d**) (90 g) from three batches, prepared by above method, was dissolved in a mixture of acetone and H<sub>2</sub>O (2 : 1, 1.2 L) at 38 °C. The slightly yellow solution was cooled to 20 °C. A small amount of crystal seeds (20 mg) was added. The solution was then cooled with an ice/i-PrOH bath to -10 °C over a period of 1.5 h and stirred at -10 °C for another hour. The white crystals were filtered and rinsed with a cold mixture (0 °C) of acetone and H<sub>2</sub>O (1:1, 50 mL). After house vacuum air drying for 3 h, the product was transferred to a glass bottle and then dried in a house vacuum oven at 60 °C until no weight loss (two days). The white crystalline product **8** was weighed 59.0 g. HPLC purity, 99.8%; Chiral purity and ee%, 100%; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 500 MHz) δ 12.09 (s, 1H), 10.03 (s, 1H), 9.69 (s, 1H), 8.31 (s, 1H), 7.96-8.02 (m, 1H), 7.39 (s, 1H), 7.09 (d, J = 3.36 Hz, 1H), 7.04 (dd, J = 8.85 and 3.36 Hz, 1H), 6.39 (dd, J = 3.97 and 2.44 Hz, 1H), 6.25 (s, 1H), 3.78-3.86 (m, 1H), 3.56-3.64 (m, 1H), 2.22-2.29 (m, 1H), 1.99-2.05 (m, 1H), 1.90-1.97 (m, 2H), 1.72-1.80 (m, 1H), 1.62 (s, 3H), 0.81-0.87 (m, 2H), 0.77 (d, J = 9.77 Hz, 1H), 0.64-0.72 (m, 1H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 126 MHz) δ 174.44, 158.56, 153.80, 149.97, 146.88, 145.64, 139.49, 134.37, 133.86, 117.23, 111.52, 108.54, 108.46, 100.97, 93.10, 65.96, 48.02, 41.06, 22.19, 21.36, 7.51, 7.42, 6.73. MS/MS (ESI+) m/z 462 (M + H<sup>+</sup>); Anal. Calcd for C<sub>23</sub>H<sub>24</sub>FN<sub>9</sub>O: 1.41 H<sub>2</sub>O: C, 56.74; H, 5.55; N, 26.89. Found: C, 56.60; H, 5.36; N, 26.92. Ave. Specific Rotation (MeOH) = -93.49

Determination of HPLC purity (99.7%): Waters 2695 HPLC with Finnigan Surveyor PDA detection and LCQ Classic MS (electrospray probe). Column: Waters; Sunfire 3.5µm ; 150 X 2.1 mm ID; (at approx. 30C). Mobile phase A: 0.05% TFA/water. Mobile phase B: 0.05% TFA/acetonitrile. Flow rate: 0.30 ml/min. Gradient: 10-40-98 step gradient (10-40% B-0-50 min; 40-98% B-50-70 min; hold 98% B-70-73 min; 98-10% B-73-73.5 min; hold 10% B-73.5-81.5 min. UV detection: 245 nm.

Determination of chiral SFC purity (100% ee) : Method was developed with a racemic mixture. Column: Chiralcel OD-H column, 4.6 x 250mm, 5µm. Solvents: 75% CO<sub>2</sub> - 25% MeOH. Temp: 35C. Pressure: 150 bar. Flow rate: 2.0 mL/min. UV monitored @ 235 nm. Injection: 1.0mg/mL methanol

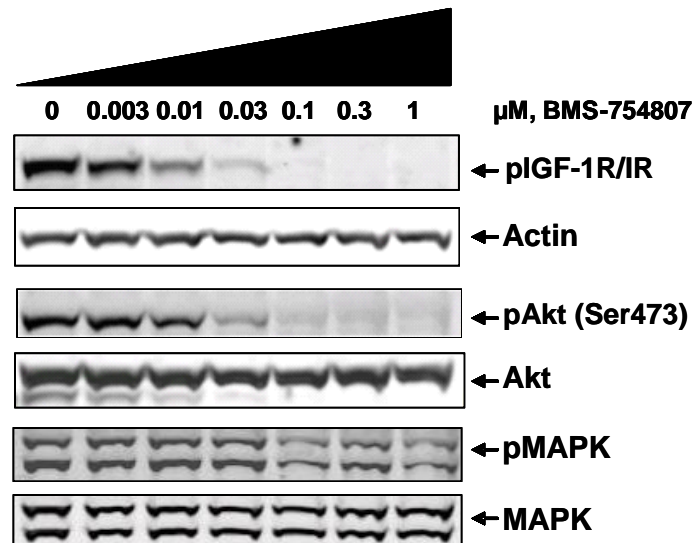
**CDK 2/cyclin E Kinase Assay:** The assays were performed in U-bottom 384-well plates. The final assay volume was 30 µl prepared from 15 µl additions of enzyme and substrates (fluoresceinated CDK2E substrate peptide and ATP) and test compounds in assay buffer (100 mM HEPES pH 7.4, 10 mM MgCl<sub>2</sub>, 0.015% Brij35 and 4 mM DTT). The reaction was initiated by the combination of bacterially expressed, CDK2E with substrates and test compounds. The reaction was incubated at room temperature for 60 min. and terminated by adding 30 µl of 35 mM EDTA to each sample. The reaction mixture was analyzed on the Caliper LabChip 3000 by electrophoretic separation of the fluorescent substrate and phosphorylated product. Inhibition data were calculated by comparison to no enzyme control reactions for 100% inhibition and vehicle-only reactions for 0% inhibition. The final concentration of reagents in the assays are ATP, 30 µM; FL-peptide, 1.5 µM; CDK2E, 0.2 nM; and DMSO, 1.6%. Dose response curves were generated to determine the concentration required inhibiting 50% of kinase activity (IC<sub>50</sub>). Compounds were dissolved at 10 mM in dimethylsulfoxide (DMSO) and evaluated at eleven concentrations, each in duplicate. IC<sub>50</sub> values were derived by non-linear regression analysis.

