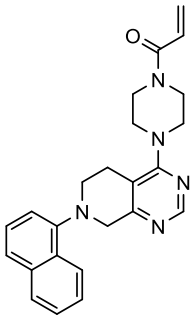
Table of Contents

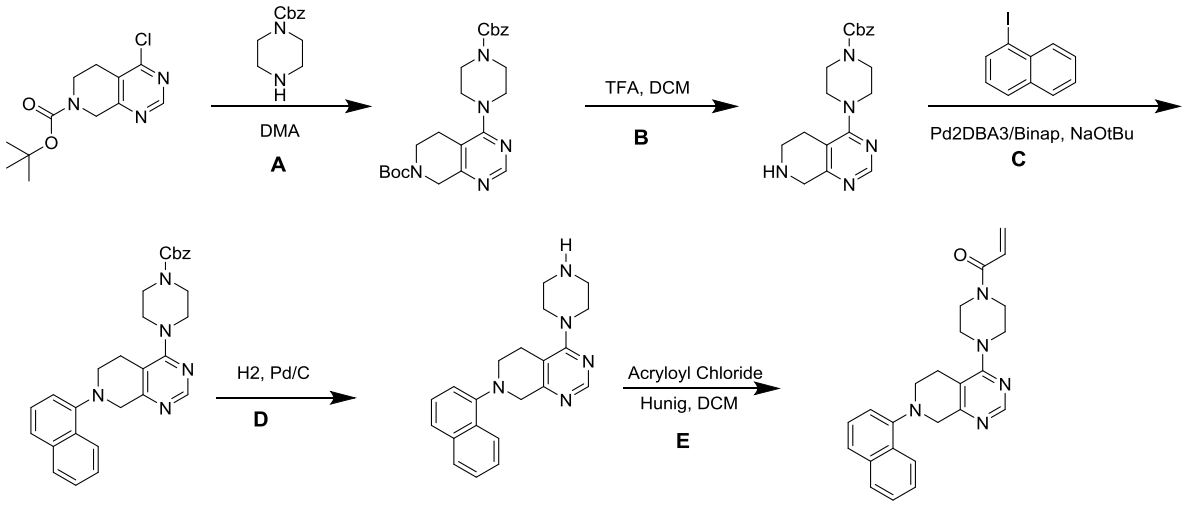
NMR spectra were recorded on a 400 MHz Varian NMR spectrometer. 1H chemical shifts are reported in δ values in ppm downfield with the deuterated solvent as the internal standard. Common abreviations for multiplicity are as follows: s = singlet, d = doublet, t = triplet, q =quartet, br = broad, app = apparent, dd = doublet of doublets, dt = doublet of triplets, td = triplet of doublets, m = multiplet. Low resolution mass spectra were obtained on an Agilent 1200 spectrometer with ESI and APCI source and a Poroshell 1200 EC-C18 4 μm column eluting over 4 minutes with 5% → 95% acetonitrile/water with both solvents containing 0.2% TFA as modifier. High resolution mass spectra were obtained on an Eksigent MicroLC 200 Plus System and a Sciex TripleTOF 6600 quadrupole time-of-flight mass spectrometer; compounds were eluted from a Eksigent C18 column (3 μm, 0.3 x 150 mm) over 20 min with 4-80% acetonitrile in water at 12 μL/min, with both solvents containing 0.1% formic acid. Reversed-phase HPLC purifications were performed using a Gilson preparative HPLC with a Gemini 10 μm NX-C18 250x21.2 millimeter column eluting over 15 minutes with 5 → 95% acetonitrile/water with both solvents containing 0.1% trifluoroacetic acid as modifier. All solvents for extraction and chromatography were HPLC grade and used without purification and all solvents for synthesis were anhydrous unless otherwise stated. All reagents were purchased and used without purification. Abreviations used in the synthetic procedures are as follows: EtOAc = ethyl acetate, ON = overnight, hr = hours, MeOH = methanol, Hex = hexanes, EtOH = ethanol, BINAP =

racemic-2,2'-bis(diphenylphosphino)-1,1'-binaphthyl, Pd2dba3 = tris(dibenzylideneacetone)dipalladium(0)

Compound 4



1-(4-(7-(naphthalen-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazin-1-yl)prop-2-en-1-one



Step A: To a solution of tert-butyl 4-chloro-5,6-dihydropyrido[3,4-d]pyrimidine-7(8H)-carboxylate (0.25 g, 0.93 mmol) in a microwave vial was added benzyl piperazine-1-carboxylate (0.41 g, 1.9 mmol), DMA (2 mL) and Cs_2CO_3 (0.60 g, 1.9 mmol) and the reaction heated to 150^{o}C for 1 hr. The reaction was next diluted with water and and etoac and the layers separated. The organics were next washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The material was next chromatographed using 10 -->80% EtOAc/hex as eluent to yield product. (0.40g , 95% yield). LC (ESI+APCI) MS mz calculated for $C_{24}H_{31}N_5O_4$ $[M+H]^+$ Calculated: 454.2, Found 454.2

Step B: To a solution of tert-butyl 4-(4-((benzyloxy)carbonyl)piperazin-1-yl)-5,6-dihydropyrido[3,4-d]pyrimidine-7(8H)-carboxylate (0.5 g, 1.1 mmol) in DCM (5 mL) was added TFA (5 mL) and the reaction stirred at rt for 2 hrs. The reaction was next concentrated in vacuo and the material partitioned between DCM and 1N NaOH and the layers separated. The organics were next washed with brine, dried over $MgSO_4$ and concentrated to give the free base which was used crude in the next reaction.

Step C: To a solution of benzyl 4-(5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazine-1-carboxylate (0.90 g, 2.5 mmol) in toluene was added sodium 2-methylpropan-2-olate (1.2 g, 13 mmol) and 1-iodonaphthalene (1.3 g, 5.1 mmol) and the reaction degassed with N_2 for 15 minutes. To the reaction was next added BINAP (0.63 g, 1.0 mmol) and $Pd_2(dba)_3$ (0.47 g, 0.51 mmol) and the reaction stirred overnight at 100^{o}C. The reaction was cooled, poured into water

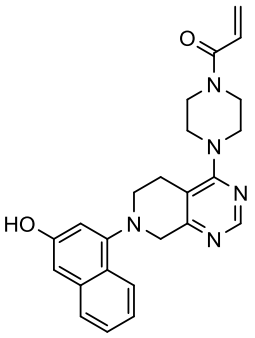
and extracted into EtOAc. The organics were next washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The material was next chromatographed using 10→80% EtOAc/Hex. as eluent to give benzyl benzyl 4-(7-(naphthalen-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazine-1-carboxylate (0.40 g, 0.83 mmol, 33 % yield). ^{1}H NMR (DMSO d_6, 400 MHz) δ 8.50 (s, 1H), 8.17 – 8.15 (m, 1H), 7.90 – 7.87 (m, 1H), 7.61 (d, J = 8.2 Hz, 1H), 7.52 – 7.47 (m, 2H), 7.43 (t, J = 7.3 Hz, 1H), 7.36 (s, 2H), 7.35 (s, 2H), 7.34 – 7.27 (m, 1H), 7.21 (d, J = 7.4 Hz, 1H), 5.09 (br s, 2H), 4.17 (s, 2H), 3.53 (br s, 4H), 3.47 (br s, 4H), 3.25 (br s, 2H), 2.96 (br s, 2H).

Step D: A solution of benzyl 4-(7-(naphthalen-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazine-1-carboxylate (0.40 g, 0.83 mmol) in ethanol was purged for 10 minutes with N_2 followed by addition of Pd/C (0.27 g, 0.25 mmol) and the reaction continued purging with N_2. The reaction was next evacuated under vacuum and backfilled with H_2 3 times. The reaction was next stirred overnight at room temperature under an atmosphere of H_2. The reaction was again purged with N_2 for 10 minutes and the slurry filtered through celite. The celite was rinsed with ethanol 2x. The combined organics were next concentrated in vacuo and the material used crude in the next reaction. (0.20g, 69%).

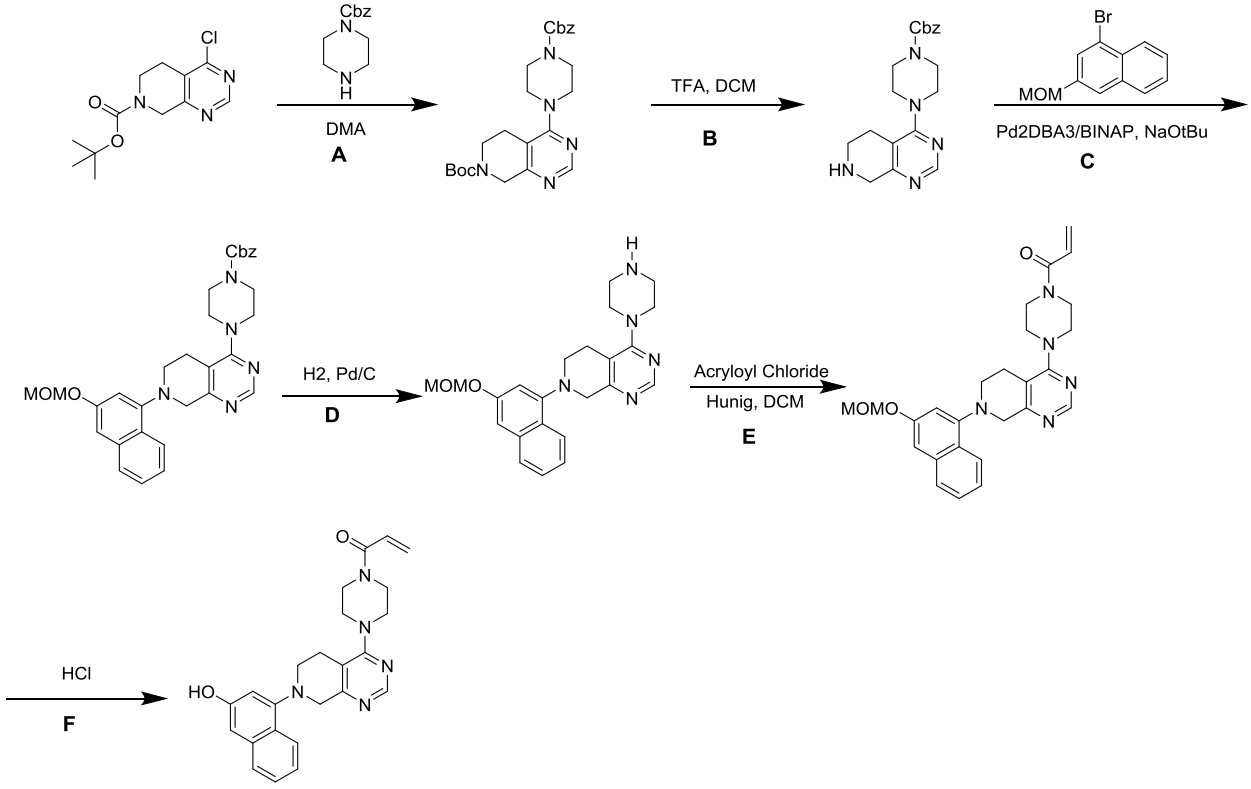
Step E: To a solution of 7-(naphthalen-1-yl)-4-(piperazin-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidine (0.20 g, 0.58 mmol) in DCM (10 mL) was added N-ethyl-N-isopropylpropan-2-amine (0.11 g, 0.87 mmol) and acryloyl chloride (0.052 g, 0.58 mmol) and the reaction stirred at room temperature for 1 hour. The reaction was next concentrated in vacuo and the material chromatograohed using 30→100% EtOAc/DCM followed by 0→10% MeOH/DCM as eluent to give 1-(4-(7-(naphthalen-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazin-1-yl)prop-2-en-1-one (0.044 g, 0.11 mmol, 19 % yield). LC (ESI+APCI) MS MS m/z 400.2 $[M+H]^+$. HRMS mz calculated for $C_{24}H_{25}N_5O$ $[M+H]^+$ 400.2132, Found:400.2135.

