Supporting Information:

Discovery and Evaluation of Pyrazolo[3,4-*d*]pyridazinone as a Potent and Orally Active Irreversible BTK Inhibitor

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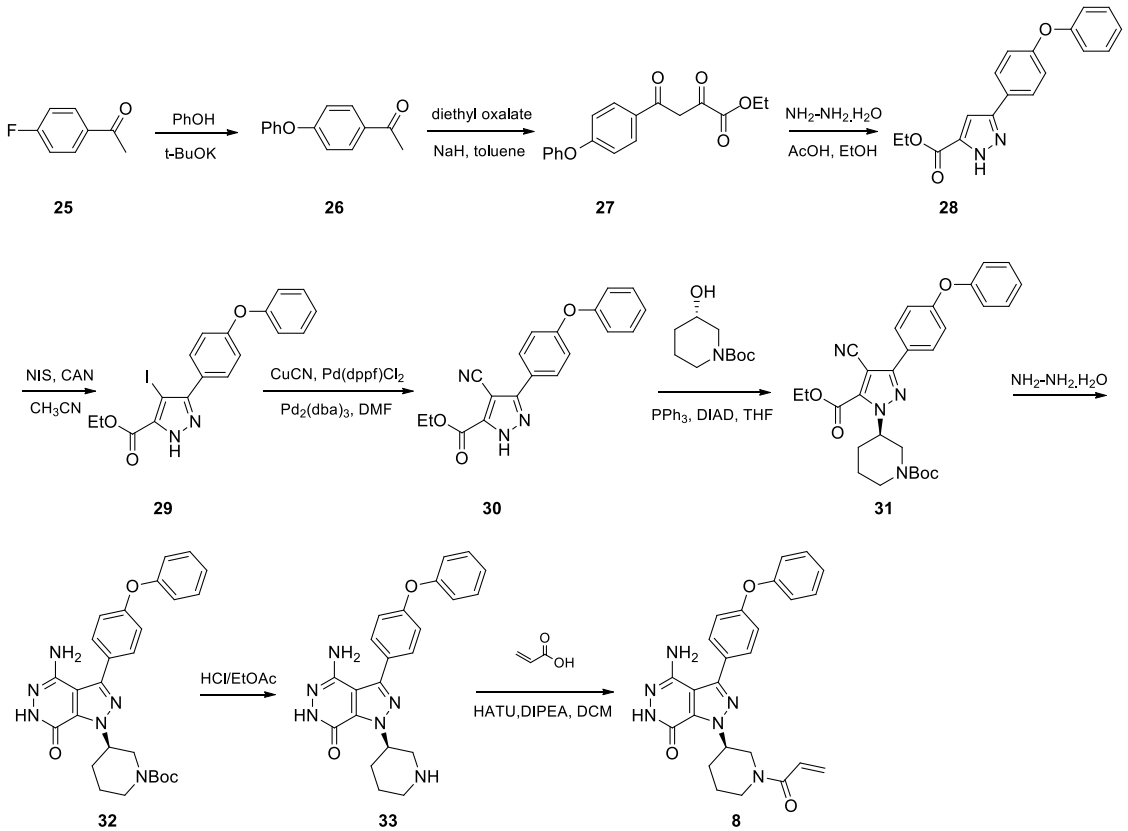
1. Chemistry Synthesis

General Information

Reagents and solvents were obtained from commercial suppliers and were used without further purification. ^{1}H NMR and ^{13}C NMR measurements were recorded at 400MHz using a Bruker AMX400 instrument. Preparative HPLC separations were performed on a Boston Green ODS 150*30*5u column using gradient mixtures of water / 0.1% TFA and water (10%) / acetonitrile (90%) / 0.1% TFA. Chromatographic purifications were performed silica gel (300-400 mesh). All of the compounds were established by a variety of LC/MS, and NMR analytical techniques, and purities were >95% for all final products. Ibrutinib was synthesized in-house, and the HPLC purity is 99%.

