Supporting Information

Pyrazolopyridine Inhibitors of B-Raf^{V600E}. Part 1: The Development of Selective, Orally Bioavailable, and Efficacious Inhibitors

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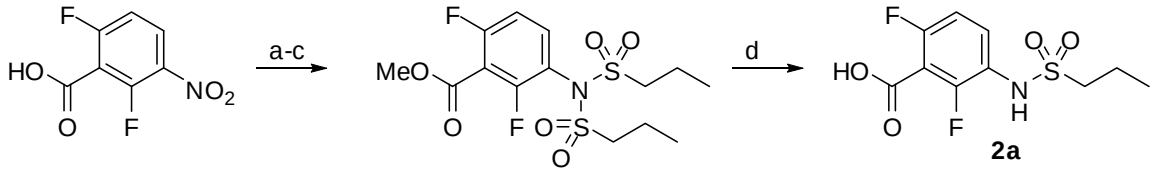
Chemistry Experimental Section

The reactions set forth below were done generally under a positive pressure of nitrogen or argon or with a drying tube in anhydrous solvents, and the reaction flasks were typically fitted with rubber septa for the introduction of substrates and reagents via syringe or cannula. Glassware was oven dried and/or heat dried. All reagents and solvents were used without further purification unless otherwise stated. Reactions were monitored by either analytical TLC or analytical HPLC. Analytical TLC was performed using glass plates pre-coated with silica gel (Manufacturer: EMD, Silica Gel 60 F_{254}, 250 μm). Analytical HPLC was performed on YMC ODS-AQ 3 μm, 120Å, 3.0 x 50 mm column using a 0.01% HFBA/1% IPA/water/acetonitrile gradient and UV detection at 220 and 254 nm. Flash column chromatography was performed on a Biotage system (Manufacturer: Dyax Corporation) having pre-packed silica gel columns (Manufacturer: Biotage, part no. FPKO-1107-15046, FPKO-1107-17026, FPKO-1107-17046, or F-1107-1804C) or on a Biotage model SP1 purification system running SPX software with prepacked silica gel columns (Manufacturer: Biotage, part no. FSKO-1107-0010, FSKO-1107-0050, FSKO-1107-0100, or FSKO-1107-03400) and UV detection at 220 and 254 nm. Mass spectra were recorded on Thermo Finnigan LCQ Duo Flow Injection APCI (±). LC/MS was performed on Advanced Materials Technology, Halo C18, 2.1 x 50mm, 2.7μm column (Part number 92812-402) using a 0.01% HFBA/1% IPA/water/acetonitrile gradient and UV detection at 220 and 254 nm. High resolution mass spectral analyses were performed on an Agilent 6520 Q-TOF ESI. Melting points were recorded on an Electrothermal melting point apparatus, model 9100. ^{1}H-NMR spectra were recorded on a Varian Mercury (400 MHz) NMR spectrometer. Chemical shifts are expressed in parts per million (ppm, δ scale) using tetramethylsilane as the reference standard. When peak multiplicities are reported, the following abbreviations are used: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), dt (doublet of triplet), br (broad). Coupling constants are reported in Hertz (Hz).

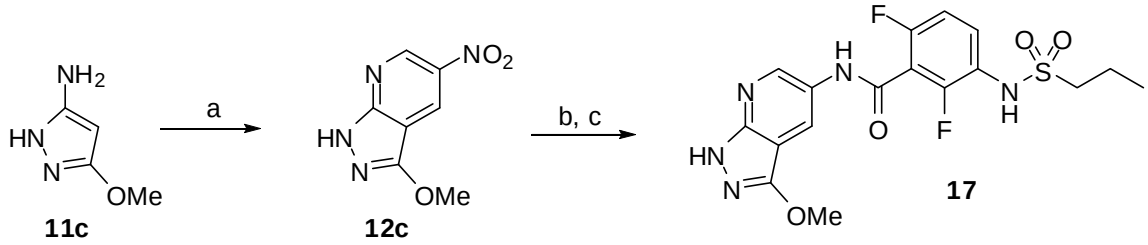
Preparation and characterization of compound 17

Scheme 1



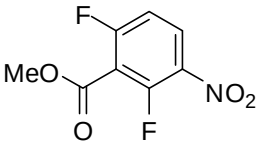
Reagents and conditions: (a) 2M $TMSCHN_2$/Hexanes, MeOH, 22 °C, 2hr, 99%; (b) H_2, 10% Pd/C, EtOH, 22 °C, 20hr, 99%; (c) propane-1-sulfonyl chloride, TEA, DCM, 22 °C, 1hr, 73%; (d) 1N NaOH, 4:1 THF/MeOH, 22 °C, 16hr, 77%.

Scheme 2



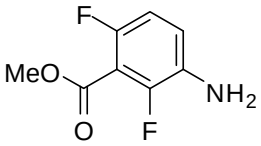
Reagents and conditions: (a) sodium nitromalonaldehyde•H_2O, water, 90 °C, 16 h, 43%; (b) H_2, 10% Pd/C, EtOAc/MeOH, 22 °C, 4hr, 82%; (e) **2a**, EDCI, HOBt, DMF, 22°C, 15 h, 78%.

a) Methyl 2,6-difluoro-3-nitrobenzoate



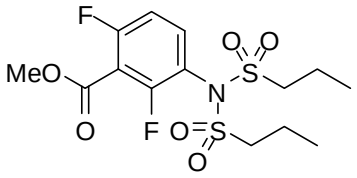
A 1 L flask was charged with 2,6-difluoro-3-nitrobenzoic acid (17.0 g, 83.7 mmol) and MeOH (170 mL, 0.5M). The flask was placed in a cold water bath and an addition funnel charged with a 2M solution of trimethylsilyl ("TMS") diazomethane in hexanes (209 mL, 419 mmol) was attached to the flask. The TMS diazomethane solution was added dropwise to the reaction over 2 hours. The volatiles were removed in vacuo to afford methyl 2,6-difluoro-3-nitrobenzoate as a tan solid (18.2 g, 99%) which was used in the next step without further purification

b) Methyl 3-amino-2,6-difluorobenzoate



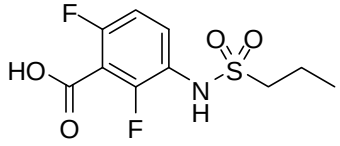
10% (wt.) Pd on activated carbon (4.46 g, 4.19 mmol) was added to a 1L flask charged with methyl 2,6-difluoro-3-nitrobenzoate (18.2 g, 83.8 mmol) under an atmosphere of N_2. Ethanol (350 mL, 0.25M) was added and H_2 was passed through the reaction mixture for 15 minutes. The reaction mixture was then left to stir under one atmosphere of H_2 for 16 hours. The mixture was then filtered through glass microfibre filter paper. The volatiles were removed in vacuo to afford methyl 3-amino-2,6-difluorobenzoate as an oil (15.66 g, 99%) which was used in the next step without further purification.

