

SUPPORTING INFORMATION

Targeting KRAS Mutant Cancers via Combination Treatment: Discovery of a Pyridopyridazinone pan-RAF Kinase Inhibitor

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General Methods.

Enzymatic assays measuring RAF kinase activity. Compounds are evaluated for potency against BRAF (416-766aa, Sigma B4062), and CRAF (Y340D Y341D, Life Technologies PV3805), using a DELFIA assay by Perkin Elmer. Phosphorylated MAP2K1, (inactive, Carna 07-141-10-3000) is directly measured by detection of Eu-anti-p-MEK1/2 (Ser 217/221), (Perkin Elmer TRF0213). Assay buffer (1X) shared by both enzymes includes the following: 50 mM HEPES, pH 7.5, 10 mM MgCl₂, 0.01% Brij 35, 2 mM TCEP, and 0.05% bovine gamma-globulins. Inactive MAP2K1 is 50 nM [final] in the assay. The assays are run at 10× ATP Km for both enzymes, which translates to 500 μM [ATP] for CRAF, and 50 μM [ATP] for BRAF. The enzyme concentration for both CRAF and BRAF is 1 nM [final]. Endpoint assays are run for 90 min at room temp, with 30 min pre-incubations of compound and enzyme. Assays are quenched and transferred to glutathione-coated plates for the capture of the GST-MAP2K1, and time-resolved fluorescence of Europium-Ab is measured following the DELFIA assay protocol. Potency is determined from the IC₅₀, and converted to Ki via the Cheng-Prusoff equation.

Cell Lines. All cell lines and compounds were obtained from the Genentech in-house repositories. Cell lines were maintained in RPMI-1640 media and supplemented with 10% heat-inactivated FBS (HyClone, SH3007003HI), 1X GlutaMAX (Gibco, 35050-061), and 1X Pen Strep (Gibco, 15140-122). The cells were maintained in a humidity-controlled environment (37 °C, 5% CO₂; Forma Scientific II). All cell lines were utilized before passage 20 and treated in exponential growth phase at 50-75% confluence.

Drug Combination Assays. Click-iT EdU Cell Proliferation Imaging assay of A549 cell line, combination studies with cobimetinib. A volume of 50 ul was dispensed in 384-well plates at 2000 cells per well, and compounds were dispensed directly to wells via the Echo acoustic dispenser. Cells were grown in the presence of compound for 24 hours. Pulse cells: Plates were dispensed with 20 μM EdU 647, 5 ul of volume dispensed to 50 μL cells in media, and incubated for 30 minutes in 37 C, 5% CO₂. All proceeding steps performed at room temp on the bench. Fix cells: An equal volume of 4% paraformaldehyde, 2% final, was dispensed to each well, and incubated for 15 minutes. Cells were washed for two cycles with PBS, final aspiration. Permeabilize Cells: Triton-X-100 at 0.5% in PBS was dispensed to each well at 50 μL and for 20 minutes. Cells were washed for two cycles with PBS, final aspiration. At 25 μL per well, a solution of 1X Alexafluor 647, 4 mM CuSO₄, 2 mg/ml Sodium Ascorbate in TBS was dispensed to the cells and incubated for 30 minutes. Cells were washed for six cycles using PBS, with final aspiration. Stain cells: a solution of 3.2 μM Hoechst 33342 in PBS was added to the cells at 25 μL per well, and incubated in the dark for 30 minutes. Cells were washed for two cycles with final aspiration. A Perkin Elmer PHENIX was used to image cellular DNA and cytoplasm (Alexa647 multiplexed with Hoechst stain), and calculate cell number. Analysis and visualization obtained via Columbus software. Genedata was used to calculate potencies and visualize the synergies of the double titrations of experimental compounds against cobimetinib.¹

Cloning, Expression and purification, crystallization of BRAF Mutant. cDNA encoding BRAF residues R444-K723 with H539K was generated in the background of 16 mutations to improve expression (I543A, I544S, I551K, Q562R, L588N, K630S, F667E, Y673S, A688R, L706S, Q709R, S713E, L716E, S720E, P722S, K723G) with a N-terminal histidine tag was generated by gene synthesis for bacterial expression. Expression was auto-induced at 16 °C. E. coli cells were lysed in 25 mM Tris pH 8.0, 150 mM NaCl, 1 mM TCEP, 5% glycerol and 10 μM of GNE-9815 with protease inhibitor tablets (Roche). The cell suspension was homogenized and passed through a microfluidizer twice. The lysate was clarified by centrifugation at 8,000 rpm for 30 min. The supernatant was loaded onto a 4 mL NiNTA column (Qiagen), then washed with 25 mM Tris pH 8.0, 150 mM NaCl, 1 mM TCEP, 5% glycerol and 15 mM imidazole. Protein was eluted with 0.3 M of imidazole in the same buffer. After verification by SDS-PAGE, the protein was concentrated to 2 mL and loaded onto a HiLoad 16/60 Superdex 200 column (GE Healthcare) pre-equilibrated with 25 mM Hepes, 150 mM NaCl, 1 mM TCEP and 5% glycerol. The peak corresponding to monomeric protein was pooled and diluted 3-fold with 25 mM Hepes, 5% glycerol and 1 mM TCEP, and directly loaded onto a 5 mL HiTrap SP HP column (GE Healthcare). The protein was eluted with a 0-500 mM NaCl gradient in 25 mM Hepes, 1 mM TCEP and 5% glycerol. The eluted protein was pooled and concentrated to 5 mg/mL.

The BRAF mutant GNE-9815 complex was crystallized using vapor diffusion. Hanging drops were set up by mixing 1 μ L of protein and 1 μ L of well solution (18% PEG 3350, and 0.2 M Na Nitrate, and 0.1 M bis-Tris propane pH 6.5) and incubated at 19 °C. Crystals grew after two days. Crystals were washed and transferred to a cryoprotectant solution of 25% glycerol, 18% PEG 3350, and 0.2 M Na Nitrate, and 0.1 M bis-Tris propane pH 6.5 prior to flash cooling in liquid nitrogen.

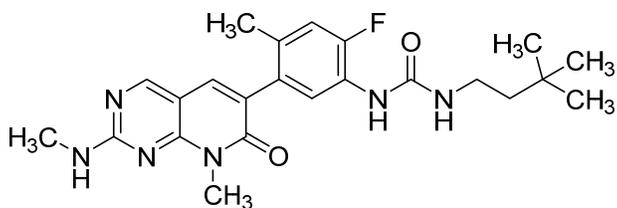
Data collection and structure determination of BRAF/GNE-9815. The diffraction data of BRAF/GNE-9815 were collected using monochromatic X-rays at Advanced Light Source (ALS) beam line 5.0.2 using a PILATUS3 6M detector. The rotation method was applied to one crystal to provide a complete data set. The crystals were kept at cryogenic temperature throughout the data collection process. Data reduction was performed using the program XDS² and the CCP4 program suites.³ Data reduction statistics are shown in Table S3. The structure was phased by molecular replacement (MR) using program Phaser.⁴ A previously published crystal structure of BRAF (PDB code 3C4C) was used as the MR search model. Manual rebuilding was performed with graphics program COOT.⁵ The structure was further refined iteratively using program REFMAC5⁶ and PHENIX⁷ using maximum likelihood target functions, anisotropic individual B-factor refinement and TLS refinement, and to achieve final statistics shown in Supplementary Table S3.

Animal studies. All individuals participating in animal care and use are required to undergo training by the institution's veterinary staff. Any procedures, including handling, dosing, and sample collection mandates training and validation of proficiency under the direction of the veterinary staff prior to performing procedures in experimental in-vivo studies. All animals were dosed and monitored according to guidelines from the Institutional Animal Care and Use Committee (IACUC) on study protocols approved by Genentech's Laboratory Animal Resource Committee at Genentech, Inc.

Xenograft tumor studies. All xenograft studies were done as previously described.⁸ Briefly, the HCT116 cells were grown in normal growth media (RPMI 1640 with L-glutamine and 10% FCS), harvested and implanted subcutaneously into the right flank of female NCR nude mice (6–8 weeks old) obtained from Taconic (Cambridge City, IN) weighing an average of 24–26 g. The mice were housed at Genentech in standard rodent micro-isolator cages and were acclimated to study conditions at least 3 days before tumor cell implantation. Only animals that appeared to be healthy, free of obvious abnormalities and harbored tumors without signs of ulceration were used for each study. Tumor volumes were determined using digital calipers (Fred V. Fowler Company, Inc.) using the formula $(L \times W \times W)/2$. Tumor growth inhibition (%TGI) was calculated as the percentage of the area under the fitted curve (AUC) for the respective dose group per day in relation to the vehicle, such that $\%TGI = 100 \times [1 - (AUC_{\text{treatment/day}})/(AUC_{\text{vehicle/day}})]$. Curve fitting was applied to Log₂ transformed individual tumor volume data using a linear mixed-effects model using the R package nlme, version 3.1–97 in R v2.12.0. Mice were weighed twice a week using a standard scale and checked daily for signs of morbidity as detailed above. Animals were euthanized within 4 hours if deemed moribund or if tumor volumes exceeded 1500 mm³.

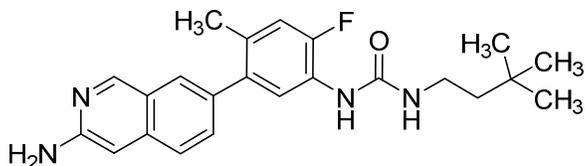
Chemistry. All metal catalysts, ligands, and bases were purchased from MilliporeSigma or Strem Chemicals, Inc. and stored in a desiccator (weighing to air). Anhydrous solvents were purchased from MilliporeSigma. Thin-layer chromatograms were performed on EMD TLC Silica gel 60 F₂₅₄ aluminum-backed plates and visualized with UV light. Flash chromatographic purifications were performed with Teledyne Isco RediSep Rf Gold silica cartridges on a Teledyne Isco Combiflash Rf. Preparative HPLC purification was conducted on C18 (Gemini-NX C18 50 \times 30 mm, 5 μ m packing, 110 Å particle size) with mixtures of acetonitrile and either 0.1% aq NH₄OH or formic acid. Nuclear magnetic resonance data was acquired on a Bruker Avance III HD 400 Ascend with a Z-GRD Prodigy BBO 5 mm cryoprobe. Chemical shift values are reported relative to internal standards and operating frequencies shown in parentheses: ¹H (400.33 MHz) – trimethylsilane = 0.00 ppm, ¹³C (100.67 MHz) – trimethylsilane = 0.00 ppm, ¹⁹F (376.52 MHz) – trichlorofluoromethane = 0.00 ppm. In cases of uncertain assignments, structural confirmation was secured through 2D NMR experiments. Reactions were monitored by HPLC-MS analysis on a Shimadzu LC-30AD with a Waters 2.1 mm \times 30 mm 1.7 μ m BEH C18 column, UV detection at 254 nm, and dual ESI/APCI to a Shimadzu LCMS-2020 single quadrupole mass analyzer. The method was conducted at a flowrate of 0.7 mL/min whereby mobile phase A was 0.1% formic acid in water and mobile phase B was acetonitrile. The method began at 2% B, ramping linearly to 98% B over

2 min. The gradient was held at 98% B for 0.2 min and then ramped down to 2% B over 0.1 min, and held at 2% B for 0.1 min. The purity of final compounds was verified by LCMS to be >95% in all cases using either of the following methods: (1) 10-min LCMS method: Experiments were performed on an Agilent 1290 UHPLC coupled with Agilent MSD (6140) mass spectrometer using ESI as ionization source. The LC separation was using a Phenomenex XB-C18, 1.7 μ m, 50 \times 2.1 mm column at a flow rate of 0.4 mL/min. Mobile phase A was water with 0.1% formic acid and mobile phase B was acetonitrile with 0.1% formic acid. The gradient started at 2% B and ended at 98% B over 7 min and held at 98% B for 1.5 min following equilibration for 1.5 min. LC column temperature was 40 $^{\circ}$ C. UV absorbance was collected at 220 nm and 254 nm and mass spectrometry full scan was applied to all experiments. (2) 30-min LCMS method: Experiments were performed on an Agilent 1290 HPLC coupled with Agilent MSD (6140) mass spectrometer using ESI as ionization source. The LC separation was done on an Agilent Zorbax Eclipse XDB-C18, 3.5 μ m, 100 \times 3.0 mm column at a flow rate of 0.7 mL/min. Mobile phase A was water with 0.1% formic acid and mobile phase B was acetonitrile with 0.1% formic acid. The gradient started at 2% B and ended at 98% B over 25.5 min and held at 98% B for 2.5 min following equilibration for 1.5 min. LC column temperature was 40 $^{\circ}$ C. UV absorbance was collected at 220 nm and 254 nm and mass spectrometry full scan was applied to all experiments.