Procedures for Preparation of Compound 8:



1-(4-phenoxyphenyl)ethanone (**26**)

To a solution of 1-(4-fluorophenyl)ethanone (150 g, 1.087 mol) and phenol (153.2 g, 1.63 mol) in DMF (1500 mL) was added potassium tert-butoxide (243 g, 2.17 mol), the mixture was stirred at 100°C for 14 hours. After the reaction, water (100 mL) was added and extracted

with EtOAc (500 mL×4), dried over Na_2SO_4, filtered and concentrated, the residue was purified by column chromatography to give **compound 26** (171 g, 74%) as yellow solid. **^{1}H NMR (400MHz, MeOD)** δ 7.96 (d, *J* = 8.0 Hz, 2H), 7.43 (t, *J* = 16.0 Hz, 2H), 7.23 (t, *J* = 16.0 Hz, 1H), 7.09 (d, *J* = 8.0 Hz, 2H), 7.02 (d, *J* = 8.0 Hz, 2H), 2.58 (s, 3H).

ethyl 2,4-dioxo-4-(4-phenoxyphenyl)butanoate (27)

To a solution of **compound 26** (150 g, 0.707 mol) and diethyl oxalate (3.38 g, 10.2 mmol) in toluene (2000 mL) was added NaH (50 g, 1.25 mmol) in batches at 85°C, the mixture was stirred for 10 mins. After the reaction, Saturated citric acid solution was added to adjust pH=5, and extracted with EtOAc (1000 mL×4), dried over Na_2SO_4, filtered and concentrated, the residue was purified by column chromatography to give **compound 27** (135 g, 60%) as yellow solid. **^{1}H NMR (400MHz, MeOD)** δ 8.00 (d, *J* = 8.0 Hz, 2H), 7.44 (d, *J* = 16.0 Hz, 2H), 7.25 (t, *J* = 16.0 Hz, 1H), 7.10-7.03 (m, 4H), 4.43-4.38 (m, 2H), 1.43 (t, *J* = 12.0 Hz, 3H).

ethyl 3-(4-phenoxyphenyl)-1H-pyrazole-5-carboxylate (**28**)

To a solution of **compound 27** (100 g, 0.32 mol) in AcOH/EtOH (400 mL, 1:1) was added NH_2-NH_2·H_2O (48 g, 0.96 mol) dropwise, the reaction mixture was stirred for 30 mins. Water (1000 mL) was added and extracted with DCM (1000 mL×3), dried over Na_2SO_4, filtered and concentrated, the residue was purified by column chromatography to give **compound 28** (75 g, 76%) as yellow solid. **^{1}H NMR (400MHz, MeOD)** δ 7.74 (d, *J* = 8.0 Hz, 2H), 7.39 (t, *J* = 16.0 Hz, 2H), 7.16 (t, *J* = 16.0 Hz, 1H), 7.07-7.05 (m, 5H), 4.44-4.39 (m, 2H), 1.43 (t, *J* = 12.0 Hz, 3H).

ethyl 4-iodo-3-(4-phenoxyphenyl)-1H-pyrazole-5-carboxylate (**29**)

A mixture of **compound 28** (25.0 g, 81.2 mmol), NIS (18.3 g, 81.2 mmol), CAN (6.7 g, 12.2 mmol) in MeCN (500 mL) was heated to 80°C for 1 hour under N_2. LCMS shown starting material was completely consumed. The mixture was added water (30 mL) and extracted by EtOAc (1000 mL×2). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was washed with DCM to give **compound 29** (25.0 g, 71%) as white solid.. **^{1}H NMR (400MHz, $CDCl_3$)** δ 11.57 (br, 1H), 7.73-7.71 (m, 2H), 7.40-7.38 (m, 2H), 7.19-7.17 (m, 1H), 7.11-7.08 (m, 4H), 4.45-4.40 (m, 2H), 1.43 (t, *J* = 12.0 Hz, 3H).

ethyl 4-cyano-3-(4-phenoxyphenyl)-1H-pyrazole-5-carboxylate (**30**)