c) Methyl 2,6-difluoro-3-(N-(propylsulfonyl)propylsulfonamido)benzoate



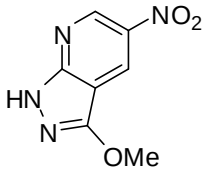
Propane-1-sulfonyl chloride (23.46 mL, 209.3 mmol) was slowly added to a solution of methyl 3-amino-2,6-difluorobenzoate (15.66 g, 83.7 mmol) and triethylamine (35.00 mL, 251.1 mmol) in CH_2Cl_2 (175 mL, 0.5M) at 0 °C. The reaction mixture was stirred for 1 hour at room temperature. Water (300 mL) was added, and the organic layer was separated, washed with water (2 X 300 mL), brine (200 mL), dried (Na_2SO_4), filtered and concentrated to an oil. The crude material was subjected to silica gel chromatography eluting with 15% ethyl acetate/hexanes to afford methyl 2,6-difluoro-3-(N-(propylsulfonyl)propylsulfonamido)benzoate (24.4 g, 73%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52-7.45 (m, 1H), 7.08-7.02 (m, 1H), 3.97 (s, 3H), 3.68-3.59 (m, 2H), 3.53-3.45 (m, 2H), 2.02-1.89 (m, 4H), 1.10 (t, *J*=7.4 Hz, 6H). *m/z* (APCI-neg) M-(SO_2Pr) = 292.2.

d) 2,6-Difluoro-3-(propylsulfonamido)benzoic acid



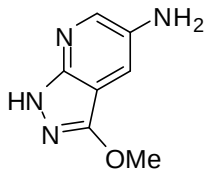
A 1N aqueous NaOH solution (150 mL, 150 mmol) was added to a solution of methyl 2,6-difluoro-3-(N-(propylsulfonyl)propylsulfonamido)benzoate (20.0 g, 50.1 mmol) in 4:1 THF/MeOH (250 mL, 0.2M). The reaction mixture was stirred at room temperature overnight. The majority of the organic solvents were then removed in vacuo. 1.0 N HCl (150 mL) was slowly added to the mixture, and the resulting solid was filtered and rinsed with water (4 X 50 mL). The material was then washed with Et_2O (4 X 15 mL) to give 2,6-difluoro-3-(propylsulfonamido)benzoic acid as a solid (10.7 g, 77% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.74 (s, 1H), 7.57-7.50 (m, 1H), 7.23-7.17 (m, 1H), 3.11-3.06 (m, 2H), 1.79-1.69 (m, 2H), 0.98 (t, *J*=7.4 Hz, 3H). *m/z* (APCI-neg) M-1 = 278.0.

e) 3-Methoxy-5-nitro-1H-pyrazolo[3,4-b]pyridine



A suspension of 3-methoxy-1H-pyrazol-5-amine (0.84 g, 7.43 mmol) (Beta Pharma, Inc.) and sodium nitromalonaldehyde monohydrate (1.23 g, 7.81 mmol) in water (40 mL) was heated to 90 ºC for 16 hours. The reaction mixture was cooled to room temperature and the pH of the aqueous layer was adjusted to 5 with acetic acid. The mixture was poured into ethyl acetate (200 mL), the layers were separated, and the organic layer was dried, filtered and concentrated. The crude product was purified by silica gel chromatography, eluting with hexanes/ethyl acetate (4:1) to give 3-methoxy-5-nitro-1H-pyrazolo[3,4-b]pyridine (0.625 g, 43% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.46 (br s, 1H), 9.30 (s, 1H), 8.96 (s, 1H), 4.07 (s, 3H); *m/z* (APCI-neg) M-1 = 193.0.

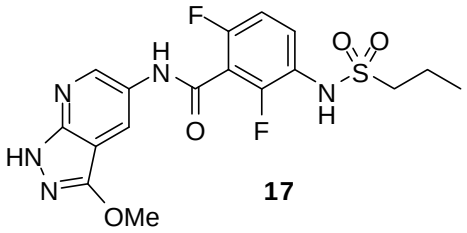
f) 3-Methoxy-1H-pyrazolo[3,4-b]pyridin-5-amine



To a solution of 3-methoxy-5-nitro-1H-pyrazolo[3,4-b]pyridine (7.3 g, 38.0 mmol) in ethyl acetate/MeOH (1:1, 240 mL) was added 10% wt Pd/C (4.03, 3.8 mmol). The

reaction mixture was hydrogenated under 30 psi of hydrogen for 16 hours. The Pd/C was removed by filtration, and the filtrate was concentrated to give 3-methoxy-1H-pyrazolo[3,4-b]pyridin-5-amine (5.1 g, 82% yield) as a solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.09 (d, *J*=2.5 Hz, 1H), 7.33 (d, *J*=2.5 Hz, 1H), 4.02 (s, 3H); *m/z* (APCI-pos) M+1 = 165.1.

g) 2,6-Difluoro-N-(3-methoxy-1H-pyrazolo[3,4-b]pyridin-5-yl)-3-(propylsulfonamido)benzamide (**17**)



17

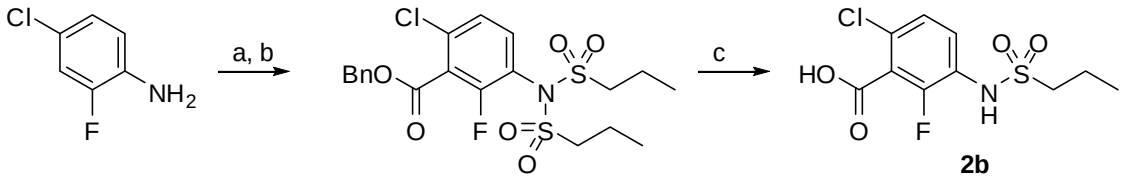
3-Methoxy-1H-pyrazolo[3,4-b]pyridin-5-amine (18.7 g, 114 mmol), 2,6-difluoro-3-(propylsulfonamido)benzoic acid (31.8 g, 114 mmol), EDCI (21.8 g, 114 mmol), HOBt-H_2O (17.4 g, 114 mmol) were dissolved in DMF (500 mL) and stirred at room temperature for 16 hours. DMF was removed by rotary evaporation to give a dark viscous mixture. A solution of 1:1 water:sat. aq. $NaHCO_3$ (500 mL) was added dropwise via addition funnel with rapid stirring. Once addition was complete, the mixture was stirred for an additional 30 minutes and the solids were collected via vacuum filtration, rinsed with water and dried under high vacuum at 50 °C for 16 hours to afford 49.0 g of a tan solid. The solid was treated with 650 mL isobutanol (~13.3 volumes) and heated to 104 °C until the mixture became homogeneous. The solution was allowed to cool slowly to room temperature. The resulting precipitates were collected and dried under high vacuum at 50 °C for 16 hours to afford 2,6-difluoro-N-(3-methoxy-1H-pyrazolo[3,4-b]pyridin-5-yl)-3-(propylsulfonamido)benzamide (**17**) as a beige solid (37.63 g, 78%). HRMS calcd. for $C_{17}H_{17}F_2N_5O_4S$: 425.0969, found: 425.0971. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.61 (s, 1H), 11.10 (s, 1H), 9.82 (s, 1H), 8.60 (s, 1H), 8.50 (s, 1H), 7.55-7.60 (m, 1H), 7.29 (t, *J*=8.60 Hz, 1H), 3.33 (s, 3H), 3.14 (t, *J*=6.00 Hz, 2H), 1.75-1.81 (m, 2H), 1.00 (t, *J*=7.00 Hz, 3H); *m/z* (APCI-pos) M+1=426.1. 98.39% Pure by HPLC at 220 nm.

h) Preparation of **17** (K^+ salt)

To a 12 L flask, compound **17** (425.2 g, 999.49 mmoles) and ethanol (6460 mL) were added to form a slurry. Potassium ethoxide (999.49 mmoles; 392.04 mL, 2.55M) was added and the slurry was heated to 40 °C. The mixture was stirred until all solids were dissolved. The solution was concentrated to ~3000 mL. Toluene (2000 mL) was added in 500 mL portions and the mixture was concentrated to ~2500 mL forming a precipitate. The solids were collected by filtration, washed with toluene and dried in a vacuum oven at 50 °C overnight to yield **17**, K^+ salt (423 g; 91%).