^{1}H NMR (DMSO d_6, 400 MHz) δ 8.50 (s, 1H), 8.18 – 8.15 (m, 1H), 7.90 – 7.86 (m, 1H), 7.61 (d, J = 8.2 Hz, 1H), 7.53 – 7.47 (m, 2H), 7.43 (t, J = 7.4 Hz, 1H), 7.21 (d, J = 6.7 Hz, 1H), 6.8 (dd, J = 16.7, 10.5 Hz, 1H), 6.13 (dd, J = 16.9, 2.3 Hz, 1H), 5.69 (dd, J = 10.5, 2.4 Hz, 1H), 4.17 (br s, 2H), 3.67 (br s, 4H), 3.48 (br s, 4H), 3.25 (br s, 2H), 2.97 (br s, 2H).

Compound 8



1-(4-(7-(3-hydroxynaphthalen-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazin-1-yl)prop-2-en-1-one



Step A: In 2 mL of dimethyl acetamide were combined tert-butyl 4-chloro-5,8-dihydropyrido[3,4-d]pyrimidine-7(6H)-carboxylate (1.0 g, 3.7 mmol), triethylamine (1.0 mL, 7.4 mmol), and benzyl 1-piperazinecarboxylate (0.86 mL, 4.4 mmol). The reaction vessel was sealed and the reaction mixture was heated to 90 °C with stirring. After 5 hours the reaction was diluted with brine and extracted with methyl t-butyl ether. The combined organic layers were washed sequentially with saturated ammonium chloride and brine, dried over $MgSO_4$, and concentrated under reduced pressure to a thick oil. The oil was chromatographed (RediSep®, 24 g) eluting with1:1 ethyl acetate/Hexanes to give tert-butyl 4-(4-((benzyloxy)carbonyl)piperazin-

1-yl)-5,8-dihydropyrido[3,4-d]pyrimidine-7(6H)-carboxylate (1.3 g, 2.9 mmol, 77 % yield). LC (ESI+APCI) MS mz calculated for $C_{24}H_{31}N_5O_4$ $[M+H]^+$ Calculated: 454.2, Found 454.2

Step B : To a solution of tert-butyl 4-(4-((benzyloxy)carbonyl)piperazin-1-yl)-5,8-dihydropyrido[3,4-d]pyrimidine-7(6H)-carboxylate (1.6 g, 3.5 mmol) in dichloromethane (12 mL) was added trifluoroacetic acid (2.7 mL, 35mmol) and the reaction was stirred at room temperature for 3 hours. The reaction was concentrated under vacuum and the residue was taken up in dichloromethane. The solution was washed sequentially with 1M NaOH and brine, dried over Na_2SO_4, filtered and concentrated under vacuum. The crude product was purified by column chromatography (Biotage Isolera, 24G Isco RediSep® Gold, 10 to 20% methanol/dichloromethane) to afford the product (1.1 g, 89%) as an off-white foam. LC (ESI+APCI) MS mz calculated for $C_{19}H_{23}N_5O_2$ $[M+H]^+$ Calculated: 353.2, Found 354.2

Step C: To a vial was added tris(dibenzylideneacetone)dipalladium (0) (0.0069 g, 0.0075 mmol), racemic-2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (0.0096 g, 0.015 mmol) and toluene (0.62 mL, 0.19 mmol). Argon was bubbled through the mixture for 5 minutes and then the vial was capped and the mixture was heated to 100 °C for 15 minutes. The mixture was cooled to ambient temperature and then sodium tert-butoxide (0.036 g, 0.37 mmol) was added followed by 1-bromo-3-(methoxymethoxy)naphthalene (0.050 g, 0.19 mmol) and benzyl 4-(5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazine-1-carboxylate (0.13 g, 0.37 mmol). The vial was capped and the mixture heated to 100 °C for 20 hours. The mixture was cooled to ambient temperature, diluted with dichloromethane and filtered through GF/F paper. The filtrate was concentrated and purified by column chromatography (Biotage Isolera, 12G Isco RediSep®, 10-50% ethyl acetate/dichloromethane) to afford the product (0.062 g, 61%) as an off-white foam. LC (ESI+APCI) MS mz calculated for $C_{31}H_{33}N_5O_4$ $[M+H]^+$ Calculated: 540.3, Found 540.3

Step D: To a solution of benzyl 4-(7-(3-(methoxymethoxy)naphthalen-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazine-1-carboxylate (0.061 g, 0.11 mmol) in ethanol (1.1 mL, 0.11 mmol) and tetrahydrofuran (1.1 mL, 0.11 mmol) was added palladium (0.024 g, 0.011 mmol) (Degussa Type, 10 wt.%, 50% H_2O). An atmosphere of H_2 was introduced into the reaction vessel by vacuum, and then the reaction mixture was maintained under an atmosphere of H_2. The mixture was stirred at ambient temperature for 2.5 hours, then diluted with methanol and filtered through GF/F paper. The colorless filtrate was concentrated under vacuum with

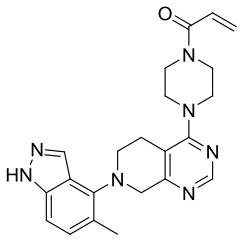
toluene to provide an off-white foam (0.048 g, 105%) that was used directly in the next step. LC (ESI+APCI) MS mz calculated for $C_{23}H_{27}N_5O_2$ $[M+H]^+$ Calculated: 406.2, Found 406.2

Step E: To a suspension of 7-(3-(methoxymethoxy)naphthalen-1-yl)-4-(piperazin-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidine (0.046 g, 0.11 mmol) in dichloromethane (1.1 mL, 0.11 mmol) at ambient temperature was added acryloyl chloride (1.2 mL, 0.12 mmol) (freshly prepared 0.1 M solution in dichloromethane) followed by triethylamine (0.032 mL, 0.23 mmol). The reaction was stirred at ambient temperature for 1 hour. The mixture was concentrated and the product was purified by column chromatography (Biotage Isolera, 12G Isco RediSep®, ethyl acetate) to afford the product (0.042 g, 79%) as an off-white solid foam. LC (ESI+APCI) MS mz calculated for $C_{26}H_{29}N_5O_3$ $[M+H]^+$ Calculated: 460.2, Found 460.2

Step F: To a solution of 1-(4-(7-(3-(methoxymethoxy)naphthalen-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazin-1-yl)prop-2-en-1-one (0.034 g, 0.074 mmol) in ethyl acetate (0.74 mL, 0.074 mmol) was added hydrochloric acid (5 to 6 N solution in 2-propanol (0.44 mL, 2.2 mmol). The mixture was stirred at ambient temperature for 5 hours. The mixture was diluted with ethyl acetate (10 mL), filtered through a polypropylene filter and the collected solid was washed with ethyl acetate and hexanes to provide the product as the HCl salt. The impure material was treated with 1 mL of ammonium hydroxide/methanol to quench the acid and the mixture was concentrated. The residue was dissolved in 10% methanol/dichloromethane and purified by column chromatography (Biotage Isolera, 12G Isco RediSep®, 2 to 5% methanol/ethyl acetate) to afford the product (0.008 g, 25%) as an off-white solid. LC (ESI+APCI) MS m/z 416.2 $[M+H]^+$. HRMS mz calculated for $C_{24}H_{25}N_5O_2$ $[M+H]^+$ 416.2081, Found 416.2071.

^{1}H NMR (CD_3OD, 400 MHz) δ 8.49 (s, 1H), 8.07 (app d, J = 8.2 Hz, 1H), 7.61 (app d, J = 8.2 Hz, 1H), 7.35 (m, 1H), 7.25 (m, 1H), 6.80 (m, 3H), 6.23 (dd, J = 16.8, 1.6 Hz, 1H), 5.77 (dd, J = 10.6, 2.0 Hz, 1H), 4.22 (br s, 2H), 3.80 (app t, J = 4.7 Hz, 4H), 3.63 (br s, 4H), 3.35 (br s, 2H), 3.03 (br s, 2H).