A mixture of **compound 29** (2.17 g, 5.0 mmol), CuCN (0.9 g, 10 mmol) in DMF (22 mL) was added $Pd(dppf)Cl_2$ (366 mg, 0.5 mmol) and $Pd_2(dba)_3$ (458 mg, 0.5 mmol) under N_2 was heated to 100°C overnight. LCMS showed starting material was completely consumed. The mixture was cooled to room temperature and was added H_2O and was extracted with EtOAc (150 mL×2). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by column chromatography to give **compound 30** (1.48 g, 89%) as oil. **^{1}H NMR (400MHz, $CDCl_3$)** δ 7.93-7.91 (m, 2H), 7.43-7.39 (m, 2H), 7.22-7.18 (m, 1H), 7.12-7.08 (m, 4H), 4.51-4.45 (m, 2H), 1.47 (t, *J* = 12.0 Hz, 3H).

(R)-tert-butyl 3-(4-cyano-5-(ethoxycarbonyl)-3-(4-phenoxyphenyl)-1H-pyrazol-1-yl)piperidine-1-carboxylate (**31**)

To a solution of **compound 30** (0.72 g, 2.16 mmol), PPh_3 (0.57 g, 2.16 mmol) in anhydrous THF (6 mL) was added drop-wise DIAD (0.44 g, 0.64 mmol), followed by a solution of (*S*)-N-Boc-piperidin-3-ol (0.36 g, 1.80 mmol) at 0°C under N_2. The mixture was allowed to warm up to room temperature and stirred overnight. TLC (PE/EA=5:1) showed the starting material was completely consumed. The mixture was added drop-wise a saturated NH_4Cl solution and was extracted with EtOAc (200 mL×3). The combined organic layer was dried (Na_2SO_4), filtered and concentrated. The residue was purified by column chromatography to afford **compound 31** (0.65 g, 59%) as white solid. **^{1}H NMR (400MHz, $CDCl_3$)** δ 7.99-7.97 (m, 2H), 7.42-7.38 (m, 2H), 7.20-7.16 (m, 1H), 7.12-7.08 (m, 4H), 5.22-5.21 (m, 1H), 4.54-4.49 (m, 2H), 4.35-4.09 (m, 2H), 3.43-3.37 (m, 1H), 2.92-2.86 (m, 1H), 2.21-2.19 (m, 2H), 1.94-1.90 (m, 1H), 1.72-1.69 (m, 1H), 1.53-1.48 (m, 12H).

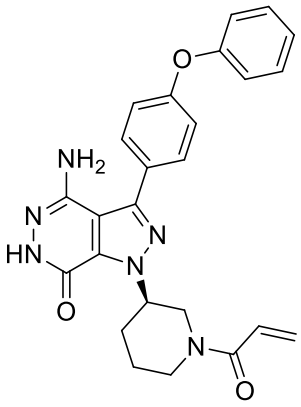
(R)-tert-butyl 3-(4-amino-7-oxo-3-(4-phenoxyphenyl)-6,7-dihydro-1H-pyrazolo[3,4-d]pyridazin-1-yl)piperidine-1-carboxylate (**32**)

A mixture of **compound 31** (340.4 mg, 0.66 mmol) in NH_2-NH_2·H_2O (6.0mL) was heated to reflux for 1.5 hours. The mixture was washed with water (20 mL) and extracted by a solution of DCM/MeOH (20 mL×2, 10:1). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated to give **compound 32** (300 mg, 93%) as white solid. **^{1}H NMR**

(400MHz, $CDCl_3$) δ 10.61 (br, 1H), 7.57-7.55 (m, 2H), 7.35-7.33 (m, 2H), 7.10-7.07 (m, 1H), 7.05-7.01 (m, 4H), 5.44-5.37 (m, 1H), 5.05 (br, 2H), 4.21-4.19 (m, 1H), 4.03-4.00 (m, 1H), 3.41-3.39 (m, 1H), 2.83-2.78 (m, 1H), 2.16-2.12 (m, 2H), 1.89-1.84 (m, 1H), 1.71-1.67 (m, 1H), 1.35 (s, 9H).