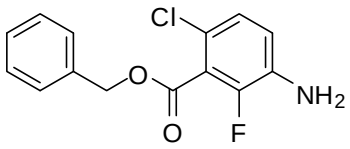
Preparation and characterization of compound 19

Scheme 3



Reagents and conditions: (a) 1.05 equiv. n-BuLi, THF, -78 °C, 1,2-bis(chlorodimethylsilyl)ethane, 1h, then 1.05 equiv. n-BuLi, 22 °C, 1h, then 1.05 equiv. n-BuLi, benzyl chloroformate, -78 °C, 1hr, 45%; (b) propane-1-sulfonyl chloride, TEA, DCM, 22 °C, 1hr, 72%; (d) 1N KOH, 4:1 THF/MeOH, 22 °C, 16hr, 68%.

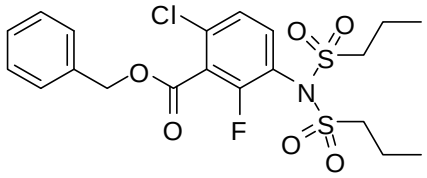
a) Benzyl 3-amino-6-chloro-2-fluorobenzoate



A flame dried flask equipped with a stir bar and rubber septum was charged with 4-chloro-2-fluoroaniline (5.00 g, 34.35 mmol) and dry THF (170 mL). This solution was chilled to -78°C, and n-BuLi (14.7 mL, 1.07 eq. of 2.5M solution in hexanes) was then added over a 15 minute period. This mixture was stirred at -78°C for 20 minutes, and then a THF solution (25 mL) of 1,2-bis(chlorodimethylsilyl)ethane (7.76 g, 1.05 eq.) was added slowly (over a 10 minute period) to the reaction mixture. This was stirred for 1 hour, and then 2.5M n-BuLi in hexanes (15.11 mL, 1.1 eq.) was added slowly. After allowing the mixture to warm to room temperature for one hour, the mixture was chilled

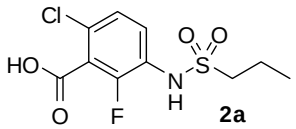
back to -78°C. A third allotment of n-BuLi (15.66 mL, 1.14 eq.) was added slowly, and the mixture was stirred at -78°C for 75 minutes. Benzyl chloroformate (7.40 g, 1.2 eq.) was then added slowly, and the mixture was stirred at -78°C for one hour. The cooling bath was then removed. The mixture was allowed to warm for 30 minutes and then quenched with water (70 mL) and concentrated HCl (25 mL). The mixture was allowed to continue to warm to room temperature. The mixture was then extracted with ethyl acetate. The extracts were washed twice with a saturated $NaHCO_3$ solution, once with water, dried over sodium sulfate and concentrated. The resulting residue was flashed on a 65 Biotage (30% ethyl acetate/hexanes) to produce benzyl 3-amino-6-chloro-2-fluorobenzoate (4.3 g, 45%) as an oil. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.37-7.48 (m, 5H), 7.07 (dd, *J* = 8.2 Hz, 1H), 6.87 (t, *J* = 8.2 Hz, 1H), 5.61 (br s, 2H), 5.40 (s, 2H).

b) Benzyl 6-chloro-2-fluoro-3-(N-(propylsulfonyl)propylsulfonamido)benzoate



Benzyl 3-amino-6-chloro-2-fluorobenzoate (4.3 g, 15.37 mmol) was dissolved in dry dichloromethane (270 mL). Triethylamine (5.36 mL, 2.5 eq.) was added, and the mixture was chilled to 0°C. Propane-1-sulfonyl chloride (3.63 mL, 32.3 mmol, 2.1 eq.) was then added via syringe, and a precipitate resulted. Once the addition was complete, the mixture was allowed to warm to room temperature, and the starting material was consumed as determined by TLC (3:1 hexanes:ethyl acetate). The mixture was then diluted with dichloromethane (200 mL), washed with 2M aqueous HCl (2 X 100 mL), saturated $NaHCO_3$ solution, dried over sodium sulfate and concentrated. The resulting residue was purified on a 65 Biotage chromatography system (40% ethyl acetate/hexanes) to produce benzyl 6-chloro-2-fluoro-3-(N-(propylsulfonyl)propylsulfonamido)benzoate (5.5 g, 72%) as an oil that slowly solidified upon standing. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.28-7.45 (m, 7H), 5.42 (s, 2H), 3.58-3.66 (m, 2H), 3.43-3.52 (m, 2H), 2.02-1.89 (m, 4H), 1.08 (t, *J*=8.0 Hz, 6H).

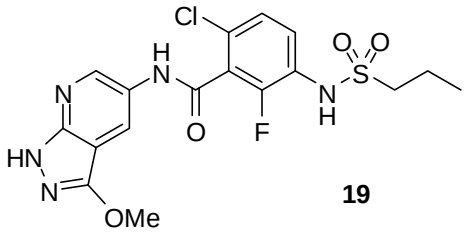
c) 6-Chloro-2-fluoro-3-(propylsulfonamido)benzoic acid (**2a**)



2a

Benzyl 6-chloro-2-fluoro-3-(N-(propylsulfonyl)propylsulfonamido)benzoate (5.4 g, 10.98 mmol) was dissolved in THF (100 mL) and 1M aqueous KOH (100 mL). This mixture was refluxed for 16 hours and then allowed to cool to room temperature. The mixture was then acidified to a pH of 2 with 2M aqueous HCl and extracted with ethyl acetate (2 X). The extracts were washed with water, dried over sodium sulfate and concentrated to a solid that was triturated with hexanes/ether to give 6-chloro-2-fluoro-3-(propylsulfonamido)benzoic acid (2.2 g, 68%) as a solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.93 (s, 1H), 7.49 (t, *J*=8.0 Hz, 1H), 7.38 (dd, *J* = 8.0 Hz, 1H), 3.11-3.16 (m, 2H), 1.68-1.78 (m, 2H), 0.97 (t, *J* = 8.2 Hz, 3H).

6-Chloro-2-fluoro-N-(3-methoxy-1H-pyrazolo[3,4-b]pyridin-5-yl)-3-(propylsulfonamido)benzamide (**19)**



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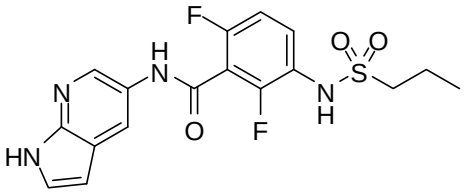
HRMS calcd. for $C_{17}H_{17}F_2N_5O_4S$: 441.0674, found: 441.0679. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.60 (s, 1H), 11.07 (s, 1H), 9.97 (br s, 1H), 8.58 (s, 1H), 8.48 (s, 1H), 7.52-7.57 (m, 1H), 7.44-7.46 (m, 1H), 4.02 (s, 3H), 3.15-3.19 (m, 2H), 1.73-1.79 (m, 2H), 0.99 (t, *J*=7.00 Hz, 3H); *m/z* (APCI-pos) M+1 = 442.1, 444.0.