Compound 5



1-(4-(7-(5-methyl-1H-indazol-4-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazin-1-yl)prop-2-en-1-one

Steps A-C: benzyl 4-(7-(5-methyl-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-indazol-4-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazine-1-carboxylate: Synthesized according to compound 8 Steps A-C substituting 4-bromo-5-methyl-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-indazole in place of 1-bromo-3-(methoxymethoxy)naphthalene in Step C

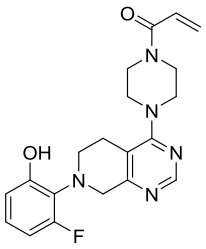
Step D: To a solution of benzyl 4-(7-(5-methyl-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-indazol-4-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazine-1-carboxylate (0.16 g, 0.26 mmol) in dichloromethane (10 mL) was added 2,2,2-trifluoroacetic acid (0.89 g, 7.8 mmol) followed by anisole (0.028 g, 0.26 mmol), and the reaction was stirred at room temperature for 3 hours at room temperature. The reaction was concentrated under vacuum and the concentrated material was taken up in ethyl acetate and washed with basic brine. The combined organic layers were dried over $MgSO_4$ and concentrated under vacuum. The crude material was chromatographed using 0 to 10% methanol/dichloromethane as the eluent to give benzyl 4-(7-(5-methyl-1H-indazol-4-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazine-1-carboxylate (0.05g, 38%). LC (ESI+APCI) MS mz calculated for $C_{27}H_{29}N_7O_2$ $[M+H]^+$ Calculated: 484.2, Found 484.2

Step E: 7-(5-methyl-1H-indazol-4-yl)-4-(piperazin-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidine: Prepared according to the method of compound 8, Step D.

Step F: 1-(4-(7-(5-methyl-1H-indazol-4-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazin-1-yl)prop-2-en-1-one: Prepared according to the method of compound 8, Step E. LC (ESI+APCI) MS m/z 404.2 $[M+H]^+$. HRMS mz calculated for $C_{22}H_{25}N_7O$ $[M+H]^+$ 404.2193, Found 404.2182.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.68 (s, 1H), 8.17 (s, 1H), 7.33(s, 2H), 6.57 (dd, J = 18.0, 10.6 Hz, 1H), 6.37 (dd, J = 16.8, 1.6 Hz, 1H), 5.83 (dd, J =10.6, 1.6 Hz, 1H), 4.53 (br s, 2H), 4.00 (br s, 4H), 3.90 (br s, 2H), 3.82 (br s, 2H), 3.54 (br s, 2H), 2.95 (br s, 2H), 2.39 (s, 3H).

Compound 6

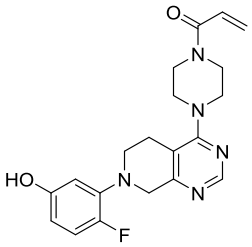


1-(4-(7-(2-fluoro-6-hydroxyphenyl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazin-1-yl)prop-2-en-1-one

Synthesized according to the method of compound 8 using 2-bromo-1-fluoro-3-(methoxymethoxy)benzene in place of 1-bromo-3-(methoxymethoxy)naphthalene in Step C. LC (ESI+APCI) MS m/z 384.2 $[M+H]^+$. HRMS mz calculated for $C_{20}H_{22}N_5O_2F$ $[M+H]^+$ 384.1830, Found 384.1824.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.47 (s, 1H), 6.99-6.93 (m, 1H), 6.63 (d, J = 8.2 Hz, 1H), 6.54 – 6.47 (m, 2 H), 6.25 (dd, J = 16.8, 0.9 Hz, 1H), 5.72 (dd, J = 10.7, 0.8 Hz, 1H), 4.32 (br s, 2H), 3.92 (br s, 4h), 3.73 (br s, 4H), 3.25 (br s, 2H), 2.85 (br s, 2h).

Compound 7

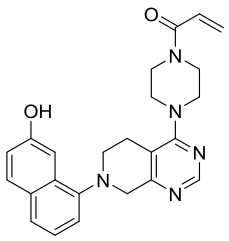


1-(4-(7-(2-fluoro-5-hydroxyphenyl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazin-1-yl)prop-2-en-1-one

Synthesized according to the method of compound 8 using 2-bromo-1-fluoro-4-(methoxymethoxy)benzene in place of 1-bromo-3-(methoxymethoxy)naphthalene in Step C. LC (ESI+APCI) MS m/z 384.2 $[M+H]^+$. HRMS mz calculated for $C_{20}H_{22}N_5O_2F$ $[M+H]^+$ 384.1830, Found 384.1831.

^{1}H NMR (CD_3OD, 400 MHz) δ 8.45 (s, 1H), 6.84 (dd, J = 12.3, 8.7 Hz, 1H), 6.57 (dd, J = 16.8, 10.9 Hz, 1H), 6.45 (dd, J = 7.3, 2.7 Hz, 1H), 6.35 (dt, J = 8.3, 3.5Hz, 1H), 6.23 (dd, J = 16.9, 1.6 Hz, 1H), 5.73 (dd, J = 10.6, 1.9 Hz, 1H), 4.14 (s, 2H), 3.73 (br s, 2H), 3.65 (br s. 2H), 3.55 (br s, 4H), 3.29 (2H, under methanol signal), 2.79 (t, J = 5.1Hz, 2H).

Compound 9

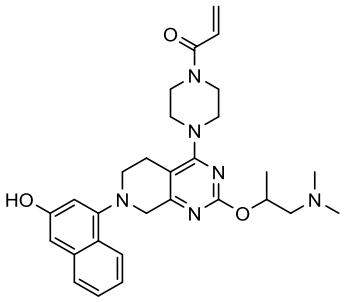


1-(4-(7-(7-hydroxynaphthalen-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazin-1-yl)prop-2-en-1-one

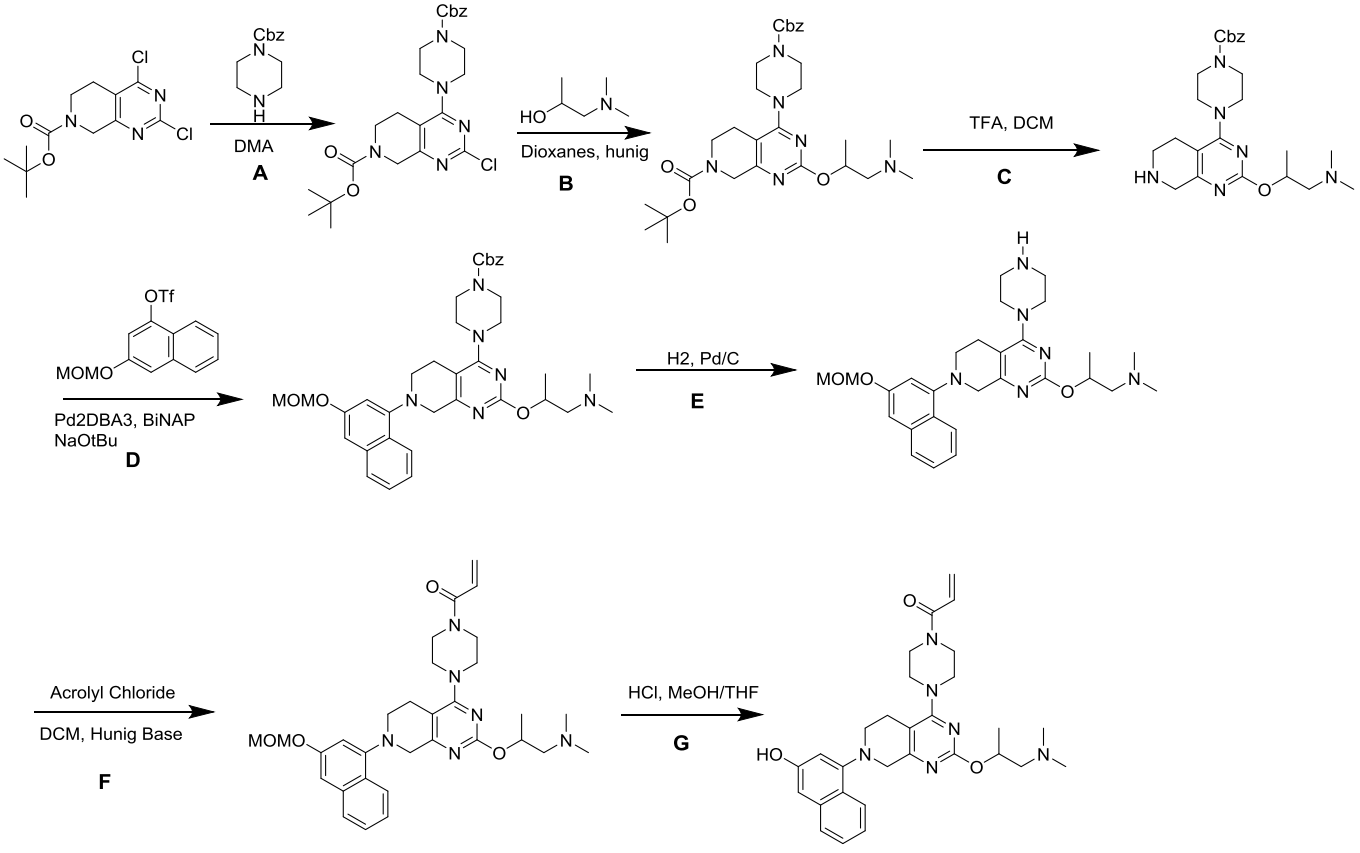
Synthesized according to the method of compound 8 using 1-bromo-7-(methoxymethoxy)naphthalene in place of 1-bromo-3-(methoxymethoxy)naphthalene in Step C. LC (ESI+APCI) MS m/z 416.1 $[M+H]^+$. HRMS mz calculated for $C_{24}H_{25}N_5O_2$ $[M+H]^+$ 416.2081, Found 416.2065.

^{1}H NMR (DMSO d_6, 400 MHz) δ 9.66 (s, 1H), 8.50 (s, 1H), 7.73 (d, J = 9.0 Hz, 1H), 7.49 (d, J = 7.8 Hz, 1H), 7.41 (d, J = 2.7 Hz, 1H), 7.19 (t, J = 8.1 Hz, 1H), 7.12 (d, J = 7.4 Hz, 1H), 7.01 (dd, J = 8.7, 2.7 Hz, 1H), 6.81 (dd, J = 16.6 , 10.2 Hz, 1H), 6.12 (dd, J = 16.7, 2.3 Hz, 1H), 5.70 (dd, J = 10.1, 2.3 Hz, 1H), 4.12 (s, 2H), 3.68 (br s, 4H), 3.48 (br s, 4H), 3.2 (br s, 2H), 2.95 (br s, 2H).