**IGF1- Receptor Tyrosine Kinase Assay:** The assays were performed in U-bottom 384-well plates. The final assay volume was 30 µl prepared from 15 µl additions of enzyme and substrates (fluoresceinated IGF1R substrate peptide and ATP) and test compounds in assay buffer (100 mM HEPES pH 7.4, 10 mM MnCl<sub>2</sub>, 0.015% Brij35 and 4 mM DTT). The reaction was initiated by the combination of IGF1-receptor with substrates and test compounds. The reaction was incubated at room temperature for 60 min. and terminated by adding 30 µl of 35 mM EDTA to each sample. The reaction mixture was analyzed on the Caliper LabChip 3000 by electrophoretic separation of the fluorescent substrate and phosphorylated product. Inhibition data were calculated by comparison to no enzyme control reactions for 100% inhibition and vehicle-only reactions for 0% inhibition. The final concentration of reagents in the assays are ATP, 25 µM; FL-peptide, 1.5 µM; IGF1-Receptor, 14 nM; and DMSO, 1.6%. Dose response curves were generated to determine the concentration required inhibiting 50% of kinase activity (IC<sub>50</sub>). Compounds were dissolved at 10 mM in dimethylsulfoxide (DMSO) and evaluated at eleven concentrations, each in duplicate. IC<sub>50</sub> values were derived by non-linear regression analysis.

**Insulin Receptor Tyrosine Kinase Assay:** The assays were performed in U-bottom 384-well plates. The final assay volume was 30 µl prepared from 15 µl additions of enzyme and substrates (fluoresceinated InsR substrate peptide and ATP) and test compounds in assay buffer (100 mM HEPES pH 7.4, 10 mM MnCl<sub>2</sub>, 0.015% Brij35 and 4 mM DTT). The reaction was initiated by the combination of Insulin Receptor with substrates and test compounds. The reaction was incubated at room temperature for 60 min. and terminated by adding 30 µl of 35 mM EDTA to each sample. The reaction mixture was analyzed on the Caliper LabChip 3000 by electrophoretic separation of the fluorescent substrate and phosphorylated product. Inhibition data were calculated by comparison to no enzyme control reactions for 100% inhibition and vehicle-only reactions for 0% inhibition. The final concentration of reagents in the assays are ATP, 25µM; FL-peptide, 1.5 µM; Insulin Receptor, 14 nM; and DMSO, 1.6%. Dose response curves were generated to determine the concentration required inhibiting 50% of kinase activity (IC<sub>50</sub>). Compounds were dissolved at 10 mM in dimethylsulfoxide (DMSO) and evaluated at eleven concentrations, each in duplicate. IC<sub>50</sub> values were derived by non-linear regression analysis.

**Cellular Proliferation Assays:** Cells were grown at their optimal density in RPMI medium+GlutaMax™ supplemented with 10% heat-inactivated fetal bovine serum, 10mM Hepes, penicillin and streptomycin. Cell proliferation was evaluated by incorporation of [<sup>3</sup>H]-thymidine (GE Healthcare) into DNA after exposure of cells for 72hrs. Results are expressed as an IC<sub>50</sub>, which is the drug concentration required to inhibit cell proliferation by 50% compared to untreated control cells.

**Western blot analysis: Effect of BMS-754807 on IGF-1R, Akt and MAPK activity in IGF-Sal Cells**



**IGF-1R Sal in vivo model:** A salivary gland adenocarcinoma that developed spontaneously in a transgenic mouse (MCI-19) was excised and cut into fragments of about 20 mg. Tumor fragments were implanted s.c. into the ventral thoracic region of a group of six female, athymic BALB/c nu/nu mice (Harley Sprague-Dawley, Indianapolis, IN), using a 13-gauge trocar. Once established, the salivary gland-derived tumor line was designated IGF1R-Sal and was propagated as a tumor xenograft in nude mice. Tumors were passaged every 2 weeks, at which time the tumor reached 500 to 1,000 mm<sup>3</sup> in size. For treatment studies, nude mice bearing IGF1R-Sal tumors of about 100 mm<sup>3</sup> in size were sorted into groups of five for treatment with vehicle (80% polyethylene glycol 400 in water) alone or the test article. Compounds were administered either on a bid schedule (oral doses 8 hours apart) or on a once a day schedule orally (qd) for 4 consecutive days. Tumors were measured at the start and end of treatment. Activity was measured as %tumor growth inhibition (%TGI). The %TGI was determined using the following formula  $(Ct-Tt)/(Ct-Co)$  where Ct is defined as the median tumor size of the control group at the end of treatment, Co is defined as the median tumor size of the control group at the start of treatment, and Tt is defined as the median tumor size of the treated group at the end of treatment.