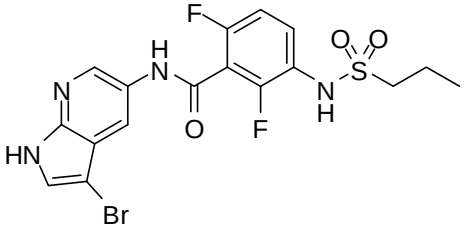
Preparation and characterization of compounds 9 and 10

2,6-Difluoro-3-(propylsulfonamido)-N-(1H-pyrrolo[2,3-b]pyridin-5-yl)benzamide (**9**)



1H-Pyrrolo[2,3-b]pyridin-5-amine (MolBridge) (9.97 g, 74.88 mmol), 2,6-difluoro-3-(propylsulfonamido)benzoic acid (23.00 g, 82.37 mmol), EDCI (15.79 g, 82.37 mmol), and HOBt-H_2O (11.13 g, 82.37 mmol) were charged to a 2 L round-bottomed flask. DMF (350 mL) was added and the reaction was stirred at room temperature overnight. The solution was partitioned between water and EtOAc. The aqueous layer was extracted with EtOAc (3 X), and the combined organics were washed with water (3 X), brine, dried over Na_2SO_4 and concentrated to a slurry. DCM (500 mL) was added, and the slurry was filtered, washed with DCM and dried under vacuum providing 2,6-difluoro-3-(propylsulfonamido)-N-(1H-pyrrolo[2,3-b]pyridin-5-yl)benzamide (15.49 g, 52.5%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.65 (br s, 1H), 10.85 (s, 1H), 9.79 (br s, 1H), 8.34-8.37 (m, 2H), 7.51-7.57 (m, 1H), 7.48-7.50 (m, 1H), 7.25-7.30 (t, *J*=9.06 Hz, 1H), 6.46-6.48 (m, 1H), 3.11-3.15 (m, 2H), 1.75-1.80 (m, 2H), 1.01 (t, *J*=7.42 Hz, 3H); *m/z* (APCI-pos) M+1 = 395.1.

N-(3-Bromo-1H-pyrrolo[2,3-b]pyridin-5-yl)-2,6-difluoro-3-(propylsulfonamido)benzamide (**10**)

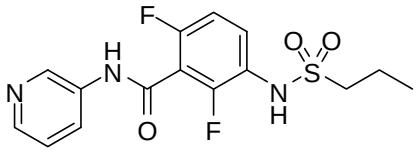


2,6-Difluoro-3-(propylsulfonamido)-N-(1H-pyrrolo[2,3-b]pyridin-5-yl)benzamide (0.500 g, 1.268 mmol) was charged to a 100 mL round-bottom flask. $CHCl_3$ (25 mL) was added to form a slurry. N-Bromosuccinimide (0.271 g, 1.52 mmol) was added and stirred for 20 minutes. The solids were filtered, washed with DCM, and dried under vacuum providing N-(3-bromo-1H-pyrrolo[2,3-b]pyridin-5-yl)-2,6-difluoro-3-(propylsulfonamido)benzamide (0.427 g, 71.2%) as a white solid. HRMS calcd. for $C_{17}H_{15}BrF_2N_4O_3S$: 472.0016, found: 472.0016. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.12

(br s, 1H), 11.04 (s, 1H), 9.81 (br s, 1H), 8.41-8.43 (m, 1H), 8.34-8.35 (m, 1H), 7.74-7.76 (m, 1H), 7.53-7.59 (q, *J*=7.83 Hz, 1H), 7.25-7.30 (d, *J*=8.48 Hz, 1H), 3.11-3.15 (d, *J*=7.48 Hz, 2H), 1.75-1.80 (m, 2H), 0.98-1.02 (t, *J*=7.46 Hz, 3H); *m/z* (APCI-pos) M+1 = 473.0, 475.0. 95.37% Pure by HPLC at 220 nm.

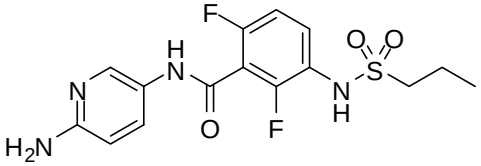
Characterization of compounds 3-5, 8, 13-18

2,6-Difluoro-3-(propylsulfonamido)-N-(pyridin-3-yl)benzamide (**3**)



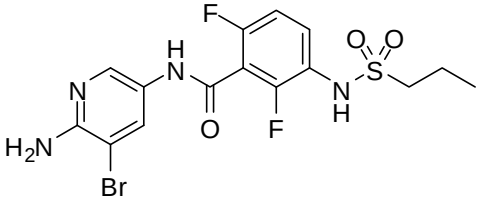
^{1}H NMR (400 MHz, MeOD) δ 8.81 (s, 1H), 8.34 (d, *J*=4.88 Hz, 1H), 8.23 (d, *J*=8.42 Hz, 1H), 7.67 (dd, *J*=8.42 Hz, 1H), 7.45-7.84 (m, 1H), 7.13 (t, *J*=9.07 Hz, 1H), 3.11 (t, *J*=7.46 Hz, 2H), 1.84-1.89 (m, 2H), 1.03-1.07 (t, *J*=7.50 Hz, 3H); *m/z* (APCI-pos) M+1=356.0.

N-(6-Aminopyridin-3-yl)-2,6-difluoro-3-(propylsulfonamido)benzamide (**4**)



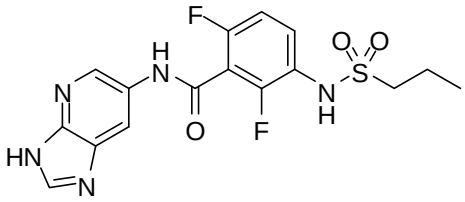
^{1}H NMR (400 MHz, CD_3OD) δ 8.17 (s, 1H), 7.76 (d, *J*=9.45 Hz, 1H), 7.66-7.59 (m, 1H), 7.10 (t, *J*=8.47 Hz, 1H), 6.62 (d, *J*=8.63, 1H), 3.09 (t, *J*=7.78, 2H), 1.91-1.81 (m, 2H), 1.05 (t, *J*=7.41, 3H); *m/z* (APCI-pos) M+1=371.1.