(R)-4-amino-3-(4-phenoxyphenyl)-1-(piperidin-3-yl)-1H-pyrazolo[3,4-d]pyridazin-7(6H)-one (**33**)

To a solution of **compound 32** (300 mg, 0.60 mmol) in EtOAc (2 mL) was added drop-wise a solution of HCl (g) in EtOAc (6 mL, saturated) at ice-H_2O bath. The reaction mixture was stirred at room temperature under N_2. LCMS showed starting material was completely consumed. The mixture was concentrated to afford **compound 33** (263.1 mg, crude) as yellow solid, which was used next step without further purification.



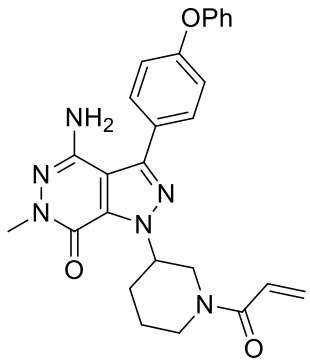
8

(R)-1-(1-acryloylpiperidin-3-yl)-4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyridazin-7(6H)-one (**8**)

A mixture of **compound 33** (142.4 mg, 0.32 mmol), acrylic acid (25.8 mg, 0.352 mmol), DIPEA (168 mg, 1.30 mmol), HATU (138 mg, 0.352 mmol) in DCM (7.2 mL) was stirred at room temperature under N_2 for 2 hours. LCMS showed starting material was completely consumed. The mixture was washed with water (20 mL) and extracted by DCM (20 mL×2). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by pre-HPLC to give **compound 8** (14.8 mg, 10%) as white solid, HPLC purity 100%. **^{1}H NMR (400MHz, $CDCl_3$)** δ 10.13-10.06 (m, 1H), 7.57-7.55 (m, 2H), 7.33-7.32 (m, 2H), 7.13-7.11 (m, 1H), 7.08-7.02 (m, 4H), 6.58-6.55 (m, 1H), 6.25-6.17 (m, 1H), 5.64-5.56 (m, 1H), 5.42-5.40 (m, 1H), 4.80 (br, 2H), 4.57-4.51 (m, 1H), 4.25-4.23 (m,

1H), 3.91-3.89 (m, 1H), 3.56-3.53 (m, 1H), 3.51-3.42 (m, 1H), 3.14-3.10 (m, 1H), 2.81-2.78 (m, 1H), 2.36-2.17 (m, 3H), 1.94-1.91 (m, 1H), 1.72-1.68 (m, 1H). **^{13}CNMR (100MHz, $CDCl_3$)** δ 165.79 , 158.92, 156.19，154.19, 146.48, 146.04，133.39, 130.85 , 130.00，127.84，125.80，124.15，119.72, 118.75, 111.80, 56.93, 55.79，51.18, 46.31, 45.83，42.19, 31.34，30.29, 24.89，23.81. LC-MS (ESI) m/z: 457 $[M+H]^+$. HRMS (ESI) calcd for $C_{25}H_{24}N_6O_3$ $[M+H]^+$: 457.1910; found, 457.1989.

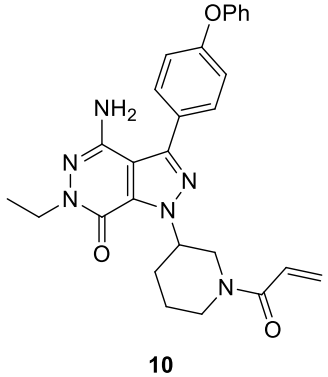
Procedures for Preparation of Compounds 9-24 (were prepared according the similar procedure as 8):



9

1-(1-acryloylpiperidin-3-yl)-4-amino-6-methyl-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d] pyridazin-7(6H)-one (9)