1-(3,3-Dimethylbutyl)-3-(2-fluoro-4-methyl-5-(8-methyl-2-(methylamino)-7-oxo-7,8-dihydropyrido[2,3-d]pyrimidin-6-yl)phenyl)urea (2)

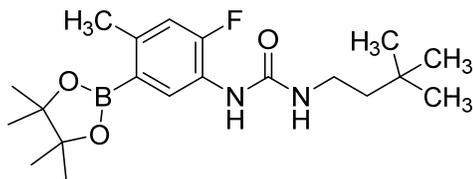
Into a vial was weighed 1-(5-bromo-2-fluoro-4-methylphenyl)-3-(3,3-dimethylbutyl)urea⁹ (100 mg, 0.302 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) dichloromethane adduct (12.5 mg, 0.0151 mmol), bis(pinacolato)diboron (84.3 mg, 0.332 mmol), and potassium acetate (88.9 mg, 0.906 mmol). Under a stream of nitrogen gas, the vessel was charged with anhydrous 1,4-dioxane (1.5 mL) and the vial was sealed. The reaction mixture was stirred at 100 $^{\circ}$ C for 17 h and then cooled to rt. While still under nitrogen, 6-bromo-8-methyl-2-(methylamino)pyrido[2,3-d]pyrimidin-7(8*H*)-one (81.3 mg, 0.302 mmol), chloro(2-dicyclohexylphosphino-2',4',6'-triisopropyl-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II) (12.7 mg, 0.0151 mmol), 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (7.3 mg, 0.0151 mmol), potassium phosphate tribasic monohydrate (215 mg, 0.906 mmol), tetrahydrofuran (1 mL), and water (0.15 mL) were added and the vial sealed again. The reaction mixture was stirred at 80 $^{\circ}$ C for 3 h. After cooling to rt, the mixture was filtered through Celite, rinsing with dichloromethane and methanol. The mother liquor was concentrated to dryness and the residue purified by preparative HPLC to afford the title compound as a white solid (43.9 mg, 33% over 2 steps, >99% HPLC purity); ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.74 – 8.51 (m, 1H), 8.16 (d, *J* = 2.4 Hz, 1H), 7.93 (d, *J* = 8.7 Hz, 1H), 7.86 – 7.58 (m, 2H), 7.07 (d, *J* = 12.2 Hz, 1H), 6.45 (t, *J* = 5.6 Hz, 1H), 3.57 (br s, 3H), 3.13 – 3.00 (m, 2H), 2.92 (br d, *J* = 4.8 Hz, 3H), 2.05 (s, 3H), 1.42 – 1.24 (m, 2H), 0.89 (s, 9H); LCMS *m/z* calcd for C₂₃H₂₉FN₆O₂ [M+H]⁺: 441.2. Found 441.2.



1-(5-(3-Aminoisoquinolin-7-yl)-2-fluoro-4-methylphenyl)-3-(3,3-dimethylbutyl)urea (3)

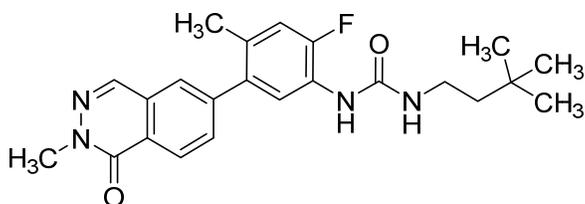
Into a vial was weighed 1-(5-bromo-2-fluoro-4-methylphenyl)-3-(3,3-dimethylbutyl)urea⁹ (88.0 mg, 0.266 mmol), 7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoquinolin-3-amine (86.1 mg, 0.319 mmol), chloro(2-dicyclohexylphosphino-2',4',6'-triisopropyl-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II) (11.2 mg,

0.0133 mmol), 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (6.5 mg, 0.0133 mmol), and potassium phosphate tribasic monohydrate (189 mg, 0.797 mmol). The vial was purged with nitrogen gas, charged with degassed tetrahydrofuran (1.3 mL) and distilled water (0.13 mL), then sealed, and the reaction mixture was stirred at 80 °C for 20 h. After cooling to rt, the mixture was concentrated to dryness and the reaction residue thus obtained was purified preparative HPLC to afford the title compound as a yellow solid (26.6 mg, 25%, 96% HPLC purity); ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.85 (s, 1H), 8.20 (d, *J* = 2.4 Hz, 1H), 8.06 (d, *J* = 8.6 Hz, 1H), 7.69 (d, *J* = 1.7 Hz, 1H), 7.56 (d, *J* = 8.6 Hz, 1H), 7.40 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.13 (d, *J* = 12.1 Hz, 1H), 6.65 (s, 1H), 6.47 (t, *J* = 5.6 Hz, 1H), 5.94 (br s, 2H), 3.12 – 3.01 (m, 2H), 2.18 (s, 3H), 1.38 – 1.31 (m, 2H), 0.89 (s, 9H); LCMS *m/z* calcd for C₂₃H₂₇FN₄O [M+H]⁺: 395.2. Found 395.2.



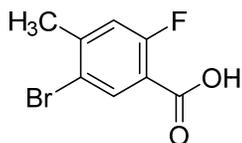
1-(3,3-Dimethylbutyl)-3-(2-fluoro-4-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)urea

Into a pressure flask was weighed 1-(5-bromo-2-fluoro-4-methylphenyl)-3-(3,3-dimethylbutyl)urea⁹ (2.00 g, 6.04 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) dichloromethane adduct (124 mg, 0.151 mmol), bis(pinacolato)diboron (1.69 g, 6.64 mmol), and potassium acetate (1.78 g, 18.1 mmol). Under a stream of nitrogen gas, the vessel was charged with anhydrous 1,4-dioxane (30 mL) and the pressure flask was sealed. The reaction mixture was stirred at 100 °C for 113 h. After cooling to rt, the mixture was concentrated and the residue purified by flash chromatography (100:0 – 70:30 heptane/EtOAc) to afford the title compound as a white solid (881 mg, 39%); ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.33 (d, *J* = 9.5 Hz, 1H), 8.06 (d, *J* = 2.4 Hz, 1H), 6.98 (d, *J* = 12.8 Hz, 1H), 6.40 (t, *J* = 5.6 Hz, 1H), 3.13 – 3.04 (m, 2H), 2.39 (s, 3H), 1.38 – 1.32 (m, 2H), 1.29 (s, 12H), 0.91 (s, 9H).



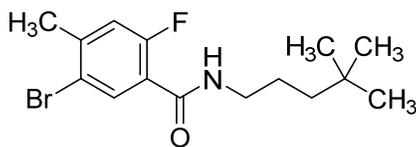
1-(3,3-Dimethylbutyl)-3-(2-fluoro-4-methyl-5-(2-methyl-1-oxo-1,2-dihydrophthalazin-6-yl)phenyl)urea (4)

Into a vial was weighed 6-bromophthalazin-1(2*H*)-one¹⁰ (158 mg, 0.661 mmol), 1-(3,3-dimethylbutyl)-3-(2-fluoro-4-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)urea (250 mg, 0.661 mmol), chloro(2-dicyclohexylphosphino-2',4',6'-triisopropyl-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II) (27.9 mg, 0.0330 mmol), 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (16.1 mg, 0.0330 mmol), and potassium phosphate tribasic monohydrate (470 mg, 1.98 mmol). The vial was purged with nitrogen gas, charged with degassed tetrahydrofuran (6.6 mL) and distilled water (0.7 mL), then sealed, and the reaction mixture was stirred at 80 °C for 18 h. After cooling to rt, the mixture was concentrated to dryness. The reaction residue thus obtained was purified by flash column chromatography (100:0 – 95:5 CH₂Cl₂/MeOH) and by reverse phase HPLC to afford the title compound as a white solid (235 mg, 86%, >99% HPLC purity); ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.46 (s, 1H), 8.33 – 8.26 (m, 2H), 8.12 (d, *J* = 8.5 Hz, 1H), 7.89 (d, *J* = 1.5 Hz, 1H), 7.80 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.20 (d, *J* = 12.4 Hz, 1H), 6.56 – 6.45 (m, 1H), 3.75 (s, 3H), 3.14 – 3.03 (m, 2H), 2.17 (s, 3H), 1.40 – 1.29 (m, 2H), 0.89 (s, 9H); LCMS *m/z* calcd for C₂₃H₂₇FN₄O₂ [M+H]⁺: 411.2. Found 411.2.



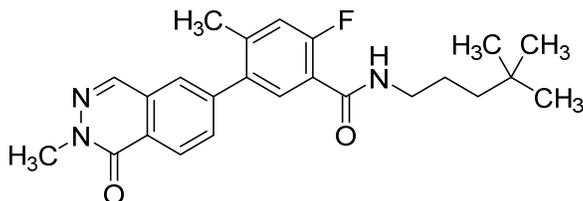
5-Bromo-2-fluoro-4-methylbenzoic acid

While stirring rapidly, bromine (21.6 mL) was added slowly to iron powder (2.72 g, 48.7 mmol) at 0 °C. Then, over 30 min, 2-fluoro-4-methylbenzoic acid (5.00 g, 32.4 mmol) was added and the reaction mixture stirred for 5 min before removing the ice bath. After stirring 20 min, the reaction mixture was poured onto an icy aqueous solution of sodium thiosulfate, affording a creamy orange mixture that was extracted 2× with dichloromethane. The organics were then washed with water, then brine, and dried over magnesium sulfate. After concentration to dryness, the crude solid was dissolved in minimal dichloromethane and heptane was added. Rotary evaporation of the dichloromethane afforded a slurry that was filtered. The white solid was rinsed with heptane and collected to afford the title compound (6.02 g, 80%); ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.43 (br s, 1H), 7.97 (d, *J* = 7.0 Hz, 1H), 7.40 (d, *J* = 11.5 Hz, 1H), 2.39 (s, 3H).