N-(6-Amino-5-bromopyridin-3-yl)-2,6-difluoro-3-(propylsulfonamido)benzamide (**5**)



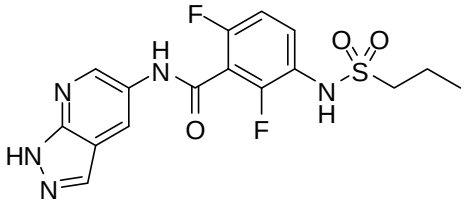
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, *J*=2.55, 1H), 8.16 (br s, 1H), 8.03 (d, *J*=2.55, 1H), 7.01-6.96 (m, 1H), 5.19 (br s, 2H), 3.08-3.03 (m, 2H), 1.92-1.82 (m, 2H), 1.65 (br s, 1H), 1.03 (t, *J*=7.44, 3H); *m/z* (APCI-pos) M+1=449.0, 451.0

2,6-Difluoro-N-(3H-imidazo[4,5-b]pyridin-6-yl)-3-(propylsulfonamido)benzamide (**8**)



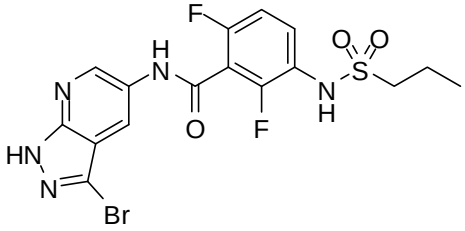
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.63 (br s, 1H), 11.03 (br s, 1H), 9.81 (br s, 1H), 8.43-8.53 (m, 3H), 7.53-7.59 (m, 1H), 7.26-7.30 (m, 1H), 3.11-3.15 (m, 2H), 1.74-1.80 (m, 2H), 0.98-1.02 (m, 3H); *m/z* (APCI-pos) M+1 = 394.3.

2,6-Difluoro-3-(propylsulfonamido)-N-(1H-pyrazolo[3,4-b]pyridin-5-yl)benzamide (**13**)



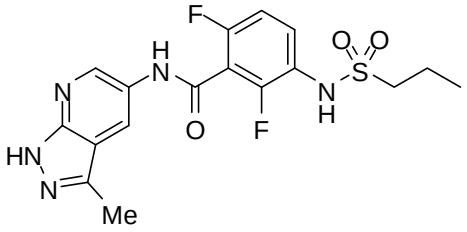
^{1}H NMR (400 MHz, CD_3OD) δ 8.70 (s, 1H), 8.65 (d, *J*=2.12, 1H), 8.13 (s, 1H), 7.63-7.69 (m, 1H), 7.12-7.17 (m, 1H), 3.10-3.14 (m, 2H), 1.84-1.91 (m, 2H), 1.06 (t, *J*=7.6 Hz, 3H); *m/z* (APCI-neg) M-1 = 394.2.

N-(3-Bromo-1H-pyrazolo[3,4-b]pyridin-5-yl)-2,6-difluoro-3-(propylsulfonamido)benzamide (**14**)



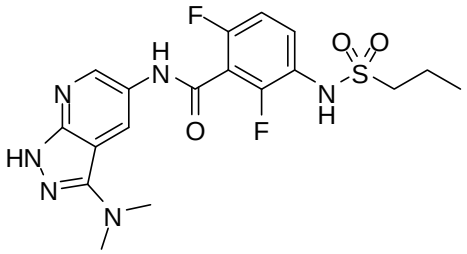
^{1}H NMR (400 MHz, CD_3OD) δ 8.68 (d, *J*=2.34 Hz, 1H), 8.56 (d, *J*=2.34 Hz, 1H), 7.64-7.70 (m, 1H), 7.17-7.17 (m, 1H), 3.10-3.14 (m, 2H), 1.84-1.91 (m, 2H), 1.06 (t, *J*=7.73 Hz, 3H); *m/z* (APCI-neg) M-1 = 472.2, 474.2.

2,6-Difluoro-N-(3-methyl-1H-pyrazolo[3,4-b]pyridin-5-yl)-3-(propylsulfonamido)benzamide (**15**)



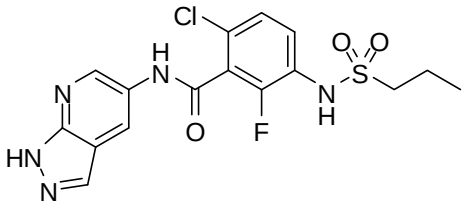
^{1}H NMR (400 MHz, DMSO-d_6) δ 13.24 (s, 1H), 11.08 (s, 1H), 9.81 (s, 1H), 8.61-8.59 (m, 1H), 8.56-8.54 (m, 1H), 7.59-7.53 (m, 1H), 7.30-7.26 (m, 1H), 3.32 (s, 3H), 3.15-3.11 (m, 2H), 1.82-1.73 (m, 2H), 1.00 (t, *J*=7.5 Hz, 3H). *m/z* (APCI-pos) M+1 = 410.1.

N-(3-(Dimethylamino)-1H-pyrazolo[3,4-b]pyridin-5-yl)-2,6-difluoro-3-(propylsulfonamido)benzamide (**16**)



^{1}H NMR (400 MHz, CD_3OD) δ 8.75 (s, 1H), 8.53 (s, 1H), 7.63-7.69 (m, 1H), 7.12-7.16 (m, 1H), 3.10-3.14 (m, 8H), 1.84-1.90 (m, 2H), 1.06 (t, *J*=7.6 Hz, 3H); *m/z* (APCI-pos) M+1 = 439.1.

6-Chloro-2-fluoro-3-(propylsulfonamido)-N-(1H-pyrazolo[3,4-b]pyridin-5-yl)benzamide (**18**)



^{1}H NMR (400 MHz, CD_3OD) δ 8.68 (s, 1H), 8.56 (s, 1H), 7.63-7.68 (m, 1H), 7.36-7.38 (m, 1H), 3.13-3.17 (m, 2H), 1.84-1.89 (m, 2H), 1.06 (t, *J*=7.6 Hz, 3H); *m/z* (APCI-neg) M-1 = 410.2, 412.2.

Kinase selectivity of compounds 17 and 19

Selectivity of compounds **17** and **19** were determined against a panel of 228 kinases at 1 μM (Invitrogen). Five of 228 kinases produced >50% inhibition and the IC_{50} for each were determined and reported below.

Kinase	IC_{50} nM (17)	IC_{50} nM (19)
$B\text{-}Raf^{V600E}$	4.8	1.7
$B\text{-}Raf^{WT}$	2.2	1.0
C-Raf	6.0	1.9
SRMS (Srm)	17	6
PTK6 (Brk)	84	53
FGR	143	20
LCK	356	112
FLT3	448	439

Kinases screened with <50% inhibition at 1 μM:

LYN B
LYN A
BLK
LTK (TYK1)
CSK
CSF1R (FMS)
SRC N1
YES1
KIT
FYN
NTRK3 (TRKC)
MUSK
SRC
NTRK1 (TRKA)
ALK
ERBB2 (HER2)
HCK
FRK (PTK5)
PRKG2 (PKG2)
CLK2
MAPK8 (JNK1)
MAPK12 (p38 gamma)
RPS6KA4 (MSK2)
PRKD1 (PKC mu)
CAMK2B (CaMKII beta)
MARK1 (MARK)
MAP2K2 (MEK2)

PRKCB2 (PKC beta II)
NTRK2 (TRKB)
FGFR2
TEK (Tie2)
IGF1R
PRKCZ (PKC zeta)
PRKCN (PKD3)
ERBB4 (HER4)
(GSK3 alpha
CDK9/cyclin T1
BTK
PDGFR beta
AURKC (Aurora C)
CK2 alpha 1
CLK1
MAPK13 (p38 delta)
FGFR3
PRKCG (PKC gamma)
MAP3K8 (COT)
ROS1
EPHB2
EPHA1
RET
PAK6
RPS6KA5 (MSK1)
BMX
MARK2