Compound 9 was obtained by column chromatography (6.5 mg, 8%) as white solid, HPLC purity 93.55%. **^{1}H NMR (400MHz, $CDCl_3$)** δ 7.56 (d, *J* = 8.0 Hz, 2H), 7.32 (s, 2H), 7.10-7.00 (m, 4H), 6.54 (s, 1H), 6.21 (s, 1H), 5.60-5.52 (m, 2H), 4.79 (s, 1H), 4.49 (s, 1H), 4.19 (s, 2H), 3.92 (s, 1H), 3.61 (s, 3H), 3.40 (s, 1H), 3.12 (s, 1H), 2.80 (s, 1H), 2.24 (s, 2H), 1.93 (d, *J* = 12.0 Hz, 1H), 1.688 (s, 1H). (ESI) m/z: 471 $[M+H]^+$.

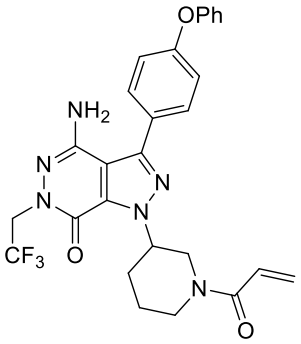


10

1-(1-acryloylpiperidin-3-yl)-4-amino-6-ethyl-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]py

ridazin-7(6H)-one (**10**)

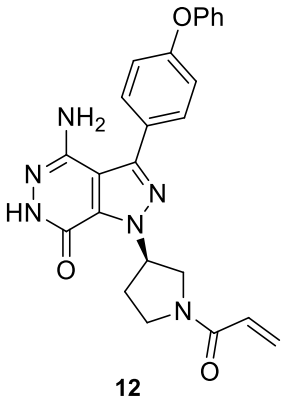
Compound 10 was obtained by prep-HPLC (14.79 mg, 28%) as white solid, HPLC purity 92.73%. **^{1}H NMR (400MHz, DMSO-*d*$_6$)** δ 7.68 (d, *J* = 8.0 Hz, 2H), 7.44 (t, *J* = 8.0 Hz, 2H), 7.22-7.09 (m, 5H), 6.92-6.73 (m, 1H), 6.14 (t, *J* = 16.0 Hz, 1H), 5.75-5.57 (m, 1H), 5.46 (br. s., 1H), 5.32 (br. s., 2H), 4.56-4.54 (m, 1H), 4.36-4.20 (m, 1H), 4.02 (d, *J* = 16.0 Hz, 3H), 3.57-3.55 (m, 1H), 2.94 (br. s., 1H), 2.18 (br. s., 2H), 1.92 (br. s., 1H), 1.52 (br. s., 1H), 1.27 (t, *J* = 12.0 Hz, 3H). (ESI) m/z: 485 $[M+H]^+$.



11

1-(1-acryloylpiperidin-3-yl)-4-amino-3-(4-phenoxyphenyl)-6-(2,2,2-trifluoroethyl)-1H-pyrazolo[3,4-d]pyridazin-7(6H)-one (**11**)

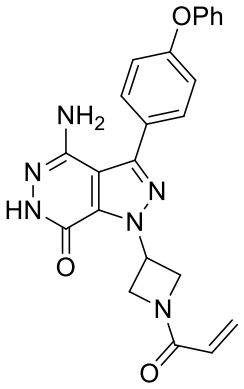
Compound 11 was obtained by prep-HPLC (8.29 mg, 14%) as white solid, HPLC purity 99.47%. **^{1}H NMR (400MHz, DMSO-*d*$_6$)** δ 7.75-7.61 (m, 2H), 7.44 (brs, 2H), 7.13 (brs, 5H), 6.95-6.68 (m, 1H), 6.09 (brs, 1H), 5.80-5.30 (m, 5H), 4.79 (brs, 2H), 4.57 (brs, 1H), 4.39-4.16 (m, 1H), 4.03 (brs, 1H), 3.59 (brs, 1H), 2.98 (brs, 1H), 2.20 (brs, 2H), 1.94 (brs, 1H), 1.54 (brs, 1H). (ESI) m/z: 539 $[M+H]^+$.