5-Bromo-*N*-(4,4-dimethylpentyl)-2-fluoro-4-methylbenzamide

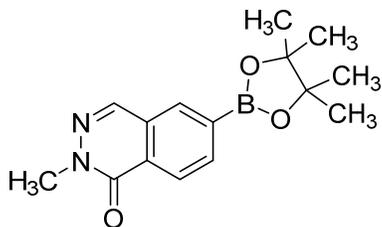
To a solution of 5-bromo-2-fluoro-4-methylbenzoic acid (1.50 g, 6.44 mmol) and HATU (3.75 g, 9.66 mmol) in *N,N*-dimethylformamide (22 mL) was added triethylamine (3.59 mL, 25.7 mmol) and 4,4-dimethylpentan-1-amine hydrochloride (1.03 g, 6.44 mmol). The reaction mixture was stirred for 2 h and then diluted with isopropyl acetate and washed with satd aqueous sodium bicarbonate. The organics were dried over magnesium sulfate, filtered, concentrated, and purified by flash chromatography (100:0 – 85:15 heptane/*i*PrOAc) to afford the title compound as a white solid (1.71 g, 80%); ¹H NMR (400 MHz, CDCl₃) δ 8.25 (d, *J* = 7.3 Hz, 1H), 7.01 (d, *J* = 12.2 Hz, 1H), 6.66 (s, 1H), 3.60 – 3.35 (m, 2H), 2.42 (s, 3H), 1.63 – 1.56 (m, 2H), 1.30 – 1.18 (m, 2H), 0.89 (s, 9H).



N-(4,4-Dimethylpentyl)-2-fluoro-4-methyl-5-(2-methyl-1-oxo-1,2-dihydrophthalazin-6-yl)benzamide (5)

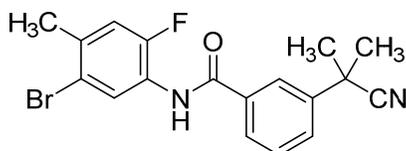
Into a vial was weighed 5-bromo-*N*-(4,4-dimethylpentyl)-2-fluoro-4-methylbenzamide (100 mg, 0.303 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) dichloromethane adduct (12.5 mg, 0.0151 mmol), bis(pinacolato)diboron (84.6 mg, 0.333 mmol), and potassium acetate (89.2 mg, 0.909 mmol). Under a stream of nitrogen gas, the vessel was charged with anhydrous 1,4-dioxane (1.5 mL) and the vial was sealed. The reaction mixture was stirred at 100 °C for 68 h and then cooled to rt. While still under nitrogen, 6-bromo-2-methylphthalazin-1(2*H*)-one¹⁰ (72.4 mg, 0.303 mmol), chloro(2-dicyclohexylphosphino-2',4',6'-triisopropyl-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II) (12.8 mg, 0.0151 mmol), 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (7.4 mg, 0.0151 mmol), potassium phosphate tribasic monohydrate (216 mg, 0.909 mmol), and water (0.30 mL) were added and the vial sealed again. The reaction mixture was stirred at 80 °C for 22 h. After cooling to rt, the mixture was filtered through Celite, rinsing with dichloromethane. The mother liquor was concentrated to dryness and the residue purified by preparative HPLC to afford the title compound as a white solid (99.8 mg, 81% over 2 steps, >99% HPLC purity); ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.44 (s, 1H), 8.36 – 8.24 (m, 2H), 7.97 (d, *J* = 1.5 Hz, 1H), 7.87 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.53 (d, *J* = 7.4 Hz, 1H), 7.33 (d, *J*

= 11.5 Hz, 1H), 3.75 (s, 3H), 3.25 – 3.16 (m, 2H), 2.30 (s, 3H), 1.54 – 1.41 (m, 2H), 1.25 – 1.15 (m, 2H), 0.86 (s, 9H); LCMS m/z calcd for $C_{24}H_{28}FN_3O_2$ $[M+H]^+$: 410.2. Found 410.2.



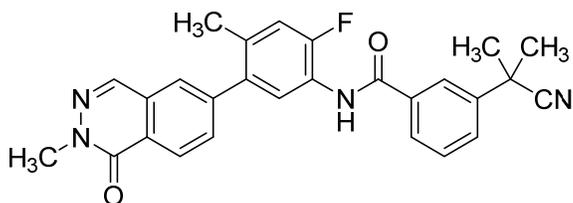
2-Methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phthalazin-1(2H)-one

Under nitrogen, a mixture of 6-bromo-2-methylphthalazin-1(2H)-one¹⁰ (500 mg, 2.09 mmol), bis(pinacolato)diboron (744 mg, 2.93 mmol), potassium acetate (411 mg, 4.18 mmol), and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (77 mg, 0.10 mmol) in 1,4-dioxane (15 mL) was stirred at 80 °C for 12 h. The reaction mixture was cooled to rt and concentrated to dryness. The residue thus obtained was purified by flash chromatography (100:0 – 50:50 petroleum ether/EtOAc) to afford the title compound as a brown solid (470 mg, 79%); LCMS m/z : 286.9 (M+H⁺).



N-(5-bromo-2-fluoro-4-methylphenyl)-3-(2-cyanopropan-2-yl)benzamide

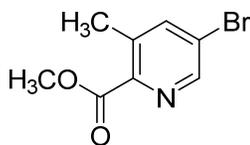
A solution of 3-(2-cyanopropan-2-yl)benzoic acid (3.42 g, 17.2 mmol) and HATU (7.99 g, 20.6 mmol) in *N,N*-dimethylformamide (57 mL) was cooled to 0 °C and then triethylamine (7.2 mL, 51.5 mmol) and 5-bromo-2-fluoro-4-methylaniline (3.50 g, 17.2 mmol) were added. The cooling bath was removed and the reaction mixture was allowed to warm to rt while stirring for 17 h. The reaction mixture was diluted with isopropyl acetate and washed with satd aqueous sodium bicarbonate and then brine. The organics were dried over magnesium sulfate, filtered, concentrated, and purified by flash chromatography (100:0 – 80:20 heptane/*i*PrOAc) to afford the title compound as a yellow solid (5.15 g, 80%); ¹H NMR (400 MHz, CDCl₃) δ 8.66 (d, *J* = 7.5 Hz, 1H), 8.00 (dd, *J* = 2.0, 2.0 Hz, 1H), 7.95 (br s, 1H), 7.79 – 7.71 (m, 2H), 7.55 (dd, *J* = 7.8, 7.8 Hz, 1H), 7.04 (d, *J* = 11.5 Hz, 1H), 2.38 (s, 3H), 1.79 (s, 6H).



3-(2-Cyanopropan-2-yl)-N-(2-fluoro-4-methyl-5-(2-methyl-1-oxo-1,2-dihydrophthalazin-6-yl)phenyl)benzamide (6)

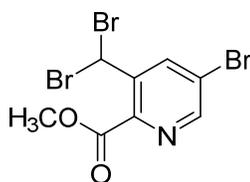
Under nitrogen, a mixture of *N*-(5-bromo-2-fluoro-4-methylphenyl)-3-(2-cyanopropan-2-yl)benzamide (79 mg, 0.21 mmol), 2-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phthalazin-1(2H)-one (50 mg, 0.17 mmol), potassium phosphate tribasic (110 mg, 0.52 mmol), and [(2-dicyclohexylphosphino-3,6-dimethoxy-2',4',6'-triisopropyl-1,1'-biphenyl)-2-(2'-amino-1,1'-biphenyl)]palladium(II) methanesulfonate (158 mg, 0.17 mmol) in tetrahydrofuran (8 mL) was stirred at 80 °C for 16 h. The reaction mixture was cooled to rt and concentrated to dryness. The residue thus obtained was purified by preparative HPLC to afford the title compound as a white solid (26.7 mg, 33%, >99% HPLC purity); ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.29 (s, 1H), 8.46 (s, 1H), 8.31 (d, *J* = 8.0 Hz, 1H), 8.09 (s, 1H), 7.97-7.94 (m, 2H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.76 (d, *J* =

7.6 Hz, 1H), 7.60-7.56 (m, 2H), 7.37 (d, $J = 11.2$ Hz, 1H), 3.75 (s, 3H), 2.28 (s, 3H), 1.74 (s, 6H); LCMS m/z calcd for $C_{27}H_{23}FN_4O_2$ $[M+H]^+$: 455.2. Found 455.0.



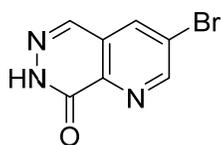
Methyl 5-bromo-3-methylpicolinate

A solution of 5-bromo-3-methylpyridine-2-carboxylic acid (50.0 g, 231 mmol) and concentrated aqueous sulfuric acid (1.5 ml) in methanol (600 ml) was stirred at 70 °C overnight. This operation was repeated 5 \times . The combined reaction mixtures were cooled to rt and the solvent was removed under reduced pressure. The residue was diluted with water (3 L) and extracted with ethyl acetate (3 L \times 3). The combined organic layers were washed with satd aqueous sodium bicarbonate (3 L \times 3), dried over anhydrous sodium sulfate and concentrated to afford the title compound as a yellow solid (300 g, 94%); 1H NMR (300 MHz, $CDCl_3$) δ 8.60 (d, $J = 1.8$ Hz, 1H), 7.81 (d, $J = 1.8$ Hz, 1H), 3.98 (s, 3H), 2.61 (s, 3H).



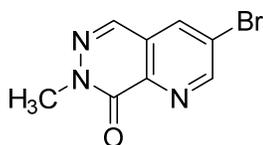
Methyl 5-bromo-3-(dibromomethyl)picolinate

A solution of methyl 5-bromo-3-methylpicolinate (100 g, 0.434 mol), *N*-bromosuccinimide (155 g, 0.869 mol), and benzoyl peroxide (5.25 g, 0.0220 mol) in carbon tetrachloride (500 ml) was stirred at 80 °C overnight. This operation was repeated 2 \times . The reaction was quenched by the addition of water (1 L) and extracted with dichloromethane (1 L \times 3). The combined organic layers were dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was by flash chromatography with ethyl acetate/petroleum ether (15:1) to afford the title compound as a yellow solid (375 g, 74%); 1H NMR (300 MHz, $CDCl_3$) δ 8.69 (d, $J = 2.1$ Hz, 1H), 8.63 (d, $J = 2.1$ Hz, 1H), 7.90 (s, 1H), 4.03 (s, 3H).



3-Bromo-7H-pyrido[2,3-d]pyridazin-8-one

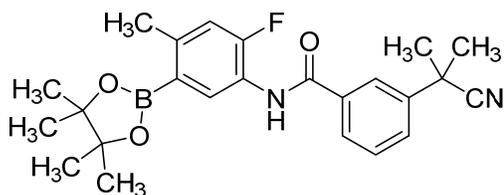
A solution of methyl 5-bromo-3-(dibromomethyl)picolinate (50.0 g, 0.129 mmol) and hydrazine hydrate (26.0 g, 0.516 mol) in methanol (750 ml) was stirred for 5 h at 70 °C. This operation was repeated 5 \times . The resulting mixtures were concentrated under vacuum and combined. The crude product was crystallized from methanol/water (9:1) to afford the title compound as yellow solid (82.8 g, 47%); LCMS m/z : 226.0 ($M+H^+$).