Compound 12



(S)-1-(4-(2-((1-(dimethylamino)propan-2-yl)oxy)-7-(3-hydroxynaphthalen-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazin-1-yl)prop-2-en-1-one



Step A: Benzyl 1-piperazinecarboxylate (1.3 mL, 6.6 mmol) and tert-Butyl 2,4-dichloro-5,6-dihydropyrido[3 4-d]pyrimidine-7(8H)-carboxylate (2 g, 6.6 mmol) were dissolved in dimethyl acetamide (10 mL) and treated with N-ethyl-N-isopropylpropan-2-amine (3.4 mL, 18 mmol). The reaction mixture was stirred at 85 °C for 2 hours. The reaction mixture was cooled to room temperature, diluted with ethyl acetate, washed with water and brine, dried over $MgSO_4$, filtered

and concentrated. The concentrate was purified by chromatography (CombiFlash®, 0%-50% ethyl acetate:Hexanes as the eluent to provide the product (2.7g, 83%). LC (ESI+APCI) MS mz calculated for $C_{24}H_{30}N_5O_4Cl$ $[M+H]^+$ Calculated: 488.2, Found 488.2

Step B: To a solution of tert-butyl 4-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-chloro-5,8-dihydropyrido[3,4-d]pyrimidine-7(6H)-carboxylate (2.0 g, 4.1 mmol) in dioxanes was added 1-(dimethylamino)propan-2-ol (8.5 g, 82 mmol) followed by N-ethyl-N-isopropylpropan-2-amine (2.6 g, 20 mmol) and the reaction stirred at 100^{o}C for ON. The reaction was next poured into water and extracted with EtOAc. The water was extracted 1 more time with EtOAc. The combined organics were washed with water, brine, dried over $MgSO_4$ and concentrated in vacuo. The material was next purified using 0→10% MeOH/DCM as eluent to give tert-butyl 4-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((1-(dimethylamino)propan-2-yl)oxy)-5,8-dihydropyrido[3,4-d]pyrimidine-7(6H)-carboxylate (1.2 g, 2.2 mmol, 53 % yield). LC (ESI+APCI) MS mz calculated for $C_{25}H_{42}N_6O_5$ $[M+H]^+$ Calculated: 555.3, Found 555.3

Step C: To a solution of tert-butyl 4-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((1-(dimethylamino)propan-2-yl)oxy)-5,8-dihydropyrido[3,4-d]pyrimidine-7(6H)-carboxylate (1.2 g, 2.2 mmol) in DCM was added 2,2,2-trifluoroacetic acid (4.9 g, 43 mmol) and the reaction stirred for 1 hr at rt. The reaction was next concentrated in vacuo and the residue partitioned between EtOAc and 1N NaOH. The organics were separated and the organics washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The material was used crude in the next reaction.

Step D: To a vial was added Tris(dibenzylideneacetone)dipalladium (0) (17 mg, 0.019 mmol), racemic-2,2'-Bis(diphenylphosphino)-1,1'-binaphthyl (24 mg, 0.039 mmol) and toluene (800 µl). Ar was bubbled through the mixture for 5 minutes and then the vial was capped and the mixture was heated to 100 °C for 15 minutes. The mixture was cooled to ambient temperature and then sodium t-butoxide (46 mg, 0.48 mmol) was added followed by 3-(methoxymethoxy)naphthalen-1-yl trifluoromethanesulfonate (80 mg, 0.24 mmol) and benzyl 4-(2-((1-(dimethylamino)propan-2-yl)oxy)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazine-1-carboxylate (110 mg, 0.24mmol). The vial was then capped again and the mixture was heated to 100 °C where it stirred for 18 hours. The mixture was then cooled and concentrated. The material was purified by silica gel (isolera, 0-12% MeOH in DCM to provide benzyl 4-(2-((1-(dimethylamino)propan-2-yl)oxy)-7-(3-(methoxymethoxy)naphthalen-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-

yl)piperazine-1-carboxylate (51 mg, 0.08 mmol, 34% yield). LC (ESI+APCI) MS mz calculated for $C_{36}H_{44}N_6O_5$ $[M+H]^+$ Calculated: 641.3, Found 641.3

Step E: To a solution of benzyl 4-(2-((1-(dimethylamino)propan-2-yl)oxy)-7-(3-(methoxymethoxy)naphthalen-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazine-1-carboxylate (51 mg, 0.080 mmol) in EtOH (800 µl) and THF (800 µl) was added Palladium (85 mg, 0.040 mmol) (Degussa Type, 10 wt%, 50% H_2O) and then an atmosphere of H_2 was introduced via vacuum followed by balloon pressure. The mixture was then stirred at ambient temperature for 3 hours. The mixture was then diluted with MeOH and filtered through GF/F paper. The colorless filtrate was concentrated to provide 2-((7-(3-(methoxymethoxy)naphthalen-1-yl)-4-(piperazin-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-2-yl)oxy)-N,N-dimethylpropan-1-amine (39 mg, 0.077 mmol, 97 % yield) which was used crude in the next reaction.

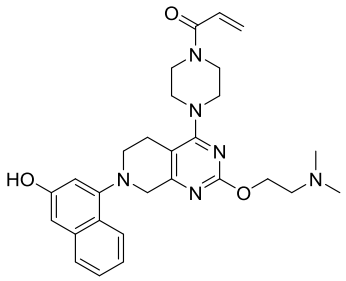
Step F: To a suspension of 2-((7-(3-(methoxymethoxy)naphthalen-1-yl)-4-(piperazin-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-2-yl)oxy)-N,N-dimethylpropan-1-amine (39 mg, 0.077 mmol) in CH_2Cl_2 (800µl) at ambient temperature was added acryloyl chloride (920 µl, 0.092 mmol) (freshly prepared 0.1M solution in DCM) followed by Triethylamine (21 µl, 0.15 mmol). The reaction was then stirred at ambient temperature for 20 min. The mixture was then concentrated and the product was then purified via column chromtography (Biotage Isolera, 12G Isco RediSep, 0-15% MeOH in DCM) to afford 1-(4-(2-((1-(dimethylamino)propan-2-yl)oxy)-7-(3-(methoxymethoxy)naphthalen-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazin-1-yl)prop-2-en-1-one (31 mg, 0.055 mmol, 72 % yield). LC (ESI+APCI) MS mz calculated for $C_{31}H_{40}N_6O_4$ $[M+H]^+$ Calculated: 561.3, Found 561.3

Step G: To a stirred solution of 1-(4-(2-((1-(dimethylamino)propan-2-yl)oxy)-7-(3-(methoxymethoxy)naphthalen-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazin-1-yl)prop-2-en-1-one (31 mg, 0.055 mmol) in 350 uL of methanol with a few drops of THF to aid solubility in a capped reaction vial was added HCl (230 µl, 1.38 mmol) (6M aqueous). The mixture was heated to 55 °C for 3 hr. The reaction was cooled and the reaction was concentrated. Saturated Bicarbonate solution was added and the reaction was extracted with 10% MeOH in DCM (3x 10 ml). The organic layers were combined and concentrated. The residue was purified by silica gel (isolera, 2-20% MeOH in DCM with 1%NH_4OH) to provide 1-

(4-(2-((1-(dimethylamino)propan-2-yl)oxy)-7-(3-hydroxynaphthalen-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazin-1-yl)prop-2-en-1-one (16 mg, 0.031 mmol, 56% yield) LC (ESI+APCI) MS m/z 517.3 $[M+H]^+$. HRMS mz calculated for $C_{29}H_{36}N_6O_3$ $[M+H]^+$ 517.2922, Found 517.2924.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.9 (d, J = 8.2 Hz, 1H), 7.54 (d, J = 8.2 Hz, 1H), 7.34 (t, J = 7.0 Hz, 1H), 7.25 (m, 1H), 6.73 (s, 1H), 6.57-6.49 (m, 2H), 6.32 (d, J =18.6 Hz, 1H), 5.73 (d, J = 9.7 Hz, 1H), 5.44 (m, 1H), 4.01 (s, 2H), 3.62 (br s, 2H), 3.47 (br s, 2H), 3.31 (br s, 5 H), 3.16 (br s, 2H), 2.84 (dd, J = 12.5, 9.0 Hz, 1H), 2.58 (br s, 2H), 2.42 (m, 6H), 1.32 (d, J = 5.8 Hz, 3H).

Compound 10

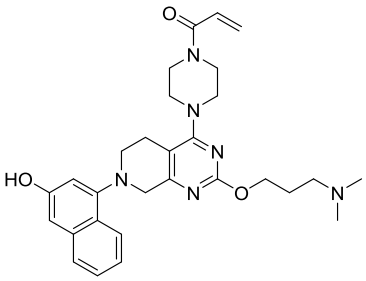


1-(4-(2-(2-(dimethylamino)ethoxy)-7-(3-hydroxynaphthalen-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazin-1-yl)prop-2-en-1-one

Synthesized according to the method of Compound 12, using 2-(dimethylamino)ethan-1-ol in place of 1-(dimethylamino)propan-2-ol (8.5 g, 82 mmol) in Step B. LC (ESI+APCI) MS m/z 503.2 $[M+H]^+$. HRMS mz calculated for $C_{28}H_{34}N_6O_3$ $[M+H]^+$ 503.2765, Found 503.2763

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.98 (d, J = 8.2 Hz, 1H), 7.60 (d, J =7.8 Hz, 1H), 7.36 (t, J = 6.7 Hz, 1H), 7.28-7.26 (m, 1H), 6.81 (s, 1H), 6.65 (s, 1H), 6.55 (dd, J = 17.2, 10.6 Hz, 1H), 6.32 (d, J = 16.8 Hz, 1H), 5.73 (d, J = 10.6 Hz, 1H), 4.45 (t, J = 5.9 Hz, 2H), 4.11 (s, 2H), 3.70 (br s, 2H), 3.55 (br s, 2H), 3.40 (br s, 4H), 3.25 (br s, 2H), 2.78 (t, J = 5.5 Hz, 2H), 2.70 (br s, 2H), 2.38 (s, 6H).