RPS6KB1 (p70S6K)
MET (cMet)
EPHA8
MAP2K1 (MEK1)
CDK7/cyclin H/MNAT1
CAMK1 (CaMK1)
HIPK4
CaMKII delta
EPHB1
CSNK1E (CK1 epsilon)
PRKCQ (PKC theta)
PAK3
PIK3CG (p110 gamma)
PKN1 (PRK1)
SGKL (SGK3)
ABL2 (Arg)
PRKX
DYRK3
STK22B (TSSK2)
KDR (VEGFR2)
ZAP70
PTK2 (FAK)
FLT4 (VEGFR3)
PDGFR alpha
CK1 gamma 2

PLK1
CDK1/cyclin B
PLK2
RPS6KA2 (RSK3)
EEF2K
PRKCH (PKC eta)
INSRR (IRR)
INSR
PAK4
PRKG1
PRKCE (PKC epsilon)
TBK1
CSNK1D (CK1 delta)
SGK2
STK24 (MST3)
HIPK1 (Myak)
CK1 alpha 1
MAPK10 (JNK3)
ABL1
PRKCI (PKC iota)
MYLK2 (skMLCK)
NEK4
CHEK1 (CHK1)
MAP2K1 (MEK1)
RPS6KA1 (RSK1)
MAPK14 (p38 alpha)

MAPK11 (p38 beta)
TAOK2 (TAO1)
PDK1
ITK
CK2 alpha 2
DYRK4
FER
CDK2/cyclin A
FGFR1
MAP2K6 (MKK6)
SGK (SGK1)
CaMKII alpha
EPHA3
CK1 gamma 3
STK23 (MSSK1)
NEK2
CDK5/p25
DYRK1B
STK22D (TSSK1)
TYRO3 (RSE)
PIM1
EPHB3
ROCK2
MAPKAPK2
SRPK1
EPHA4
MAPK1 (ERK2)
EPHA5
AMPK A2/B1/G1

AURKA (Aurora A)	PRKCA (PKC alpha)	AKT3 (PKB gamma)	PHKG2	MAPK9 (JNK2)
JAK2 JH1 JH2	MATK (HYL)	CLK3	MAP3K9 (MLK1)	SYK
EPHB4	CDC42 BPB (MRCKB)	MAPKAPK3	PIM2	NEK9
DAPK1	AXL	PRKCD (PKC delta)	ADRBK1 (GRK2)	FLT1 (VEGFR1)
CK1 gamma 1	PTK2B (FAK2)	DCAMKL2 (DCK2)	PHKG1	EGFR (ErbB1)
FES (FPS)	AKT1 (PKB alpha)	EPHA2	MAP4K5 (KHS1)	JAK1
MST1R (RON)	JAK3	PAK7 (KIAA1264)	MAPK1 (ERK2)	GRK5
RPS6KA3 (RSK2)	DAPK3 (ZIPK)	MAPKAPK2	PRKD2 (PKD2)	STK25 (YSK1)
MAPKAPK5 (PRAK)	AKT2 (PKB beta)	AURKB (Aurora B)	IKBKB (IKK beta)	PRKACA (PKA)
BRSK1 (SAD1)	MAP4K2 (GCK)	AMPK A1/B1/G1	CDC42 BPA (MRCKA)	p110 alpha/p85 alpha
PASK	PAK2 (PAK65)	SRPK2	STK4 (MST1)	NEK6
CHUK (IKK alpha)	CDK5/p35	STK3 (MST2)	MST4	NEK1
LRRK2 G2019S	NEK7	MERTK (cMER)	CAMK4 (CaMKIV)	CAMK1D (CaMKI delta)
PRKCB1 (PKC beta I)	ADRBK2 (GRK3)	FRAP1 (mTOR)	GSK3B (GSK3 beta)	CHEK2 (CHK2)
PLK3	MAPK3 (ERK1)	RPS6KA6 (RSK4)	LRRK2	IRAK4
DYRK1A	JAK2 JH1 JH2 V617F	FGFR4	GRK4	JAK2
GRK7		ACVR1B (ALK4)	ROCK1	MAP4K4 (HGK)
		GRK6	MINK1	

Biological assays

All biochemical and cellular data in the manuscript are an average of at least three individual measurements ($n \geq 3$). In all cases, individual measurements were within three-fold for each compound.

B-Raf^{V600E} enzyme assay: Full length 6x His-tagged human B-Raf^{V600E} co-expressed with CDC37 (1-378) was expressed in baculovirus-infected insect cells and purified using standard affinity tag chromatographic methods. B-Raf^{V600E} activity was assessed by measuring the incorporation of radiolabel from [γ-^{33}P]ATP into full length 6×His-tagged human wtMEK covalently modified with 5′-p-fluorosulphonylbenzoyladenosine (FSBA). The assay was carried out using 96-well polypropylene plates. The assay buffer consisted of 25 mM PIPES, pH 7.2, 10 mM $MgCl_2$, 5 mM β-glycerol phosphate, 100 μM sodium orthovanadate, 100 mM KCl, 0.01% Triton X-100, 1 mM DL-dithiothreitol, and 1% DMSO. Compounds dissolved in DMSO were varied over a ten dose, 3-fold serial dilution. Reactions containing 150 pM B-Raf^{V600E} and 1 μM FSBA-wtMEK ±compound were initiated by the addition of ATP/[γ-^{33}P]ATP (4 μM/33 μCi/mL). Incubations were carried out at 22 °C for a period of 60 min, after which the reactions were quenched by the addition of 3.3 volumes of 25% TCA. The precipitated product was captured by filtration on a Whatman glass fiber B filter plate, and excess labeled ATP was washed off using a Tomtec MACH III harvestor. Following washing and addition of scintillation cocktail, the counts per minute were determined on a Perkin Elmer TopCount System.

IC_{50} values were calculated by fitting a standard 4-parameter logistic model to the dose response curve plotted as percent of control (POC) versus concentration of compound.

pERK measurement in Malme-3M cells: Malme-3M melanoma cells were plated in 96-wells and treated with various concentrations of test compounds for 1 hr at 37 °C. The cells were fixed, permeabilized, and incubated with an anti-phospho-ERK antibody and an anti-ERK1,2 antibody. Plates were washed and fluorescently-labeled secondary antibodies were added. Plates were analyzed on a LICOR fluorescence imager. The pERK signal is normalized to the total ERK signal.

Aqueous solubility assay

The thermodynamic aqueous solubility of compounds was measured using a modified shake-flask method. Crystallinity of each compound was confirmed using a polarizing light microscope (Olympus BX51). For each compound, 0.5 mL of aqueous buffer (10 mM potassium phosphate), pH 6.5, was added to 0.5 mg of dry compound and the mixture was swirled at 350 rpm at room temperature for 24 hours. Aliquots were subsequently removed and filtered for HPLC analysis. Standards of known concentration were also prepared and analyzed for each compound in order to create a calibration curve. Analysis was accomplished using a HPLC/PDA system comprised of an Alliance 2795 Separations System (Waters) and a 2996 Photodiode Array Detector (Waters).