3-Bromo-7-methyl-pyrido[2,3-d]pyridazin-8-one

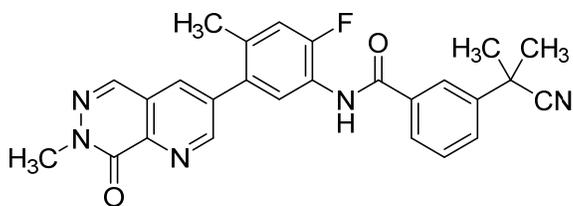
To a suspension of 3-bromo-7H-pyrido[2,3-d]pyridazin-8-one (22.6 g, 100 mmol) and cesium carbonate (39.1 g, 120 mmol) in *N,N*-dimethylformamide (200 mL), was added iodomethane (17.04 g, 120 mmol). The resulting solution was stirred at rt overnight. This operation was repeated 5 \times . The reactions were each quenched by the addition of 1 L of water and extracted with dichloromethane (1 L \times 3). The combined organic layers were washed with brine (1 L \times 3), dried over anhydrous sodium sulfate and concentrated under vacuum. The residue

was purified by flash chromatography with dichloromethane/methanol (25:1) and the obtained crude product was recrystallized from hexane to afford the title compound as a pink solid (50.5 g, 42%); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 9.11 (d, $J = 2.1$ Hz, 1H), 8.23 (d, $J = 2.1$ Hz, 1H), 8.09 (s, 1H), 3.92 (s, 3H).



3-(2-Cyanopropan-2-yl)-*N*-(2-fluoro-4-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)benzamide

Into a pressure flask was weighed *N*-(5-bromo-2-fluoro-4-methylphenyl)-3-(2-cyanopropan-2-yl)benzamide (1.00 g, 2.67 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) dichloromethane adduct (110 mg, 0.133 mmol), bis(pinacolato)diboron (744 mg, 2.93 mmol), and potassium acetate (785 mg, 8.00 mmol). Under a stream of nitrogen gas, the vessel was charged with anhydrous 1,4-dioxane (13 mL) and the pressure flask was sealed. The reaction mixture was stirred at 100 °C for 19 h. After cooling to rt, the mixture was concentrated and the residue purified by flash chromatography (100:0 – 60:40 heptane/EtOAc) to afford the title compound as a colorless foam (934 mg, 83%); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 8.62 (d, $J = 9.0$ Hz, 1H), 8.04 – 7.99 (m, 1H), 7.87 (br s, 1H), 7.80 – 7.71 (m, 2H), 7.53 (t, $J = 7.8$ Hz, 1H), 6.95 (d, $J = 12.3$ Hz, 1H), 2.53 (s, 3H), 1.78 (s, 6H), 1.34 (s, 12H).



3-(1-Cyano-1-methyl-ethyl)-*N*-[2-fluoro-4-methyl-5-(7-methyl-8-oxo-pyrido[2,3-*d*]pyridazin-3-yl)phenyl]benzamide (GNE-9815, 7)

Under nitrogen, a mixture of 3-bromo-7-methyl-pyrido[2,3-*d*]pyridazin-8-one (7.00 g, 29.2 mmol), 3-(2-cyanopropan-2-yl)-*N*-(2-fluoro-4-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)benzamide (13.6 g, 32.1 mmol), cesium carbonate (14.3 g, 43.7 mmol), and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (427 mg, 0.583 mmol) in 1,4-dioxane (120 mL) and water (20 mL) was stirred at 95 °C for 5 h. The reaction mixture was then concentrated to dryness and the residue was purified by flash column chromatography (100:0 – 0:100 petroleum ether/EtOAc) to afford a solid which was further purified by recrystallization (dichloromethane/acetonitrile, 1:6) to afford the title compound as a white solid (8.1 g, 60%, >99% HPLC purity); mp 185.0 °C; $^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$) δ 10.33 (s, 1H), 9.10 (d, $J = 2.3$ Hz, 1H), 8.47 (s, 1H), 8.45 (d, $J = 2.0$ Hz, 1H), 8.13 – 8.08 (m, 1H), 8.01 – 7.93 (m, 1H), 7.81 – 7.74 (m, 1H), 7.64 (d, $J = 7.9$ Hz, 1H), 7.61 (dd, $J = 7.9$ Hz, 1H), 7.42 (d, $J = 11.4$ Hz, 1H), 3.78 (s, 3H), 2.32 (s, 3H), 1.75 (s, 6H); $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, $\text{DMSO-}d_6$) δ 165.0, 157.9, 155.5 (d, $J_{\text{C-F}} = 252.3$ Hz), 154.0, 141.8, 141.3, 139.0, 136.6, 135.1 (d, $J_{\text{C-F}} = 7.7$ Hz), 134.8, 134.3, 132.3 (d, $J_{\text{C-F}} = 3.5$ Hz), 129.1, 128.6, 128.4, 127.1, 125.6, 124.4, 124.4 (d, $J_{\text{C-F}} = 8.4$ Hz), 123.5 (d, $J_{\text{C-F}} = 13.2$ Hz), 117.9 (d, $J_{\text{C-F}} = 20.5$ Hz), 39.4, 36.7, 28.1, 19.5; $^{19}\text{F NMR}$ (377 MHz, $\text{DMSO-}d_6$) δ -121.2 (s, 1F); HRMS m/z calcd for $\text{C}_{26}\text{H}_{22}\text{FN}_5\text{O}_2$ [$\text{M}+\text{H}$] $^+$: 456.1830. Found 456.1834. Anal. calcd for $\text{C}_{26}\text{H}_{22}\text{FN}_5\text{O}_2$: C, 68.56; H, 4.87; N, 15.38. Found C, 68.31; H, 4.51; N, 15.19.

Table S1. Kinase Selectivity Panel Data for Compounds 2-6.

Gini Coeff	0.2	0.27	0.5
Kinase Count	32	32	32
Source	ThermoFisher Selectscreen Compound 2 @0.1uM	ThermoFisher Selectscreen Compound 3 @0.1uM	ThermoFisher Selectscreen Compound 4 @0.1uM
Abl	94.4	94.2	29.8
B-Raf(V599E)	96.3	87.5	
B-Raf(V599E)	99.8	100.4	100.5
BRAF	65.6	2.5	
BRAF	101.5	99.2	101.8
Brk	22.5	19.9	5.6
CDK8/cyclinC			35.3
CLK1			5.5
CSF1R	65.2	53.0	53.6
CSK	93.1	51.9	14.2
DDR1	93.6	85.0	90.5
EphA2	100.1	69.6	26.2
Fgr	98.5	93.9	32.0
Flt1	7.3	21.9	0.1
Flt3	76.7	68.6	9.3
Flt4	42.4	59.5	12.5
JNK2			7.2
KDR	59.7	64.3	6.2
LIMK1	67.0	13.2	14.5
LIMK2	41.7	2.3	-0.3
Lck	96.9	95.8	33.3
LynB	99.2	98.5	85.7
PDGFR_alpha	77.2	91.4	86.0
PDGFR_beta	48.5	48.5	49.0
RAF1(Y340D,Y341D)	97.3	93.7	
RAF1(Y340D,Y341D)	100.9	100.7	100.3
RIPK2	86.6	98.2	78.7
Ret	89.1	87.3	38.6
Src	80.8	70.1	12.0
Srm	62.7	67.8	23.5
TNK2	21.2	6.0	0.2
Tie2	38.2	13.5	9.1
Yes	76.4	52.3	14.2
ZAK	82.9	87.1	83.9
p38_alpha(direct)	81.8	58.2	23.2
Gini Coeff	0.57	0.46	
Kinase Count	32	32	
Source	ThermoFisher Selectscreen Compound 5 @0.1uM	ThermoFisher Selectscreen Compound 6 @0.1uM	
Abl	17.0	28.5	
B-Raf(V599E)			
B-Raf(V599E)	99.2	102.0	
BRAF			

BRAF	98.8	98.0
Brk	7.0	8.0
CDK8/cyclinC	3.8	4.5
CLK1	6.0	9.5
CSF1R	67.0	53.0
CSK	8.5	23.5
DDR1	78.0	87.5
EphA2	10.0	65.0
Fgr	15.5	35.0
Flt1	4.0	6.5
Flt3	9.5	17.5
Flt4	10.5	21.0
JNK2	-1.4	26.0
KDR	13.0	32.0
LIMK1	0.3	-3.0
LIMK2	-3.1	-1.0
Lck	18.0	44.0
LynB	48.5	74.5
PDGFR_alpha	76.5	71.0
PDGFR_beta	37.5	33.0
RAF1(Y340D,Y341D)		
RAF1(Y340D,Y341D)	100.4	101.5
RIPK2	62.4	92.0
Ret	17.5	31.0
Src	2.0	10.5
Srm	12.5	20.0
TNK2	2.4	2.0
Tie2	14.0	12.0
Yes	15.5	23.5
ZAK	71.0	59.0
p38_alpha(direct)	6.0	29.5

Table S2. Kinase Selectivity Panel Data for Compound 7.

Gini Coeff	0.76
Kinase Count	226
Source	ThermoFisher Selectscreen GNE-9815 (7) @0.1uM
ACVR1B	0.0
ACVR2B	4.5
AKT1	1.0
AKT2	0.5
ALK2	-1.0
ARK5	-3.5
ASK1	-5.5
Abl	5.8
Aurora_A	5.5
Aurora_B	3.0
Axl	-10.0
B-Raf(V599E)	99.5
BMPR1A	9.5
BRAF	94.0
BTK	6.5

Blk	0.5
Bmx	9.0
BrSK1	-0.5
Brk	3.3
CAMKK1	-6.5
CAMKK2	2.5
CDK1/cyclinB	1.0
CDK2/cyclinA	0.0
CDK5/p25	8.0
CDK7/cyclinH	10.0
CDK8/cyclinC	3.8
CDK9/cyclinT1	-11.0
CHK1	4.0
CHK2	-5.5
CK1_alpha1	0.5
CK1_delta	5.5
CK1_epsilon1	-2.5
CK1_gamma1	0.5
CK1_gamma2	7.0
CK2_alpha1	-1.0
CLK1	1.5
CLK2	13.0
CLK3	1.5
CLK4	5.0
CSF1R	35.8
CSK	4.5
CaMKI	-5.5
CaMKII_beta	3.5
CaMKI_delta	0.0
CamKII_alpha	1.0
CamKIV	-12.0
DAPK1	7.0
DCAMKL2	3.5
DDR1	53.8
DMPK	2.0
DNA-PK	9.5
DRAK1	3.5
DYRK1A	-2.0
DYRK3	-1.0
DYRK4	-7.0
EGFR	0.0
EGFR(T790M,L858R)	-3.0
ERK2	0.0
ERN1	7.0
EphA1	6.5
EphA2	31.5
EphA3	6.5
EphA7	11.0
EphA8	51.0
EphB1	13.5
EphB3	-1.0
ErbB2	-3.5
ErbB4	0.0
FAK	4.0

FGFR1	-6.0
FGFR3	-4.5
FGFR4	-4.5
Fes	-2.5
Fgr	12.0
Flt1	0.8
Flt3	11.0
Flt4	0.8
Frk	34.0
GRK2	3.0
GRK3	2.0
GRK5	-2.5
GRK6	-1.0
GSK3_alpha	1.5
GSK3_beta	0.5
HIPK1	-1.0
HIPK2	1.5
HIPK4	-3.0
Hyl	-0.5
IGF1R	4.0
IKK_alpha	-2.0
IKK_beta	3.0
IKK_epsilon	-10.5
IRAK1	0.5
IRAK4	-3.0
IRR	-3.0
ITK	4.5
InsR	1.5
JAK1	6.0
JAK2	-4.5
JAK3	2.5
JNK1_alpha1	2.0
JNK2	3.0
JNK3	4.0
KDR	13.5
KHS1	7.0
Kit	14.5
LIMK1	3.3
LIMK2	-2.5
LRRK2	1.0
LTK	-10.0
Lck	12.3
Lyn	20.5
LynB	25.5
MAP4K4	4.5
MAPKAPK2	-3.5
MAPKAPK3	-1.5
MARK1	2.0
MARK3	-2.5
MEK1	-1.0
MEK3	4.5
MEKK2	4.5
MELK	5.5
MKK6	2.5