Compound 11



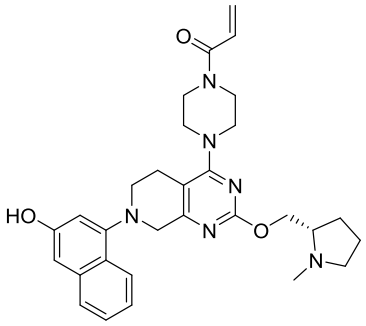
1-(4-(2-(3-(dimethylamino)propoxy)-7-(3-hydroxynaphthalen-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazin-1-yl)prop-2-en-1-one

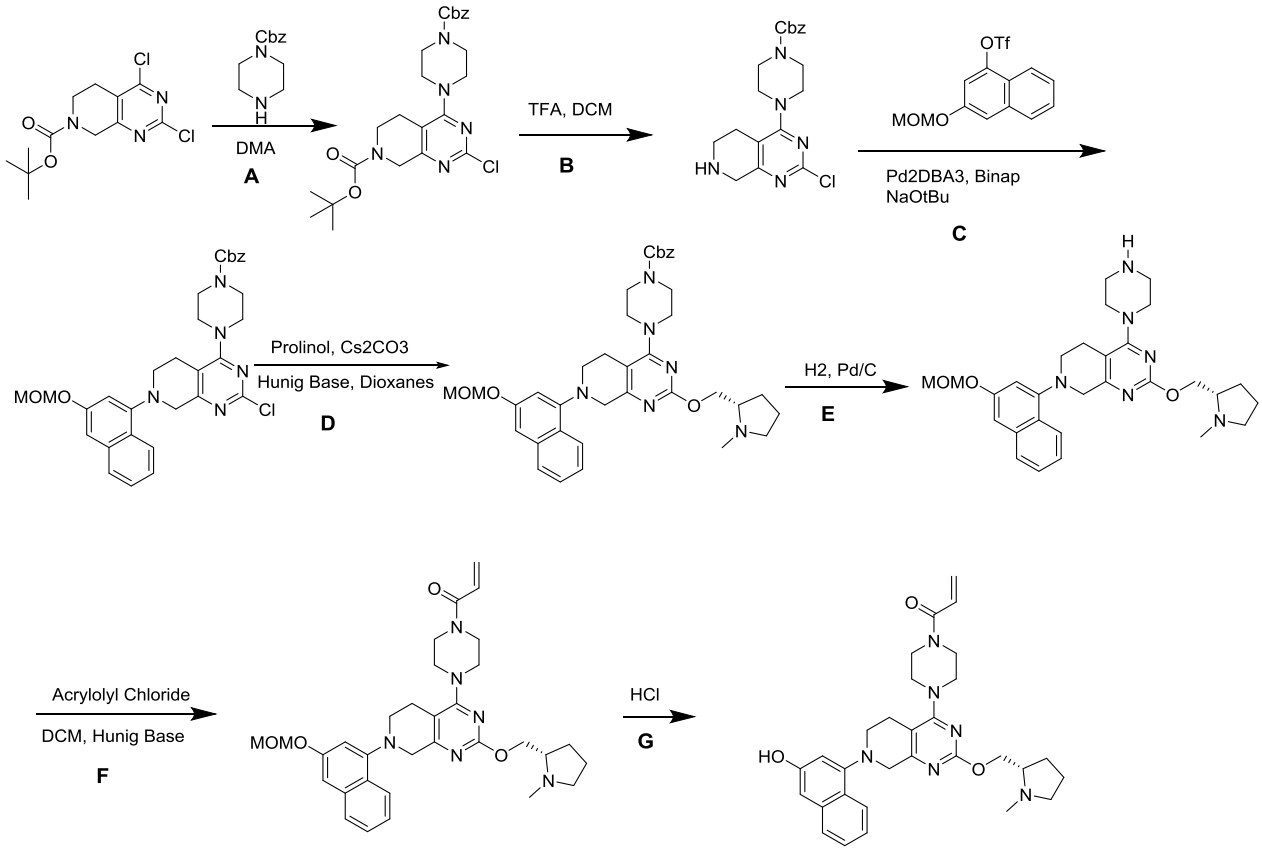
Synthesized according to the method of Compound 12, using 3-(dimethylamino)propan-1-ol in place of 1-(dimethylamino)propan-2-ol (8.5 g, 82 mmol) in Step B. LC (ESI+APCI) MS m/z 517.3 $[M+H]^+$. HRMS mz calculated for $C_{29}H_{36}N_6O_3$ $[M+H]^+$ 517.2922, Found 517.2912

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.98 (d, J = 8.6 Hz, 1H), 7.58 (d, J = 7.8 Hz, 1H), 7.35 – 7.31 (m, 1H), 7.24 -7.21 (m, 1H), 6.94 (d, J = 2.0 Hz, 1H), 6.80 (d, J = 2.0 Hz, 1H), 6.56 (dd, J = 16.4, 10.6 Hz, 1H), 6.32 (dd, J = 16.8, 2.0 Hz, 1H), 5.72 (dd, J =10.8, 1.6 Hz, 1H), 4.34 (t, J = 5.9 Hz, 2H), 4.14 (s, 2H), 3.76 (s, 2H), 3.65(s, 2H), 3.48 (br s, 4H), 3.27 (s, 2H), 2.82 (t, J = 7.4 Hz, 2H), 2.73 (br s, 2H), 2.48 (s, 6H), 2.17 – 2.11 (m, 2H).

Compound 13

(S)-7-(3-(methoxymethoxy)naphthalen-1-yl)-2-((1-methylpyrrolidin-2-yl)methoxy)-4-(piperazin-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidine





Step A: To a solution of tert-butyl 2,4-dichloro-5,8-dihydropyrido[3,4-d]pyrimidine-7(6H)-carboxylate (8.0 g, 26 mmol) in DMA (260 ml) was added benzyl piperazine-1-carboxylate (5.8 g, 26 mmol) and N-ethyl-N-isopropylpropan-2-amine (4.7 ml, 26 mmol) and the reaction stirred at room temperature for 2 hours. TLC (20% EtOAc/DCM), UV visualization, showed reaction completion. The reaction was next poured into water and extracted into DCM. The organics were next washed with water (2x), brine, dried over $MgSO_4$ and concentrated in vacuo. The concentrate was loaded onto a 220g RegiSep column and chromatagraphed on the CombiFlash (0%-10%, EtOAc:DCM). All fractions containing desired product were combined and concentrated to give tert-butyl 4-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-chloro-5,8-dihydropyrido[3,4-d]pyrimidine-7(6H)-carboxylate (9.8g, 20mmol, 76% yield) as a white foam. LC (ESI+APCI) MS mz calculated for $C_{24}H_{30}N_5O_4Cl$ $[M+H]^+$ Calculated: 488.2, Found 488.2

Step B: Tert-butyl 4-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-chloro-5,8-dihydropyrido[3,4-d]pyrimidine-7(6H)-carboxylate (9.8 g, 20 mmol) was dissolved in dichloromethane (200 ml) and treated with 2,2,2-trifluoroacetic acid (15 ml, 200 mmol). The reaction mixture stirred at

room temp for 4 hours. After completion the reaction was next concentrated in vacuo and taken up in EtOAc and the organics washed with 1M NaOH (2X), brine, dried over $MgSO_4$ and concentrated in vacuo. Benzyl 4-(2-chloro-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazine-1-carboxylate (7.4 g, 19 mmol, 95% yield) was used crude in the next reaction.

Step C: To BINAP (0.3 g, 0.4 mmol) and Pd2(dba)3 (0.2 g, 0.2 mmol) under argon was added toluene (220 ml) and the reaction bubbled with Argon for 10 minutes followed by heating to 100°C for 10 minutes. The reaction was cooled to room temperature and benzyl 4-(2-chloro-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazine-1-carboxylate (4.3 g, 11mmol) and Sodium Tert-Butoxide (2.1 g, 22 mmol) were added to the dark solution as solids. Finally, 3-(methoxymethoxy)naphthalen-1-yl trifluoromethanesulfonate (7.4 g, 22 mmol) was added (as the oil) and the reaction heated to 100°C for 1 hour. The reaction was cooled to room temperature and concentrated in vacuo. The residue was dissolved in EtOAc and the organics washed with water and brine. The combined organics were dried over Na_2SO_4 and concentrated in vacuo. The residue was then loaded on the CombiFlash and chromatographed using 0%-->50% EtOAc/Hexanes as eluent. Fractions containing clean product were combined and concentrated in vacuo to afford benzyl 4-(2-chloro-7-(3-(methoxymethoxy)naphthalen-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazine-1-carboxylate (2.6 g, 4.5 mmol, 41% yield). LC (ESI+APCI) MS mz calculated for $C_{31}H_{32}N_5O_4Cl$ $[M+H]^+$ Calculated: 574.2, Found 574.2

Step D: In a microwave tube benzyl 4-(2-chloro-7-(3-(methoxymethoxy)naphthalen-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazine-1-carboxylate (300 mg, 0.5 mmol) was dissolved in Dioxane (7 mL) and treated with cesium carbonate (510 mg, 1.6 mmol), Hunig's base (900 µl, 5 mmol) and N-Methyl-L-prolinol (96% purity) (420 mg, 3.7 mmol). The tube was then capped and microwaved at 170°C for 3 hours. The reaction was filtered through GF/F paper. The filtrate was concentrated in vacuo and the residue loaded onto a 12g RegiSep gold column and chromatagraphed on the CombiFlash (0%-15%, DCM:MeOH). All fractions contianing clean product were combined and concentrated in vacuo to give benzyl (S)-4-(7-(3-(methoxymethoxy)naphthalen-1-yl)-2-((1-methylpyrrolidin-2-yl)methoxy)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazine-1-carboxylate (220 mg, 0.34 mmol, 65% yield). LC (ESI+APCI) MS mz calculated for $C_{37}H_{44}N_6O_4$ $[M+H]^+$ Calculated: 653.3, Found 653.3

Step E**:** A solution of benzyl (S)-4-(7-(3-(methoxymethoxy)naphthalen-1-yl)-2-((1-methylpyrrolidin-2-yl)methoxy)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazine-1-carboxylate (220 mg, 0.34 mmol) in EtOH (3.4 mL) and THF (3.4 mL) was purged with N_2 for 5 minutes. To this solution was added Palladium on carbon (180 mg, 0.084 mmol) (Degussa Type, 10 wt%, 50% H2O) and was immediately capped and purged with N_2 for an additional 5 min. The solution was stirred under H_2 introduced via vacuum followed by balloon pressure. The mixture was then stirred at ambient temperature over night. The mixture was diluted with MeOH and filtered through packed celite. The filtrate was then concentrated in vacuo. (S)-7-(3-(methoxymethoxy)naphthalen-1-yl)-2-((1-methylpyrrolidin-2-yl)methoxy)-4-(piperazin-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidine (91 mg, 0.18 mmol, 52% yield) was used crude in the next reaction.