Rodent pharmacokinetics

In vivo pharmacokinetic studies were performed in male CD-1 mice (6-8 weeks of age) given food and water ad lib prior to intravenous (IV) dosing or fasted overnight prior to oral (PO) dosing and fed approximately 4 hours post dose. Intravenous dose solutions were prepared in 40% PEG400/10% EtOH/50% normal saline (except for **10** which was prepared in 40% PEG400/60% normal saline) at a concentration of 0.5 mg/mL to yield a dose of 2.5 mg/kg at a dose volume of 5 mL/kg. Oral dose solutions were prepared in 40% PEG400/10% EtOH/50% sterile water at a concentration of 3 mg/mL to yield a dose of 30 mg/kg at a dose volume of 10 mL/kg. Whole blood samples from 3 mice per time point were obtained via cardiac puncture from the mice euthanized using CO_2 and added

to polypropylene tubes containing K_2EDTA as the anticoagulant at the following time points post-dose: 0.02, 0.08, 0.25, 0.50, 1, 2, 4, and 8 hr (12 and 24 hr samples were drawn for **9**) following IV administration and 0.08, 0.25, 0.50, 1, 2, 4, and 8 hr following PO administration.

Blood samples were spun in a centrifuge and the resulting plasma was analyzed for compound concentrations following protein precipitation with acetonitrile and subsequent centrifugation. Analysis was performed using an HPLC-MS/MS system comprised of an HTC-PAL autosampler (Leap Technologies, Carrboro, NC), an Agilent 1100 or 1200 HPLC (Agilent, Palo Alto, CA) and an Applied Biosystems 4000 Q TRAP® mass spectrometer (Applied Biosystems, Foster City, CA). Chromatographic retention and separation of the analyte and internal standard was achieved using a reverse phase column in conjunction with gradient conditions using mobile phases A (aqueous 0.1% formic acid and 1% isopropyl alcohol) and B (0.1% formic acid in acetonitrile). Mass spectrometric detection of the analytes was accomplished using ESI positive ionization mode. Analyte responses were measured by multiple reaction monitoring (MRM) of transitions unique to each analyte. Labetalol was used as the internal standard. Samples with compound concentrations exceeding the upper limit of quantitation were diluted up to 10-fold using plasma from naïve animals. Pharmacokinetic (PK) parameters were calculated by established non-compartmental methods using an in-house Excel® (Microsoft Corporation, Redmond, VA) macro.

Cell Viability of compounds 17 and 19 versus PLX4032 (1)

For cell viability assays, cells were seeded at 2,000 per well and treated with compound on day 2. The relative numbers of viable cells were measured by luminescence 4 days later using CellTiter-Glo (Promega), according to the manufacturer's instructions."

Cell lines	Mutational Status		Compound, EC_{50} (μM)		
Melanoma	**B-Raf**	**PTEN**	**1**	**17**	**19**
624	V600E mutant	N	0.34	0.10	nt
888	V600E mutant	P	1.31	0.45	0.43
537MEL	WT	N	>20	>20	>20
928MEL	V600E mutant	P	0.22	0.08	nt
A2058	V600E mutant	N	>20	>20	>20
A375x1	V600E mutant	P	0.15	0.09	0.17
C32	V600E mutant	N	0.43	0.13	nt
G361	V600E mutant	P	0.41	0.34	nt
Hs 294T	V600E mutant	N	12.44	>20	nt
Hs 695T	V600E mutant	P	1.19	0.58	nt
LOX IMVI	V600E mutant	P	0.23	0.13	nt
Malme-3M	V600E mutant	P	0.10	0.03	0.28
RPMI-7951	V600E mutant	N	17.14	16.11	>20
SK-MEL-28	V600E mutant	P	0.24	0.13	nt
Colon cancer					
CL 34	V600E mutant	P	0.25	0.05	nt
COLO 201	V600E mutant	P	0.35	0.12	nt
COLO 829	V600E mutant	N	0.42	0.30	nt
COLO 205	V600E mutant	P	0.24	0.08	0.07
COLO 741	V600E mutant	P	0.77	0.31	nt
CX-1	V600E mutant	P	15.38	1.09	nt
HT-29 - TO	V600E mutant	P	1.16	0.25	0.27
RKO	V600E mutant	P	>20	1.77	0.91
SW1417	V600E mutant	P	0.69	0.14	nt
MDST8	V600E mutant	N	4.51	1.61	0.70

Abbreviations: P = present, N = null, nt = not tested.

Spray-dried dispersion of compound 17 (K^+ salt)

SDD preparation: Hydroxypropyl methylcellulose acetate succinate grade M (HPMCAS-M) was purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan), lot # 6113225. Solid molecular dispersions are reported as a % drug loading (by weight) in HPMCAS-M. For example, a 25% drug loading consists of one part (by weight) compound and three parts (by weight) HPMCAS-M. Solutions were spray dried on a

Buchi B290 spray dryer using a high-performance cyclone and small-volume sample collector. After spray drying, samples were dried at ambient (23 °C) conditions to remove the solvent. Compound 17 (K^+ salt, 1.49 g) and HPMCAS-M (4.48 g) were dissolved as a 5 wt % solution in MeOH, for a total solution wt. of 114 g. Spray drying yielded 5.43 g product (91%) as a powder which was stable upon standing and used in the preparation of suspensions for *in vivo* dosing.

Characterization: *DSC analysis and glass transition (Tg) determination* – Differential scanning calorimetry was performed on a TA instruments Q-100 modulated DSC. Tg of the solid molecular dispersion of compound **17** (K^+ salt) was 119 °C from three independent determinations. No evidence of compound crystallization was seen for temperatures near the melting point (Tm = 200 °C).

Powder x-ray diffraction (PXRD) – PXRD data were obtained using a Scintag XDS2000 apparatus with no evidence of crystallinity for compound **17** (K^+ salt).

Scanning electron microscopy (SEM) – SEM was used to characterize the spray dried particles' shape and size. The particles showed the shape of collapsed spheres with a size ranging from ~ 1 μm diameter to near 20 μm in diameter with an average diameter near 10 μm.

Compound **19** (free base) was formulated according to the procedure for **17**.

Dose escalation of compounds 17 and 19 versus COLO 205 xenografts

All procedures involving animals were performed in accordance with Genentech's Institutional Animal Care and Use Committee guidelines. To generate tumors, 100 μL of a single-cell suspension containing 5 x 10^6 COLO 205 cells (ATCC, Manassas, VA) in HBSS were injected subcutaneously into the right lateral thorax of 9-11 week old female NCR nude mice (Taconic, Oxnard, CA). Tumor volume was calculated using the mean diameter measured with vernier calipers using the formula $v = 0.5 \times a \times b^2$, where a and b are the smallest and largest perpendicular tumor diameters, respectively. Daily oral administration by gavage was initiated once tumors reached a size in the range of 125-250 mm^3 (8 days post inoculation). The spray-dried dispersions of compounds **17** and **19** were formulated in 50 mM citrate buffer (pH 4), 0.25% HPMC (E4M), 0.1% Tween-80 daily immediately prior to dosing and administered in a volume of 200 μL within 15

minutes of formulation, and was dosed at 0 (vehicle), 5, 10, 20, 30, 40, 60, 80, 100 and 125 mg/kg once daily for 21 days. Tumor volumes and body weights were measured at least twice weekly until end of study. Tumor xenograft growth data were analyzed using R version 2.9.2 (R Development Core Team 2008; R Foundation for Statistical Computing; Vienna, Austria), and the mixed models were fit within R using the linear and nonlinear mixed effects models package, version 3.1-96. The fixed effect changes in $\log_2$(Volume) by time and dose were modeled as the sum of the main effects and interaction of a natural cubic regression spline basis in time with a 2-knot natural spline basis in dose. The half-maximal and 90% maximal effect levels (ED_{90}, ED_{50}) were obtained from the %TGI dose response curve, which 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, using the formula: $\%TGI = 100 \text{ X } (1 - AUC_{dose}/AUC_{veh})$.