MKNK1	2.0
MKNK2	-0.5
MLK1	0.0
MLK2	2.0
MRCK_alpha	-0.5
MSK1	-0.5
MSSK1	0.5
MST1	-0.5
MST2	-1.5
MST3	4.5
MST4	-2.5
MYLK(smMLCK)	3.0
MYLK3(caMLCK)	4.0
Mer	1.5
Met	4.5
Mink1	9.0
MuSK	3.0
NEK1	6.0
NEK4	2.5
NEK6	-4.0
NEK9	-1.5
NLK	-1.0
PAK1	1.5
PAK3	-1.5
PAK4	11.0
PAK6	17.0
PASK	-1.0
PDGFR_alpha	15.8
PDGFR_beta	9.0
PDK1(direct)	8.0
PI3K-A	2.5
PI3K-G	-3.5
PIM1	-1.5
PKA	-4.5
PKC_alpha	7.5
PKC_beta1	1.5
PKC_delta	-11.5
PKC_epsilon	4.5
PKC_eta	6.0
PKC_theta	-7.5
PKC_zeta	-7.0
PKD1	3.0
PKG1_alpha	3.0
PLK1	4.0
PLK2	3.0
PLK3	-8.5
PRAK	-1.0
PRK1	5.5
PRKAA1	0.5
PhK_gamma1	-2.0
PhK_gamma2	1.0
PrKX	1.5
RAF1(Y340D,Y341D)	100.8
RIPK2	19.5

ROCK1	1.0
ROCK2	0.0
RSK1	-0.5
RSK2	3.5
RSK3	4.5
Ret	9.5
Ron	-1.0
Ros	4.0
Rse	0.0
SGK1	-0.5
SGK2	2.0
SGK3	-1.5
SIK2	1.0
SLK	-2.0
SPHK1	7.0
SRPK1	1.5
STK16	-4.5
STK33	0.0
Src	2.3
Srm	4.8
Syk	5.0
TAK1-TAB1	-7.5
TAO2	5.0
TBK1	2.0
TEC	-2.0
TGFBR1	6.5
TNK2	2.3
TSSK1	1.0
TTK	-8.5
TXK	6.0
TYK2	-0.5
Tie2	-2.3
Tpl2	2.0
TrkA	9.0
TrkB	3.5
WEE1	13.5
WNK2	-1.5
YSK1	1.5
Yes	9.0
ZAK	8.0
ZAP-70	-1.0
ZIPK	-3.0
eEF-2K	-0.5
mTOR	-0.5
p38_alpha(direct)	42.5
p38_beta	19.5
p38_delta	1.5
p38_gamma	2.5
p70S6K	1.0

Table S3. Crystallography statistics for Figure 3 (X-ray crystal structure of **7** bound in a mutant BRAF construct).

	BRAF + GNE-9815
PDB code	6XLO
Space group	P2 ₁
Unit cell	$a=48.1\text{\AA}$, $b=104.8\text{\AA}$, $c=60.3\text{\AA}$, $\alpha=90^\circ$, $\beta=109.7^\circ$, $\gamma=90^\circ$
Resolution	2.49 Å
Total measured reflections	64987 (8755) ¹
Completeness (%)	97.4 (94.8)
Redundancy	3.4 (3.2)
I/σ	6.1 (2.3)
Rsym ²	0.146 (0.464)
Resolution range	50-2.49 Å
Rcryst ³ / Rfree ⁴	0.235/0.267
Non-hydrogen atoms	4230
Water molecules	87
Average B	30.6 Å ²
r.m.s.d. bond lengths	0.007 Å
r.m.s.d. angles	0.86°
Ramachandran ⁵	0.907/0.093/0/0

¹Values in parentheses are of the highest resolution shell

²Rsym = $\sum |I_{hi} - I_h| / \sum I_{hi}$, where I_{hi} is the scaled intensity of the i th symmetry-related observation of reflection h and I_h is the mean value.

³Rcryst = $\sum_h |F_{oh} - F_{ch}| / \sum_h F_{oh}$, where F_{oh} and F_{ch} are the observed and calculated structure factor amplitudes for reflection h .

⁴Value of Rfree is calculated for 5% randomly chosen reflections not included in the refinement.

⁵Values following Ramachandran indication fraction of residues fall into most favored/additional allowed/generally allowed/disallowed regions of Ramachandran plot.

HPLC Spectra for compounds **2-7** and NMR Spectra for GNE-9815 (**7**)

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- ¹ (a) Greco, W. R.; Bravo, G.; Parsons, J. C. The search for synergy: a critical review from a response surface perspective. *Pharmacol. Rev.* **1995**, *47*, 331-385. (b) Chan, G., Wilson, S., Schmidt, S., Moffat, J. Unlocking the Potential of High-Throughput Drug Combination Assays Using Acoustic Dispensing. *J. Lab. Autom.* **2016**, *21*, 125 - 132.
- ² Kabsch, W. Integration, scaling, space-group assignment and post-refinement. *Acta Crystallogr. D Biol. Crystallogr.* **2010**, *66*(pt2), 133-144.
- ³ Winn, M. D.; Ballard, C. C.; Cowtan, K. D.; Dodson, E. J.; Emsley, P.; Evans, P. R.; Keegan, R. M.; Krissinel, E. B.; Leslie, A. G. W.; McCoy, A.; McNicholas, S. J.; Murshudov, G. N.; Pannu, N. S.; Potterton, E. A.; Powell, H. R.; Read, R. J.; Vagin, A.; Wilson, K. S. Overview of the CCP4 suite and current developments. *Acta Crystallogr. D Biol. Crystallogr.* **2011**, *67*(pt4), 235-242.
- ⁴ McCoy, A. J.; Grosse-Kunstleve, R. W.; Adams, P. D.; Winn, M. D.; Storoni, L. C.; Read, R. J. *Phaser* crystallographic software. *J. Appl. Cryst.* **2007**, *40*, 658-674.
- ⁵ Emsley, P.; Cowtan, K. *Coot*: model-building tools for molecular graphics. *Acta Crystallogr. D Biol. Crystallogr.* **2004**, *60*(pt12), 2126-2132.
- ⁶ Murshudov, G. N.; Vagin, A. A.; Dodson, E. J. Refinement of Macromolecular Structures by the Maximum-Likelihood Method. *Acta Crystallogr. D Biol. Crystallogr.* **1997**, *53*(pt3), 240-255.
- ⁷ Adams, P. D.; Afonine, P. V.; Bunkóczi, G.; Chen, V. B.; Davis, I. W.; Echols, N.; Headd, J. J.; Hung, L.-W.; Kapral, G. J.; Grosse-Kunstleve, R. W.; McCoy, A. J.; Moriarty, N. W.; Oeffner, R.; Read, R. J.; Richardson, D. C.; Richardson, J. S.; Terwilliger, T. C.; Zwart, P. H. *PHENIX*: a comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr. D Biol. Crystallogr.* **2010**, *66*(pt2), 213-221.
- ⁸ Hoeflich, K. P.; Merchant, M.; Orr, C.; Chan, J.; Otter, D. D.; Berry, L.; Kasman, I.; Koeppen, H.; Rice, K.; Yang, N.-Y.; Engst, S.; Johnston, S.; Friedman, L. S.; Belvin, M. Intermittent Administration of MEK Inhibitor GDC-0973 plus PI3K Inhibitor GDC-0941 Triggers Robust Apoptosis and Tumor Growth Inhibition. *Cancer Res.* **2012**, *72*, 210-219.
- ⁹ Huestis, M. P.; Dela Cruz, D.; DiPasquale, A. G.; Durk, M. R.; Eigenbrot, C.; Gibbons, P.; Gobbi, A.; Hunsaker, T. L.; La, H.; Leung, D. H.; Liu, W.; Malek, S.; Merchant, M.; Moffat, J. G.; Muli, C. S.; Orr, C. J.; Parr, B. T.; Shanahan, F.; Sneeringer, C. J.; Wang, W.; Yen, I.; Yin, J.; Siu, M.; Rudolph, J. Targeting KRAS Mutant Cancers via Combination Treatment: Discovery of a 5-Fluoro-4-(3*H*)-quinazolinone Aryl Urea pan-RAF Kinase Inhibitor. *J. Med. Chem.* **2021**, *64*, 3940-3955.
- ¹⁰ Huestis, M. P. Rhodium(III)-Catalyzed C-H Functionalization of 1-(2*H*)-Phthalazinones at C8. *J. Org. Chem.* **2016**, *81*, 12545-12552.

File ..15-01 2015-02-11 15-09-02\021115-81372177.D Tgt Mass (EZ):
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 Spec. Reported : UV Integration Inj Volume : 2 ul
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 Analysis Method : C:\Chem32\1\DATA\AGILENT-021115-01 2015-02-11 15-09-02\CAD
 Sample Info :
 Method Info : 10 min LCMS with 0.4mL/min

Integration Results for DAD1 A, Sig=254,4 Ref=off

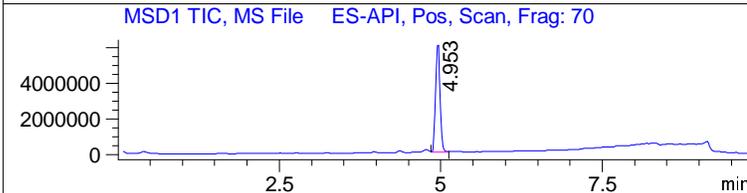
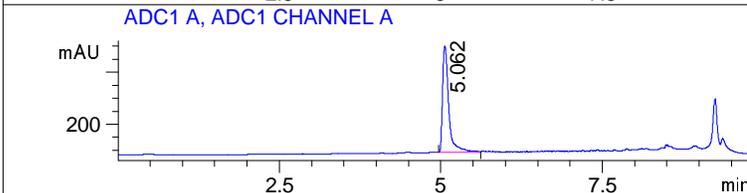
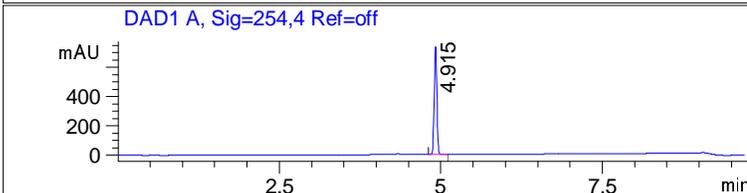
RetTim	Width	Area	Height	Area%	MS(+)
4.92	0.05	2110.38	740.13	100.00	441.2