Step F: To a suspension of (S)-7-(3-(methoxymethoxy)naphthalen-1-yl)-2-((1-methylpyrrolidin-2-yl)methoxy)-4-(piperazin-1-yl)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidine (92 mg, 0.18 mmol) in dichloromethane (2 mL) at ambient temperature was added acryloyl chloride (1.8 mL of a 0.1 M solution in DCM) followed by Hunig's base (62 µl, 0.35 mmol). The reaction was then stirred at ambient temperature for 1 hour. The mixture was then concentrated and loaded onto a 4g RegiSep gold column and chromatagraphed on the CombiFlash (0%-15%, DCM:MeOH). All fractions containing clean product were combined and concentrated in vacuo to give (S)-1-(4-(7-(3-(methoxymethoxy)naphthalen-1-yl)-2-((1-methylpyrrolidin-2-yl)methoxy)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazin-1-yl)prop-2-en-1-one (74 mg, 0.13 mmol, 73% yield). LC (ESI+APCI) MS mz calculated for $C_{32}H_{40}N_6O_4$ $[M+H]^+$ Calculated: 573.3, Found 573.3

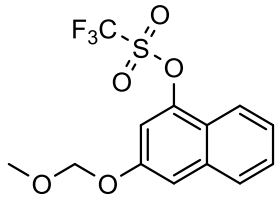
Step G: (S)-1-(4-(7-(3-(methoxymethoxy)naphthalen-1-yl)-2-((1-methylpyrrolidin-2-yl)methoxy)-5,6,7,8-tetrahydropyrido[3,4-d]pyrimidin-4-yl)piperazin-1-yl)prop-2-en-1-one (74 mg, 0.13 mmol) was dissolved in methanol (4 mL) and treated with hydrogen chloride (1 mL) (aq). The reaction was stirred at 55°C for 1 hour. The reaction mixture was concentrated in vaccuo and was resuspended in 1.5mL of MeOH. The suspension was loaded on to the Gilson (prep HPLC), which was eluted with 5-->95% ACN/0.1% TFA in water/0.1% TFA. All fractions containing clean product were combined and lyophilized overnight to give (S)-1-(4-(7-(3-hydroxynaphthalen-1-yl)-2-((1-methylpyrrolidin-2-yl)methoxy)-5,6,7,8-tetrahydropyrido[3,4-

d]pyrimidin-4-yl)piperazin-1-yl)prop-2-en-1-one (26 mg, 0.049 mmol, 38% yield). LC (ESI+APCI) MS m/z 529.3 $[M+H]^+$.HRMS mz calculated for $C_{30}H_{36}N_6O_3$ $[M+H]^+$ 529.2922, Found 529.2904.

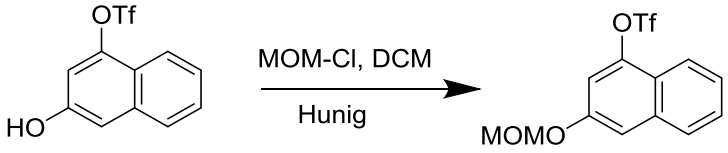
^{1}H NMR (freebase) (CD_3OD, 400 MHz) δ 8.04 (d, J = 8.5 Hz, 1H), 7.60 (d, J = 7.4 Hz, 1H), 7.36-7.32 (m, 1 H), 7.26 – 7.22 (m, 1H), 6.84 (d, J = 2.1 Hz, 1H), 6.82 – 6.74 (m, 2H), 6.23 (dd, J = 17.0, 2.0 Hz, 1H), 5.76 (dd, 10.5, 2.0 Hz, 1H), 4.38 - 4.26 (m, 2H), 4.11 (br s, 2H), 3.76 (br s, 4H), 3.63 (br s, 4H), 3.3 (m, 2H), 3.06 (quintet, J = 4.7 Hz, 1H), 2.92 (br s, 2 H), 2.72 – 2.69 (m, 1H), 2.48 (s, 3H), 2.33 (q, J = 9.0 Hz, 1H), 2.11 – 2.07 (m, 1H), 1.88 – 1.76 (m, 2 H). 1 proton missing under CD_3OD peak

^{13}C (freebase, CD_2Cl_2, 125 MHz) δ 22.1, 22.3, 27.0, 43.1, 46.4, 49.5, 57.0, 57.7, 60.7, 63.3, 73.1, 108.5, 111.9, 122.1, 123.6, 124.8, 126.3, 126.8, 127.2, 127.6, 131.1, 136.7, 153.1, 155.8, 161.2, 162.7, 164.9, 169.4.

Intermediate 1



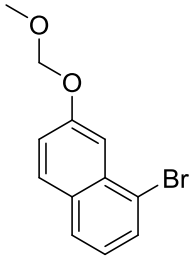
3-(methoxymethoxy)naphthalen-1-yl trifluoromethanesulfonate



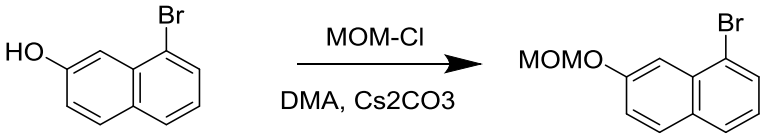
3-Hydroxynaphthalen-1-yl trifluoromethanesulfonate (13 g, 45 mmol) was dissolved in dichloromethane (100 mL) and stirred at 0 °C. To this solution was added chloro(methoxy)methane (3.7 ml, 49 mmol) and Hunig's base (12 mL, 67 mmol). The reaction was stirred at 0 °C for 4 hrs. The reaction was partitioned with 1M HCl, the layers separated and the organics washed with saturated sodium bicarbonate. The organics were dried over magnesium sulfate and concentrated under vacuum. The concentrated material was loaded onto a 120 g RediSep® gold silica gel column with dichloromethane and purified by normal phase chromatography (CombiFlash®, 0%-20% ethyl acetate/hexanes as the eluent) to give 3-(methoxymethoxy)naphthalen-1-yl trifluoromethanesulfonate (11.8 g, 78 % yield). ^{1}H NMR

($CDCl_3$, 400 MHz) δ 7.98 (d, J = 8.2 Hz, 1H), 7.78 (d, J= 7.4 Hz, 1H), 7.55 – 7.46 (m, 2H), 7.44 (d, J = 1.8 Hz, 1H), 7.24 (d, 2.4 Hz, 1H), 5.28 (s, 2H), 3.52 (s, 3H).

Intermediate 2

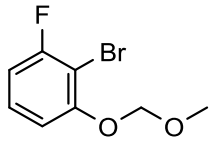


2-bromo-7-(methoxymethoxy)naphthalene

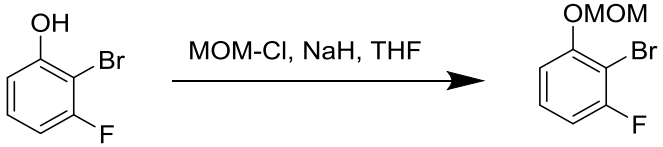


To a solution of 8-bromonaphthalen-2-ol (1.0 g, 4.5 mmol) in DMA (20 mL) was added chloro(methoxy)methane (0.51 g, 5.4 mmol) and Cs_2CO_3 (1.5 g, 4.5 mmol) and the reaction stirred ON at rt. The reatcion was next diluted with EtOAc and the organics washed with water (2x), brine, dried over MgSO4 and concentrated in vacuo. The material was chromatographed using 5→25% EtOAc/Hex as eluent to give 1-bromo-7-(methoxymethoxy)naphthalene (0.40 g, 1.5 mmol, 33 % yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.76 – 7.71 (m, 4H), 7. 26 (dd, J = 9.0, 2.3 Hz, 1H) 7.18 (dd, J = 8.2, 7.4 Hz, 1H), 5.33 (s, 2H), 3.53 (s, 3H).

Intermediate 3

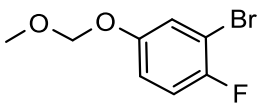


2-bromo-1-fluoro-3-(methoxymethoxy)benzene

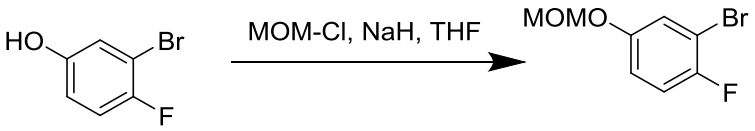


To a stirred solution of 2-bromo-3-fluorophenol (1400 mg, 7.4 mmol) in 22 mL tetrahydrofuran at room temperature under nitrogen was added NaH (330 mg, 8.2 mmol) neat as a solid portion wise. After 15 minutes, a solution had formed. Chloro(methoxy)methane (680 µL, 8.9 mmol) was added by syringe. After stirring for 2 hours, the reaction was quenched with saturated ammonium chloride solution and then partitioned between ethyl acetate (30 mL) and water (30 mL). The organics were isolated, washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product was loaded in a minimum of dichloromethane onto a 40 gram RediSep® column pre-wet with hexanes and eluted with an ethyl acetate/hexanes gradient (0% to 20% ethyl acetate). Fractions containing the product were combined and concentrated to provide the product as a clear oil (1.45g, 83%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.22 – 7.16 (m, 1H), 6.92 (dt, J = 8.3, 1.3 Hz, 1H), 6.79 (td, J = 8.2, 1.1 Hz, 1H), 5.24 (s, 2H), 3.50 (s, 3H).