Compound **17**:

Dose (mg/kg)	N Day 0	Last Day	N Last Day	Vol Last Day	AUC/Day %TGI (lower,upper)	PR	CR	STI
0	5	20	4	1875	0 (-54, 37)	0	0	5
5	5	20	5	1588	7 (-45, 42)	0	0	5
10	5	20	5	1385	31 (-8, 58)	0	0	5
20	5	20	5	1335	37 (-1, 63)	0	0	5
30	5	20	4	1078	54 (23, 73)	0	0	5
40	5	20	5	1202	50 (21, 72)	0	0	5
60	5	20	5	700	71 (48, 86)	0	0	5
80	5	20	5	735	67 (44, 82)	0	0	5
100	5	20	5	636	76 (58, 90)	0	0	5
125	5	20	5	634	76 (59, 89)	0	0	5

Compound **19**:

Dose (mg/kg)	N Day 0	Last Day	N Last Day	Last Day	%TGI (95% CI)	PR	CR	STI
0	5	17	5	1273	0 (0, 0)	0	0	5
1	5	17	5	1213	5 (3, 7)	0	0	5
2.5	5	17	5	1127	12 (7, 17)	0	0	5
5	5	17	5	999	23 (14, 31)	1	0	5
10	5	17	5	787	41 (26, 52)	0	0	5
20	5	17	5	509	65 (45, 77)	0	0	5
33	5	17	5	331	80 (63, 89)	0	0	5
48	5	17	5	263	86 (77, 93)	0	0	5
65	5	17	5	247	89 (79, 96)	0	0	5
82	5	17	5	238	91 (84, 97)	0	0	5
100	5	17	5	224	95 (85, 102)	1	0	5

Crystal Structure Determination of B-Raf with compounds 5 and 17.

B-Raf (432-726) with an N-terminal 6xHis-tag (His-B-Raf) (1) and human $p50^{Cdc37}$ were cloned into pBac4x-1 (Novagen, Inc.), with both genes under control of the polyhedrin promoter. The genes were transfected into the Baculogold Baculovirus Expression System (BD Biosciences) using standard methods, and co-expressed in *Trichoplusia ni* Hi5 insect cells. His-B-Raf was purified by immobilized metal ion chromatography using Talon resin (Clontech, Inc.). Protein eluted from the IMAC step was further purified by loading and elution from HiTrap SP Sepharose FastFlow column (GE Biosciences) and passage over a 26/60 Superdex 200 column (GE Biosciences) equilibrated in 20 mM Hepes pH 7.0, 15% (v/v) glycerol, 0.25% (w/v) CHAPS, 375 mM NaCl, 1 mM EDTA, 1 mM TCEP. Protein eluted from the Superdex 200 column was slowly concentrated to 3.9 mg/mL using an Amicon stirred cell fitted with a YM10 Ultrafiltration membrane. Protein was frozen and stored at -80 C. His-B-Raf co-crystals with a weak-binding inhibitor were grown by hanging drop vapor diffusion. 2 μL of protein-inhibitor solution were mixed with 2 μL of 100 mM Tris pH 9.0, 10% (w/v) PEG 8000 and incubated at 20 C for 2-3 weeks. The resulting co-crystals were soaked in solutions consisting of 100 mM Tris pH 9.0, 10% (w/v) PEG 8000, 7.5% (v/v) glycerol, 0.5 mM **5** or **17** (compounds added from 50 mM DMSO stocks) for 48 hours. The soaked crystals were then cryoprotected in a solution of 100 mM Tris pH 9.0, 10% PEG 8000, 30% glycerol, frozen in a stream of nitrogen vapor held at 100 K and stored in liquid nitrogen. X-ray diffraction data for the B-Raf-**5** complex were collected using a Rigaku FR-E Superbright rotating anode X-ray generator equipped with a Cu anode, Osmic confocal mirros, and an Raxis VI++ image plate detector. X-ray diffraction data for the B-Raf-**17** complex were collected at beam line 7-1 of the Stanford Synchrotron Radiation Laboratory at $\lambda = 0.979$ Å using an ADSC Quantum 315R detector. Data were processed using Mosflm (2) and Scala (3). Crystals belonged to space group $P4_12_12$ with two B-Raf molecules per asymmetric unit. Crystal structures were solved by molecular replacement in Molrep (3)

using the coordinates of a previously determined B-Raf-inhibitor complex as a search model (4). Crystallographic refinement of the structures was performed using Refmac5 (5) and the model rebuilding was performed in O (6). Each final model contained two B-Raf molecules and two inhibitor molecules. For the B-Raf-**5** complex, 79.9% of protein residues were in the most favored region and 18.6% of residues were in the additionally allowed regions of a Ramachandran plot and for the B-Raf-**17** complex, 82.6% of residues were in the most favored region and 16.3% of residues were in the additionally allowed regions.

(1) Wan, P.T.C.; Garnett, M.J.; Roe, S.M.; Lee, S.; Niculescu-Duvaz, D.; Good, V.M.; Cancer Genome Project, Jones, C.M.; Marshall, C.J.; Springer, C.J.; Barford, D.; Marais, R. *Cell* **2004**, *116*, 855-867.

(2) Leslie, A.G.W. *Joint CCP4 + ESF-EAMCD Newsletter on Protein Crystallography, No. 26* **1992**.

(3) Collaborative Computational Project, No. 4 The CCP4 Suite: Programs for crystallography. *Acta Crystallogr. D* **1994**, *50*, 760-763.

(4) Hansen, J.D.; Grina, J.; Newhouse, B.; Welch, M.; Topalov, G.; Littman, N.; Callejo, M.; Gloor, S.; Martinson, M.; Laird, E.; Brandhuber, B.J.; Vigers, G.; Morales, T.; Woessner, R.; Randolph, N.; Lyssikatos, J.; Olivero, A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4692-4695.

(5) Murshudov, G.N.; Vagin, A.A.; Dodson, E.J. *Acta Crystallogr. D* **1997**, *53*, 240-255.

(6) Jones, T.A.; Zou, J.-Y.; Cowan, S.W.; Kjeldgaard, M. *Acta Crystallogr. A* **1991**, *47*, 110-119.

Table 1. Data collection and refinement statistics

	B-Raf + **5**	B-Raf + **17**
Data Collection		
Resolution (Å)	30 – 3.4 (3.58 – 3.4)	30 – 3.3 (3.48 – 3.3)
Unit cell dimensions		
a, *b*, *c* (Å)	108.0, 108.0, 153.1	108.4, 108.4, 150.7
α, β, γ (°)	90, 90, 90	90, 90, 90
Total Reflections	39,446	63,227
Unique Reflections	12,929	13,851
Completeness (%)	99.5 (99.9)	98.5 (99.4)
R_{merge}	0.118 (0.367)	0.090 (0.326)
I / σ(I)	5.6 (2.0)	7.2 (2.3)
Refinement		
Reflections Used	12,261	13,144
R_{cryst}	0.243	0.237
R_{free}	0.307	0.289
Average B-value (Å^2)	55.3	53.1
Number of protein atoms	4,328	4,368
Number of ligand atoms	52	58
Number of solvent atoms	0	0
r.m.s.d. bonds (Å)	0.010	0.008
r.m.s.d. angles (°)	1.35	1.31