Integration Results for ADC1 A, ADC1 CHANNEL A

RetTim	Width	Area	Height	Area%	MS(+)
5.06	0.11	2764.17	407.64	100.00	441.2

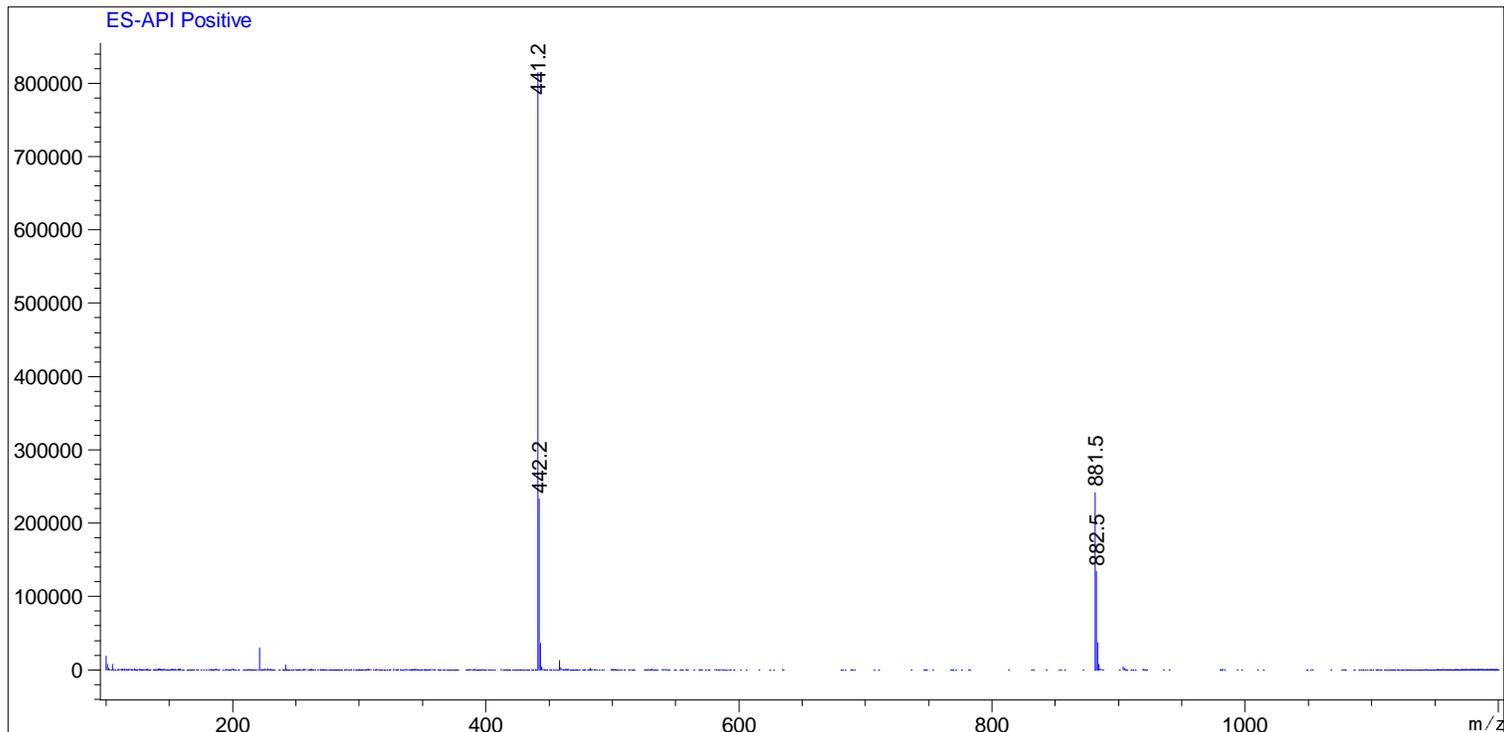
Integration Results for MSD1 TIC, MS File

RetTim	Width	Area	Height	Area%	MS(+)
4.95	0.08	29384094.00	6165433.50	100.00	441.2



Compound 2

Ret. Time: 4.92 <<<< POSITIVE SPECTRA >>>>



File ..15-01 2015-04-13 17-26-40\041315-81415925.D Tgt Mass (EZX):
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 Analysis Method : C:\Chem32\1\DATA\AGILENT-041315-01 2015-04-13 17-26-40\CAD
 Sample Info :
 Method Info : 10 min LCMS with 0.4mL/min

Integration Results for DAD1 A, Sig=254,4 Ref=off

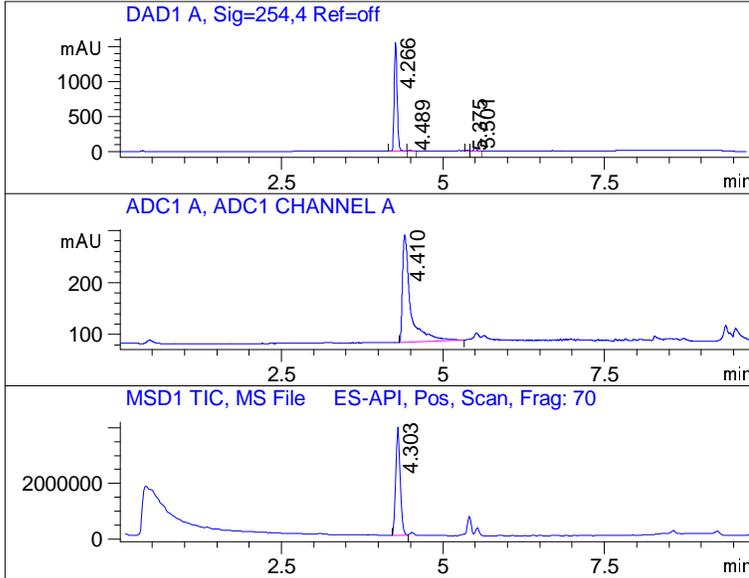
RetTim	Width	Area	Height	Area%	MS(+)
4.27	0.05	4955.75	1552.54	96.38	395.2
4.49	0.05	32.35	10.62	0.63	395.2
5.38	0.04	30.67	11.58	0.60	253.2
5.50	0.05	123.25	42.79	2.40	437.2

Integration Results for ADC1 A, ADC1 CHANNEL A

RetTim	Width	Area	Height	Area%	MS(+)
4.41	0.13	1805.36	205.87	100.00	395.2

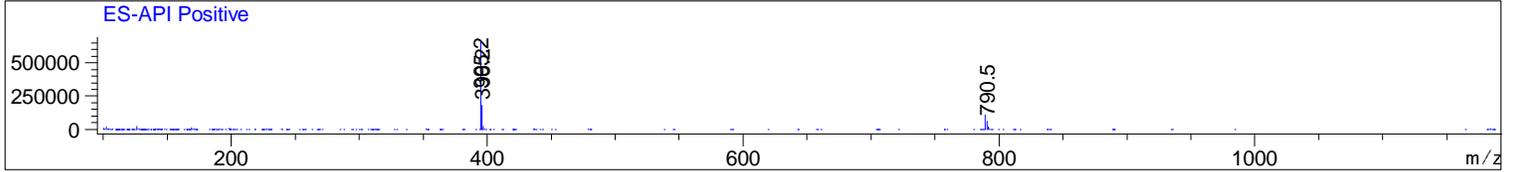
Integration Results for MSD1 TIC, MS File

RetTim	Width	Area	Height	Area%	MS(+)
4.30	0.07	18895368.00	3901962.00	100.00	395.2

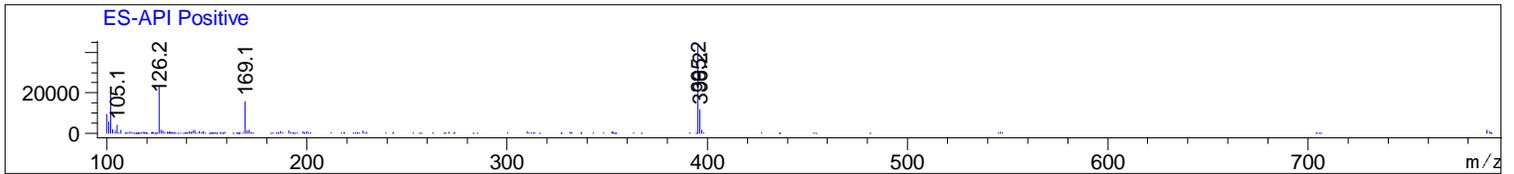


Compound 3

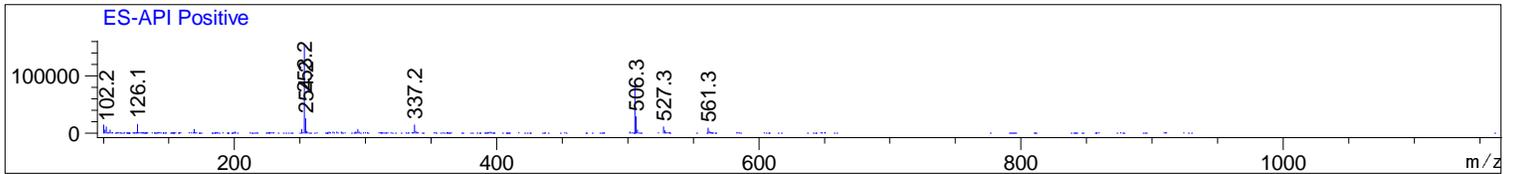
Ret. Time: 4.27 <<<< POSITIVE SPECTRA >>>>



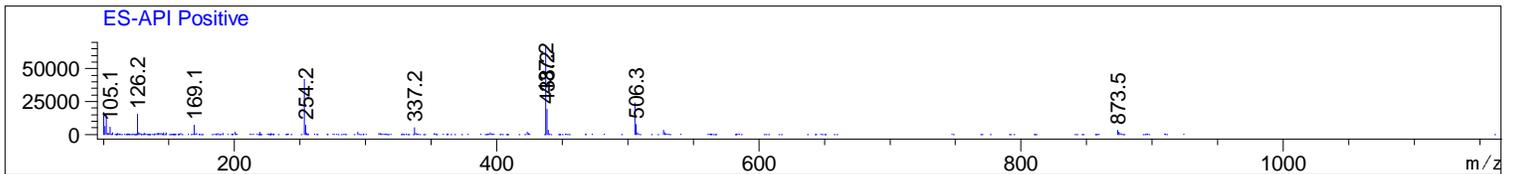
Ret. Time: 4.49



Ret. Time: 5.38



Ret. Time: 5.50



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 Sample Name : 81650905 Location : P1-A-04
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 Spec. Reported : UV Integration Inj : 1
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 Analysis Method : C:\Chem32\1\DATA\AGILENT-12-04-1 2015-12-04 14-48-00\CAD_1
 Sample Info :
 Method Info : 10 min LCMS with 0.4mL/min

Integration Results for DAD1 A, Sig=254,4 Ref=off

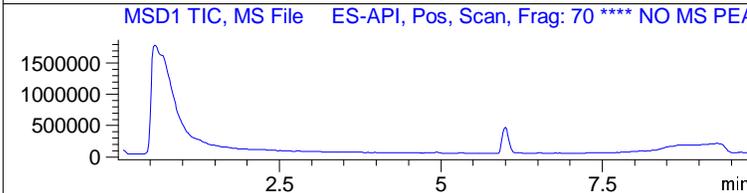
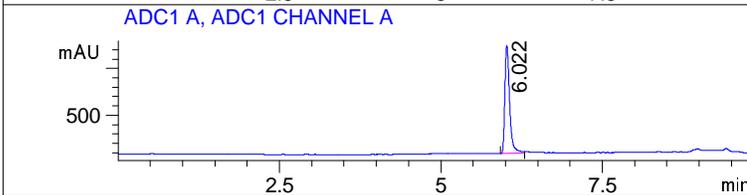
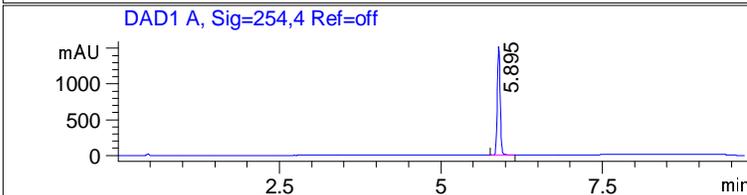
RetTim	Width	Area	Height	Area%	MS(+)
5.89	0.05	4686.86	1513.41	100.00	411.2