Intermediate 4



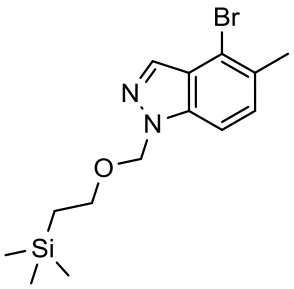
2-bromo-1-fluoro-4-(methoxymethoxy)benzene



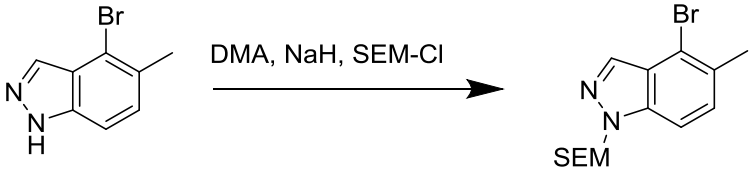
To a stirred solution of 3-bromo-4-fluorophenol (330mg, 1.7 mmol) in 5 mL tetrahydrofuran at room temperature under nitrogen was added NaH (75 mg, 1.9 mmol) neat as a solid portion wise. After 15 minutes, a solution had formed. Chloro(methoxy)methane (156 µL, 2.1 mmol) was added by syringe. After stirring for 2 hours, the reaction was quenched with saturated ammonium chloride solution and partitioned between ethyl acetate and water. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product was loaded in a minimum of dichloromethane onto a 24 gram RediSep® column pre-wet with hexanes and eluted with an ethyl acetate/hexanes gradient (0% to 20% ethyl acetate). Fractions containing the product were combined and concentrated to provide the product as a

clear oil (120 mg, 30%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.24 (dd, J = 5.6, 2.7 Hz, 1H), 7.01 (dd, J = 8.6, 8.2 Hz, 1H), 6.94-6.90 (m, 1H), 5.09 (s, 2H), 3.45 (s, 3H).

Intermediate 5

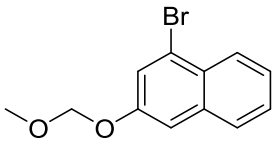


4-bromo-5-methyl-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-indazole



To a solution of 4-bromo-5-methyl-1H-indazole (0.7 g, 3.3 mmol) in dimethyl acetamide (30 mL) cooled to 0 °C was added NaH (0.19 g, 4.6 mmol) in portions and the reaction mixture was purged with nitrogen. The reaction was stirred for 20 minutes, and then (2-(chloromethoxy)ethyl)trimethylsilane (0.83 g, 5.0 mmol) was added and the reaction was stirred for 2 hours while warming to room temperature. The reaction was quenched by pouring into water and the aqueous layer was extracted into ethyl acetate. The combined organic layers were washed with water and brine, dried over $MgSO_4$ and concentrated under vacuum. The crude material was purified by chromatography using 10-50% ethyl acetate/hexanes as the eluent to give 4-bromo-5-methyl-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-indazole (0.87 g, 79%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.02 (d, J = 0.8 Hz, 1H), 7.55 (d, J = 8.6 Hz, 1H), 7.14 (d, J = 8.8 Hz, 1H), 5.67 (s, 2H), 3.62-3.58 (m, 2H), 2.44 (s, 3H), 0.97-0.90 (m, 2H), -0.04 (s, 9H).

Intermediate 6



1-bromo-3-(methoxymethoxy)naphthalene

To a RBF was added THF (2ml) followed by NaH, 60 % dispersion in mineral oil (54 mg, 1.3 mmol). The mixture was cooled to 0 °C then 4-Bromo-2-naphthol (0.25 g, 1.1 mmol) was added portionwise. Once the bubbling had ceased the resulting dark mixture was stirred at 0 °C for 30 min. Then chloro(methoxy)methane (0.094 ml, 1.2 mmol) was added and the mixture was warmed to ambient temperature where it was stirred for 3 hr. A saturated aqueous NH_4Cl solution was added and the mixture was extracted with DCM. The organic layer was dried over Na_2SO_4, filtered and concentrated. The resulting crude residue was purified by silica gel (5-10% EtOAc in hex) to provide the product as a red oil (0.22g, 72%). ^1H NMR ($CDCl_3$, 400 MHz) δ 8.18-814 (m, 1H), 7.74-7.70 (m, 1H), 7.61 (d, J = 2.3 Hz, 1H), 7.50 – 7.44 (m, 2H), 7.40 (d, J = 2.4Hz, 1H), 5.27 (s, 2H), 3.53 (s, 3H).

KRAS LCMS Modification Assay Procedure (POC Assay):

The protein concentration was adjusted to 2 µM in Assay Buffer (25 mM HEPES,150 mM NaCl, 5 mM $MgCl_2$, 10 mM Octyl β-glucopyranoside at pH 7.5). Typical final compound concentrations were 3.0, 5.0 and 25.0 µM in 20uL reactions. At each timepoint, the reactions were quenched with 20 µL of a 0.8% Formic Acid. Assay endpoints were 15, 180 and 1440 minutes. Once all reactions gad been quenched, plates were heat sealed and samples were injected into a LC/MS system for data acquisition.

Data collection took place on an Agilent 6520 Q-TOF Accurate Mass Spectrometer. Samples were injected in their liquid phase onto a C-3 reverse phase column to remove assay buffer and prepare samples for mass spectrometer. The proteins were eluted from the column using an acetonitrile gradient and fed directly into the mass analyzer. Initial raw data analysis took place in Agilent Masshunter software post data acquisition. Protein mass changes were determined by

a deconvolution of the multiple charge states of each protein using a maximum entropy deconvolution. The heights of all masses identified during raw data analysis were exported to be further analyzed in Spotfire data analysis software.

In Spotfire, each protein mass was calculated as a percent of the total signal of that sample, that percentage was then normalized to the percent signals seen in control samples the absence of compound. This normalized value was called the precent of control (POC). An increase in the POC value indicated an increase in the amount of modified protein. Because experimental samples are single reactions, the sample errors are calculated by applying total assay error for the control reactions to the POC values for each experimental sample (supplemental table X, X). The calculation is as follows:

$$Sample\ Calc.Error\ = \left(\frac{SD_{Control}}{Signal_{Control}}\right) \times Signal_{Sample}$$

Table A POC statistics for compounds in Table 1

Compound #	POC (%)	Calc. Error	Avg. Control Signal	Control Signal SD	Control Signal (n)
4	13.00	0.35	58.90	2.17	8
5	21.97	0.25	67.17	0.75	8
6	1.62	0.03	66.33	1.39	10
7	2.43	0.05	66.33	1.39	10
8	99.45	2.08	66.33	1.39	10
9	0.00	NA	70.99	2.96	12

Table B POC statistics for compounds in Table 2

Compound #	POC (%)	Calc. Error	Avg. Control Signal	Control Signal SD	Control Signal (n)
8	8.00	0.16	67.03	1.35	7
10	52.50	1.51	66.11	1.9	8
11	22.83	0.43	64.77	1.21	2
12	20.83	0.39	64.77	1.21	2
13	84.91	3.38	64.98	2.59	6

G12C Cell Assay

This general procedure was used for the H358 KRAS-G12C, MIA PaCa-2 KRAS-G12C, AGS KRAS-G12D, RKO KRAS-WILD TYPE and SNU-C5 KRAS-WILD TYPE cell assays. H358 and SNU-C5 cells were run in RPMI media and MIA PaCa-2, AGS and RKO cells were run in DMEM media. For assessment of cellular inhibition potency, cells were harvested according to a standard protocols, counted and added to flat-bottom 96-well assay plates (Greiner; Cat# 655946) at 5X104 cells/well in 100 µL/well of growth medium containing 10% FBS. Plates were then incubated at room temperature for 60 minutes prior to an overnight incubation at 37°C with 5% CO2. The following day, cells were treated for 3 hours at 37°C, 5% CO2 with compound, prepared as a 10-point, 1:3 dilution series with final DMSO concentration of 0.5%. Control wells contained either 0.5% DMSO alone (no inhibition control) or 1 µM Tremetinib (complete inhibition control). To determine levels of phosphorylated ERK the plates were tested using an In Cell Western protocol as follows. Following compound incubation, growth medium was discarded and cells were fixed with 4% formaldehyde in PBS for 20 minutes. Cells were washed with PBS, and permeabilized with 100% methanol for 10 minutes. Plates were washed with PBS containing 0.05% Tween-20 and subsequently blocked for 1 hour with LI-COR Blocking Buffer (LI-COR Biosciences; Cat# 927-40000). Plates were then incubated at room temperature for 2 hours with 50 ul of primary antibodies Phospho-ERK1/2 (Cell Signaling Technologies; Cat# 9101) and GAPDH (Millipore; Cat# MAB374) in LI-COR blocking buffer containing 0.05%Tween-20. Plates were washed with PBS containing 0.05% Tween-20 then incubated at room temperature for 1 hour with 50 ul of secondary antibodies anti-rabbit AlexaFluor 680 (Life Technologies; Cat# A21109) and anti-mouse IRDye 800CW (LI-COR; Cat# 926-32210) in LI-COR blocking buffer containing 0.05%Tween-20. Plates were analyzed by reading on an Aerius infrared scanner. For each well, the phospho-ERK 1/2 signal was normalized to the GAPDH signal. IC50 values were then calculated using a 4-parameter fit in BioAssay software.

Table C Cell assay statistics for compounds in the H358 assay

Compound	N	IC50 uM	S.dev
4	1	>16	
5	1	>16	
6	1	>16	
7	1	>16	

8	2	7.6	1.9
9	1	>16	
10	1	1.8	
11	2	1.5	0.6
12	2	0.53	0.012
13	2	0.07	0.012

Compound **13** was run in MIA PaCa-2 KRAS-G12C, AGS KRAS-G12D, RKO KRAS-WILD TYPE and SNU-C5 KRAS-WILD TYPE cell assays with an N=1 so there is no standard deviation.

Liver Microsomal Incubation

A 100 mM potassium phosphate assay buffer solution (KPB) was prepared as follows. Both KH2PO4 and K2HPO4 were dissolved separately in reagent grade water resulting in final concentrations 100 mM. A 75:25 mixture v/v of K2HPO4:KH2PO4 was prepared and the pH of the solution was adjusted to 7.4 using diluted HCl or diluted NaOH solutions. A stock solution of the test article(s) was prepared at 10 mM (active compound) in DMSO. The stock solution was diluted immediately before use to 2.5 µM using the KPB solution to create the working standard. All test compounds were completely soluble by visual inspection at room temperature. The NADPH-regenerating solution (NRS) was prepared on the day of analysis by diluting one volume of 17 mg/mL NADP+ with one volume of 78 mg/mL glucose 6 phosphate (both prepared in KPB, pH 7.4) and 7.9 volumes of 20 mM MgCl2. The final concentrations of NADP+ and glucose-6-phosphate were 1.7 mg/mL and 7.8 mg/mL, respectively. Immediately prior to use, the NRS was activated by the addition of 10 µL of glucose-6-phosphate dehydrogenase (150 Units/mL in KPB, pH 7.4) per mL of NRS stock solution. Liver microsomes were diluted to 2.5 mg protein/mL using KPB.

For each test article or positive control (i.e., dextromethorphan, diazepam, diltiazem, phenacetin, tolbutamide, and verapamil), 20 µL of 2.5 µM working standard solution of test compound and 20 µL of microsomes (2.5 mg protein/mL) were added to each well of a 96-well polypropylene plate (Costar, VWR, West Chester, PA) in duplicate. The plates were placed in an incubator at

37 °C for 5 minutes before adding the start solution. A 10-µL aliquot of the NRS solution was added to each original well to initiate metabolism. The concentration of the test compound during incubation was 1 µM. One incubation plate was prepared for each time point (i.e., 0 and 20 minutes). Incubations were conducted at 37 °C and 100% relative humidity. At each time point, the appropriate incubation plate was removed from the incubator and a solution containing internal standard (150 µL, 0.25 µM labetalol in 60% acetonitrile) was added to each well. The plate was immediately spun in a centrifuge at 2,095 x g for 7 minutes at room temperature using an Allegra benchtop centrifuge (Beckman Coulter, Fullerton, CA). A 200-µL aliquot of the supernatant was transferred from each well to a 96-well shallow plate (Costar). The plates were sealed using reusable plate mats.

Hepatocyte Incubations

A stock solution of the test article(s) was prepared at 10 mM (active compound) in DMSO. The in vitro stability of each test article or positive control was assessed in the presence of hepatocytes as follows: Cryopreserved hepatocytes were thawed, isolated from shipping media and diluted to a density of 1 x 106 viable cells/mL, according to the supplier's guidelines, using Dulbecco's Modified Eagle Medium, 1X, high glucose (DMEM, Invitrogen, Carlsbad, CA). Viability was determined by trypan blue exclusion using a hemocytometer (3500 Hausser, VWR, West Chester, PA). The 10 mM stock solution of test article(s) or control compound was diluted to 2 µM using supplemented DMEM to create the working standard. A 20-µL aliquot of test compound or control (antipyrine, diazepam, diltiazem, lorazepam, propranolol, verapamil, and 7-ethyl-10-hydroxycamptothecin [SN-38]) was added to each test well of a 96-well polypropylene plate (Costar, VWR, West Chester, PA) immediately followed by the addition of 20 µL of the hepatocyte suspension. One incubation plate was prepared for each time point (i.e., 0, 60 and 120 minutes) with samples being prepared in duplicate. Incubations were conducted at 37 °C and 100% relative humidity. At each time point, the appropriate incubation plate was removed from the incubator and a solution containing internal standard (200 µL, 0.25 µM labetalol in 60% acetonitrile) was added to each well. The plate was mixed at 700 rpm for 1 minute on a plate shaker (IKA MTS 2/4 Digital Microtiter Shaker, VWR) and immediately spun in a centrifuge at 2,095 x g for 10 minutes at room temperature using an Allegra benchtop centrifuge (Beckman

Coulter, Fullerton, CA). A 200-µL aliquot of the supernatant was transferred from each well to a 96-well shallow plate (Costar). The plates were sealed using reusable plate mats.

Analytical Quantitation Of Hepacyte and Microsomal Incubations

The LC-MS/MS system was comprised of an HTS-PAL autosampler (Leap Technologies, Carrboro, NC), an HP1200 HPLC (Agilent, Palo Alto, CA), and an API4000 triple quadrupole mass spectrometer (PE Sciex, a division of Applied Biosystems, Foster City, CA). Chromatographic separation of the analyte and internal standard was achieved at room temperature using a C18 column (Kinetex®, 30 x 3.0 mm, 2.6 µm particle size, Phenomenex, Torrance, CA) in conjunction with gradient conditions using mobile phases A (aqueous 0.1% formic acid with 1% isopropyl alcohol) and B (0.1% formic acid in acetonitrile). The total run time, including re-equilibration, for a single injection was 2 minutes. Mass spectrometric detection of the analytes was accomplished using the ESI+ ionization mode. Ion current was optimized during infusion of a stock solution of each test article. Analyte responses were measured by multiple reaction monitoring (MRM) of transitions unique to each compound.

Data were acquired and peak areas were calculated for test compounds and the internal standard using Analyst 1.6.2 software (Sciex). For the liver microsomal and hepatocyte stability assessments, peak area tables were exported to BioAssay Enterprise (CambridgeSoft, Cambridge, MA), where the average analyte to internal standard peak area ratios were used to calculate percent remaining (%REM), half-life (t1/2), predicted hepatic clearance (CLh) and predicted hepatic extraction ratio (ER).

Institutional Animal Care and Use Committee Statement

All mouse studies were conducted in compliance with all applicable regulations and guidelines of the Institutional Animal Care and Use Committee (IACUC) from the National Institutes of Health (NIH). Mice were maintained under pathogen-free conditions, and food and water was provided ad libitum.

Anti-Tumor Efficacy Study

6 – 8-week-old female athymic nude-Foxn1nu mice (Envigo, San Diego) were injected subcutaneously with tumor cells in 100 µL of PBS and Matrigel matrix in the right hind flank

with 5.0e6 cells (Corning #356237; Discovery Labware, MA) 50:50 cells:Matrigel. Mouse health was monitored daily, and caliper measurements began when tumors were palpable. Tumor volume measurements were determined utilizing the formula 0.5 x L x W^2 in which L refers to length and W refers to width of each tumor. When tumors reached an average tumor volume of ~150 mm^3, mice were randomized into treatment groups. Mice were treated by intraperitoneal injection with either vehicle consisting of 10% research grade Captisol® (CyDex Pharmaceuticals, KS) in 50 mM citrate buffer pH 5.0 or Compound **13** at indicated doses. Animals were administered Compound **13** or vehicle and monitored daily, tumors were measured 3 times per week and body weights were measured 2 times per week. Compound **13** was generally well tolerated and treatment did not result in any appreciable body weight loss over the duration of the study.

K-Ras G12C Engagement

A LCMS-based K-Ras G12C engagement assay was developed to quantitatively measure the interaction of an inhibitor with its intended protein target. The decrease of the cysteine 12-containing peptide from tryptic digests of K-Ras G12C-mutant tumors following compound treatment was quantified relative to a control peptide, representing total K-Ras. Tumor fragments were harvested from mouse xenograft models, transferred to 2-mL tubes containing Lysing Matrix A, and homogenized in 1 mL of lysis buffer (6 M guanidine-HCl, 50 mM HEPES, pH 7.5, 5 mM TCEP) with a FastPrep-24™ Instrument. Following centrifugation to remove particulate, the protein concentration of the supernatant was determined using a Bradford assay. Tumor lysates were normalized based on protein concentration by transferring a volume containing 200 µg to a clean 1.4 mL Matrix™ tube and adding lysis buffer to a total of 175 µL. The internal standard, $^{13}C^{15}N$ recombinant K-Ras G12C, was added (7.5 µL of a 4.8 µg/mL solution in lysis buffer). Cysteine residues were alkylated by adding 20 mM iodoacetamide (20 µL of a 200 mM solution in lysis buffer), and incubating at 37°C for 30 min in the dark. Following alkylation, 100 µL of the reaction was exchanged into 1 M guanidine-HCl, 50 mM HEPES, pH 7.5, using a 96-well Zeba™ spin plate. A trypsin/Lys-C mix (1 µg) was added to the tumor lysates, and the digest was allowed to proceed for 18 hr at 37°C. Peptides were desalted using a Strata-X 10 mg C18 96-well plate and a vacuum manifold and the solvent was removed by evaporation. Peptides were solubilized in 0.1% formic acid, 5% acetonitrile, 95% water, for

LCMS analysis. Samples were analyzed using a HPLC-MS system comprised of an Eksigent MicroLC 200 Plus System and a TripleTOF 6600 quadrupole time-of-flight mass spectrometer. The precursor ions and MS/MS fragment ions for K-Ras G12C peptides were optimized using recombinant K-Ras G12C. A targeted MS/MS method was created for the following four peptides using the 4 most intense fragment ions for each: LVVVGACGVGK light (529.8050^{+2}), LVVVGACGVGK heavy (557.8610^{+2}), DSEDVPMVLVGNK light (701.8478^{+2}), and DSEDVPMVLVGNK heavy (738.9245^{+2}). Chromatograms with 4 fragment ions for each peptide were integrated and the total fragment ion peak area for each light peptide was divided by the total fragment ion peak area for each heavy peptide (derived from recombinant K-Ras G12C internal standard). The K-Ras G12C engagement was calculated using the following equation: % Engagement = 100 * (1-{(^{Treated}KRAS-G12C$_{L/H}$)*(^{Vehicle} K-Ras-ALL$_{L/H}$/ ^{Treated} K-Ras-ALL$_{L/H}$)}/ ^{Vehicle} K-Ras-G12C$_{L/H}$). The ^{Vehicle} K-Ras-ALL light-to-heavy ratio was the mean of all vehicle control replicates for the DSEDVPMVLVGNK peptide, and the ^{Vehicle} K-Ras-G12C light-to-heavy ratio was the mean of all vehicle control replicates for the LVVVGACGVGK peptide. The individual % engagement values were calculated for all individual tumors from the vehicle and treated mice prior to calculation of the means and standard deviations for each group.