Integration Results for ADC1 A, ADC1 CHANNEL A

RetTim	Width	Area	Height	Area%	MS(+)
6.02	0.08	5940.06	1144.87	100.00	411.2

Integration Results for MSD1 TIC, MS File

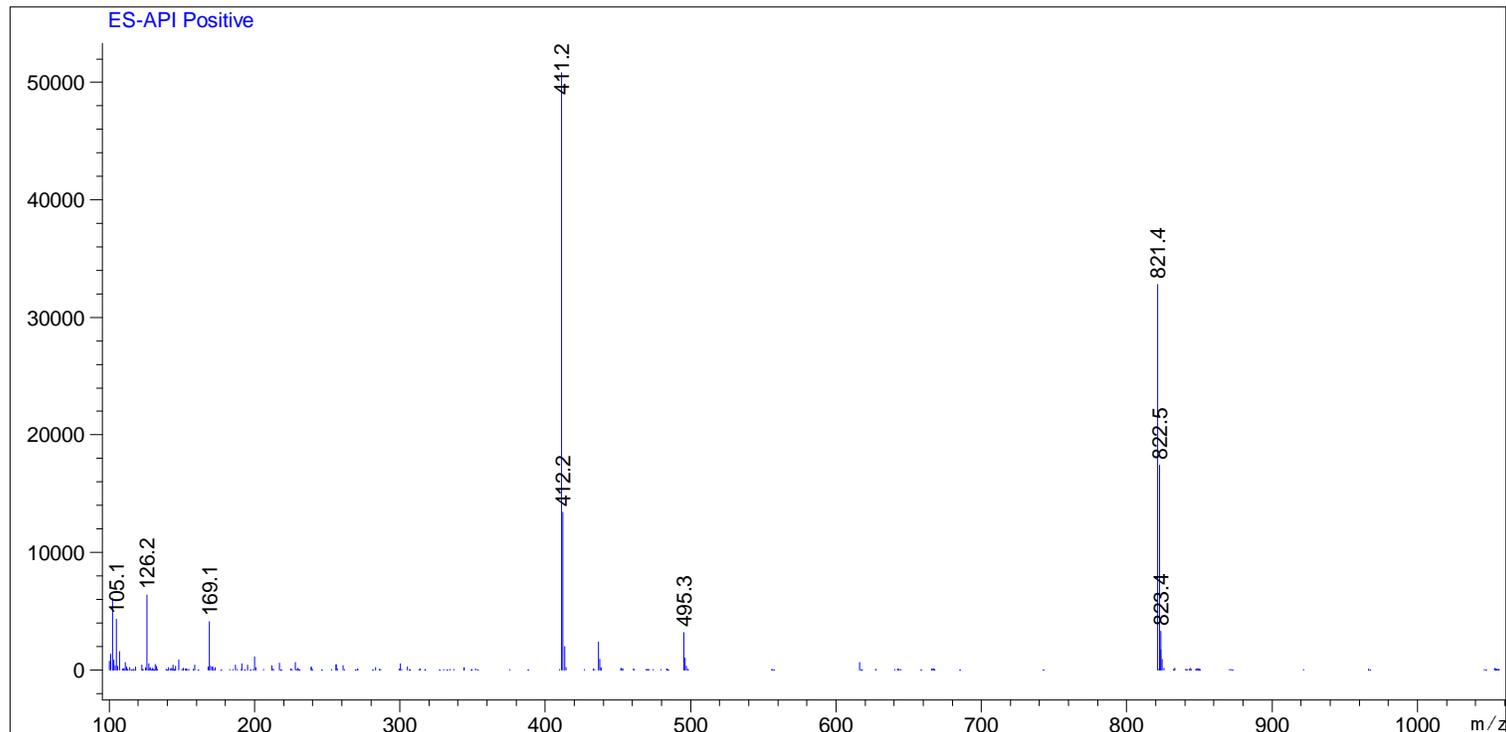
RetTim	Width	Area	Height	Area%	MS(+)



Compound 4

Ret. Time: 5.89

<<<< POSITIVE SPECTRA >>>>



File ..315-2 2015-10-26 08-54-23\102315-81606927.D Tgt Mass (EZ):
 Injection Date : 26 Oct 15 10:11 am -0800 Seq. Line : 8
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 Acq. Operator : Baiwei Inj Volume : 2 ul
 Spec. Reported : UV Integration Inj Volume : 2 ul
 Acq. Method : C:\Chem32\1\DATA\AGILENT-102315-2 2015-10-26 08-54-23\CAD
 Analysis Method : C:\Chem32\1\DATA\AGILENT-102315-2 2015-10-26 08-54-23\CAD
 Sample Info :
 Method Info : 10 min LCMS with 0.4mL/min

Integration Results for DAD1 A, Sig=254,4 Ref=off

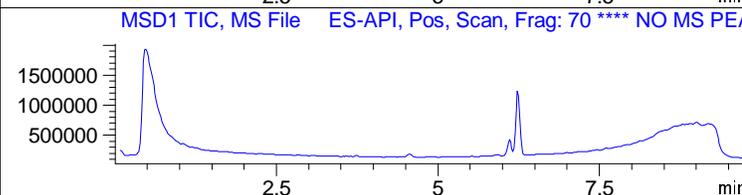
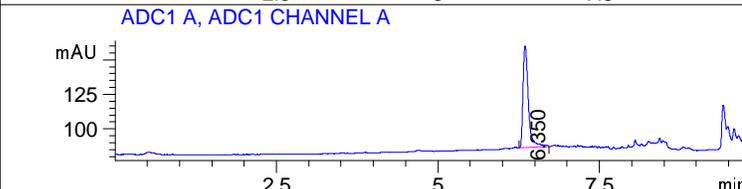
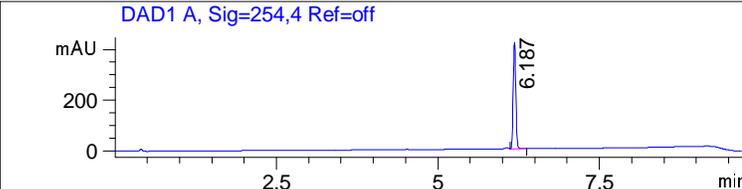
RetTim	Width	Area	Height	Area%	MS(+)
6.19	0.05	1200.54	419.80	100.00	819.5

Integration Results for ADC1 A, ADC1 CHANNEL A

RetTim	Width	Area	Height	Area%	MS(+)
6.35	0.09	428.46	73.51	100.00	100.1

Integration Results for MSD1 TIC, MS File

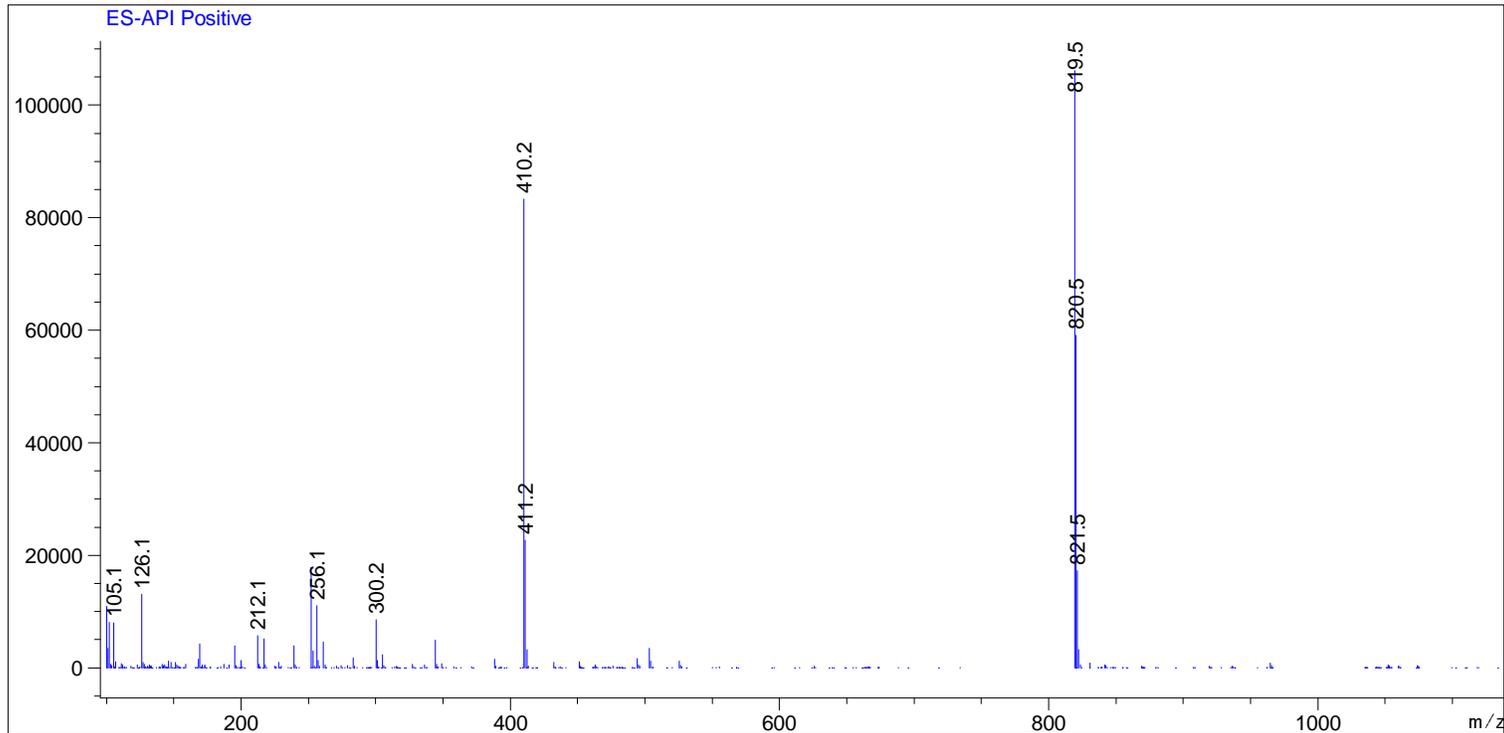
RetTim	Width	Area	Height	Area%	MS(+)



Compound 5

Ret. Time: 6.19

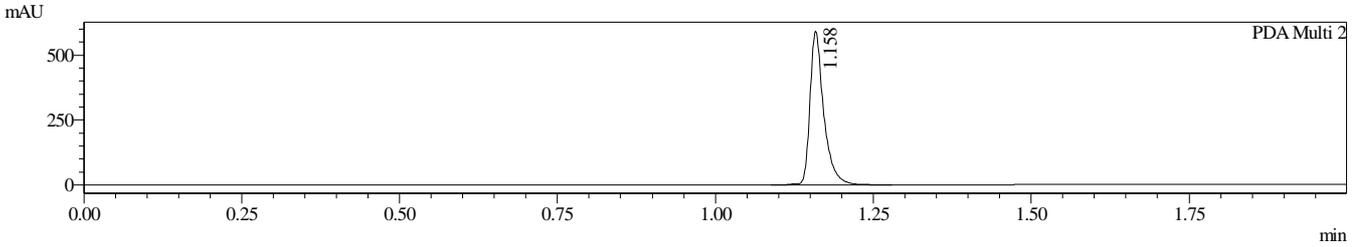
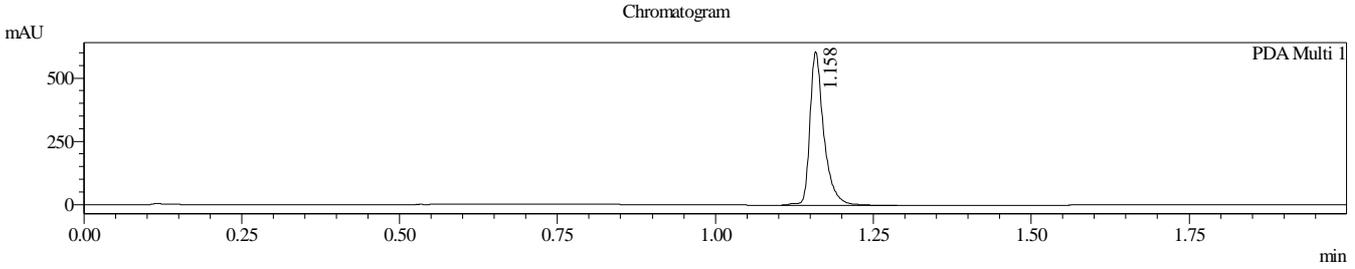
<<<< POSITIVE SPECTRA >>>>



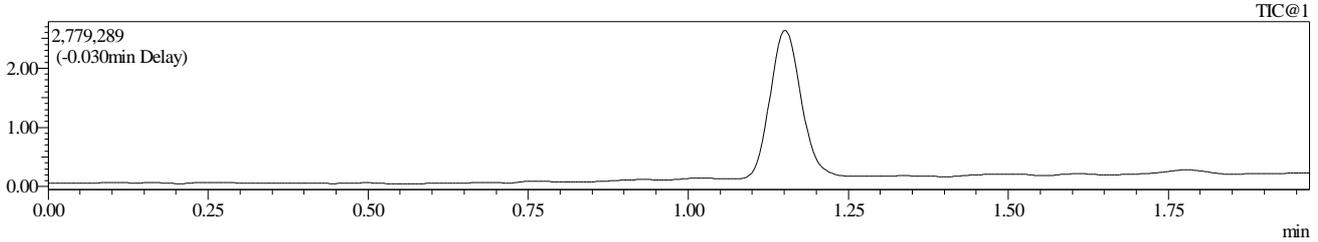
LCMS REPORT

Compound ID : GNT_B424_560-1
Sample ID : GNEWX-341-240-1E1
Injection Date : 1/13/2016 12:27:17 PM
Injection Vol : 1ul
Location : tray1 vial94
Acq Method : D:\method\10-80AB_2min_220&254.lcm
Org DataFile : D:\data\1601\160113\GNEWX-341-240-1E1.lcd
Instrument & Column: LCMS-V(12#-102) Ximate 3um,C18,2.1*30mm S/N3U

Compound 6



1 PDA Multi 1 / 220nm 4nm
2 PDA Multi 2 / 254nm 4nm
Segment#1 (x1,000,000)



Integration Result

PDA Ch1 220nm 4nm

Peak#	Ret. Time	Height	Height %	USP Width	Area	Area %
1	1.158	568528	100.000	0.039	930008	100.000

PDA Ch2 254nm 4nm

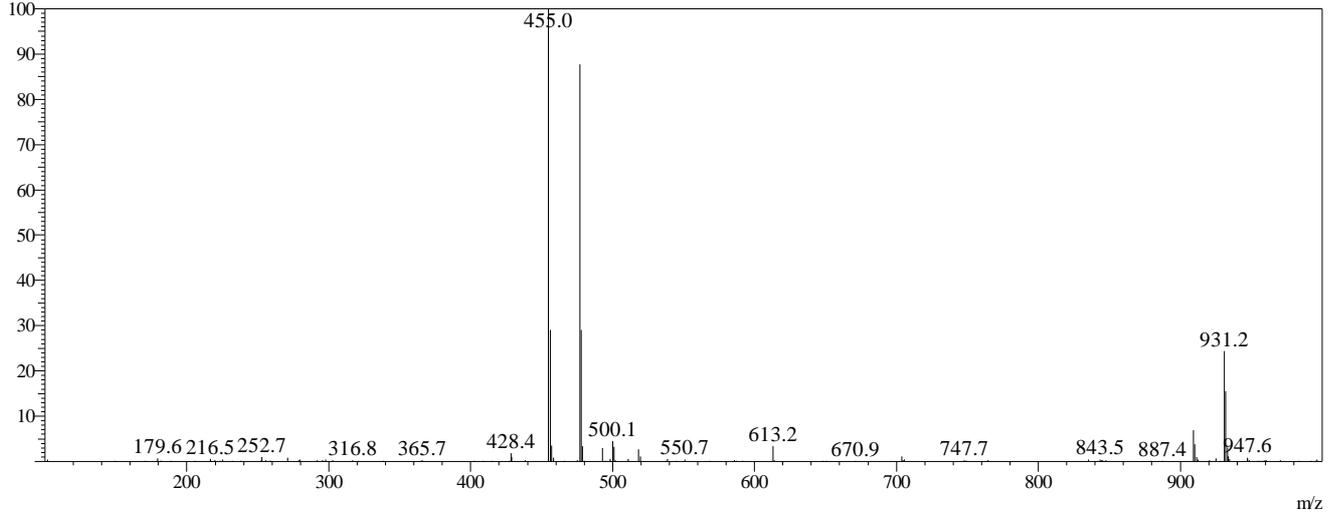
Peak#	Ret. Time	Height	Height %	USP Width	Area	Area %
1	1.158	554905	100.000	0.039	899794	100.000

Operator: _____

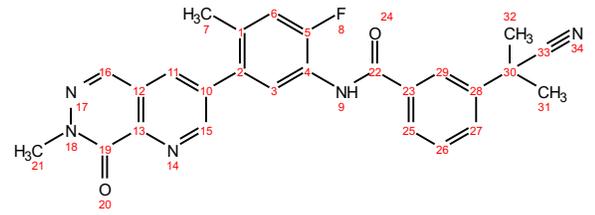
Date: _____

MS Spectrum Graph

RetTime: 1.158 DataFile: D:\data\1601\160113\GNEWX-341-240-1E1.lcd

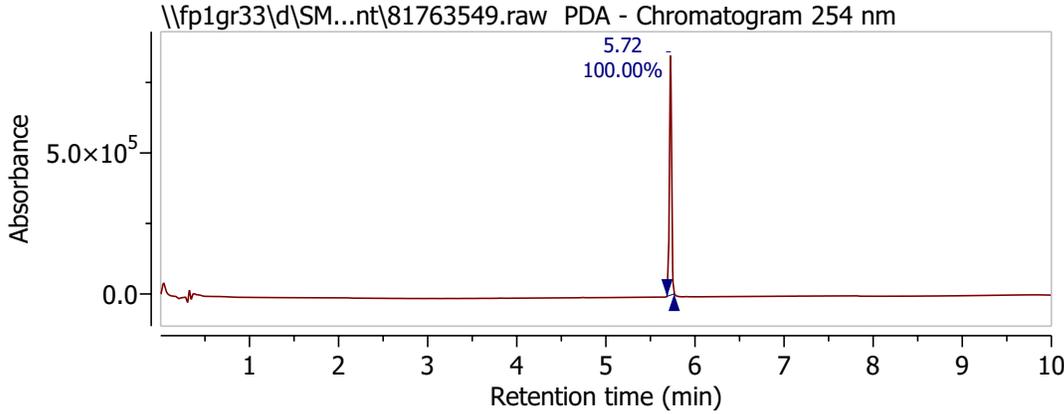


Molecule matched at RT 5.91 min. Adduct/Loss = H⁺. MF=C₂₆H₂₂FN₅O₂.
 Matched m/z=456.1834. Predicted m/z=456.1830. Error = 0.87ppm.



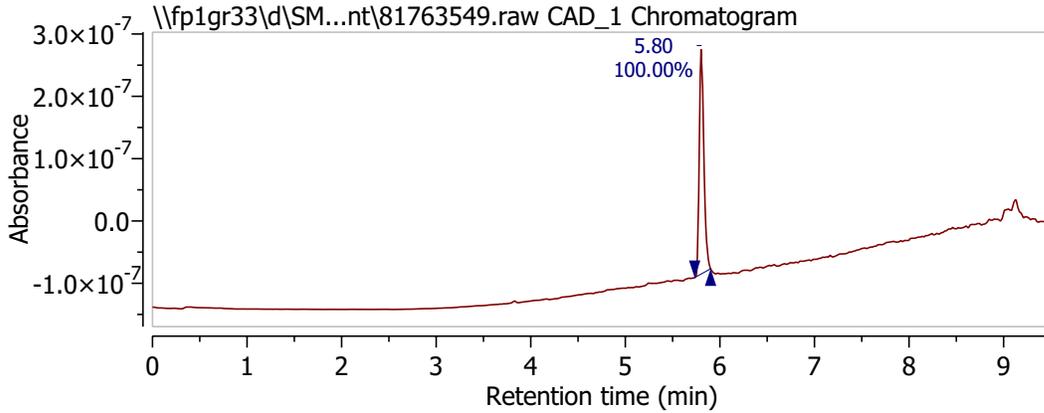
Compound 7

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 Acquired Time : 10:50:27
 Sample Name : 81763549
 Operator : Kewei
 Instrument : Thermo
 Sample Position : BB2
 Method : D:\Kewei\methods\Kewei_LCMS_10Min_Positive.meth



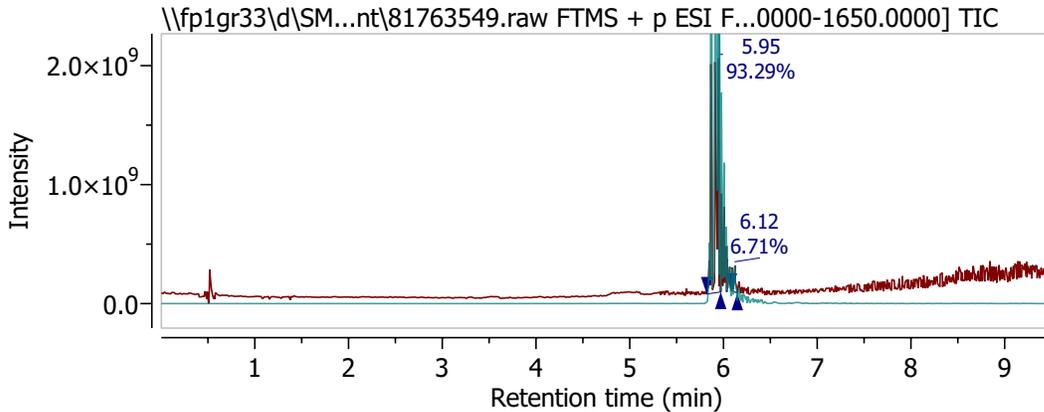
DAD, Sig=254 (>=1.5%)

RT	Area	Area %	MS+
5.72	7133031.50	100.00	456.18



CAD_1 (>=5%)

RT	Area	Area %	MS+
5.80	24248.73	100.00	456.18



TIC (>=2%)

RT	Area	Area %	MS+
5.95	22722589874.00	93.29	456.18
6.12	1634669600.00	6.71	456.18