12

(R)-1-(1-acryloylpyrrolidin-3-yl)-4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyridazin-7(6H)-one (**12**)

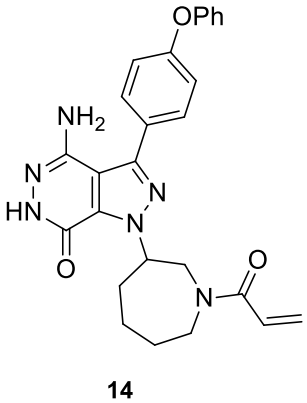
Compound 12 was obtained by prep-HPLC, HPLC purity 97.6%. **^{1}H NMR (400MHz, MeOD)** δ 7.65-7.63 (m, 2H), 7.40-7.36 (m, 2H), 7.17-7.15 (m, 1H), 7.09-7.05 (m, 5H), 6.61-6.60 (m, 1H), 6.29-6.23 (m, 1H), 5.76-5.73 (m, 1H), 4.92-4.91 (m, 1H), 4.15-3.73 (m, 4H), 2.60-2.51 (m, 2H). (ESI) m/z: 443 $[M+H]^+$.



13

1-(1-acryloylazetidin-3-yl)-4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyridazin-7(6H)-one (**13**)

Compound 13 was obtained by column chromatography, HPLC purity 99%. **^{1}H NMR (400MHz, MeOD)** δ 7.72-7.70 (m, 2H), 7.41-7.37 (m, 2H), 7.16-7.07 (m, 5H), 6.42-6.35 (m, 1H), 6.29-6.29 (m, 1H), 5.77-5.74 (m, 1H), 4.60-4.58 (m, 1H), 3.73-3.71 (m, 1H), 3.30-3.23 (m, 2H), 3.21-2.66 (m, 1H). (ESI) m/z: 428 $[M+H]^+$.

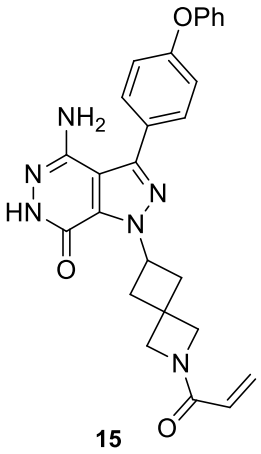


14

1-(1-acryloylazepan-3-yl)-4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyridazin-7(6H)-one (14)

Compound 14 was obtained by prep-HPLC (36.3 mg, 14%) as white solid, HPLC purity 100%. **^{1}H NMR (400MHz, $CDCl_3$)** δ 9.41-9.34 (m, 1 H), 7.66-7.64 (m, 2H), 7.43-7.41 (m, 2H), 7.23-7.21 (m, 1H), 7.17-7.11 (m, 4H), 6.82-6.79 (m, 1H), 6.65–6.60 (m, 1H), 6.43-6.39 (m, 1H), 5.78-5.73 (m, 2H), 4.48-4.44 (m, 2H), 4.27-4.16 (m, 1H), 4.40-3.90 (m, 1H),

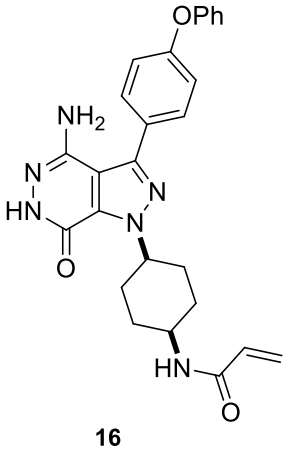
3.88-3.85 (m, 1H), 3.65-3.60 (m, 1H), 3.28-3.25 (m, 1H), 2.36-2.34 (m, 1H), 2.23-2.21 (m, 1H), 2.06-2.03 (m, 1H), 1.86-1.82 (m, 1H), 1.67-1.64 (m, 1H). (ESI) m/z: 471 $[M+H]^+$.



15

1-(2-acryloyl-2-azaspiro[3.3]heptan-6-yl)-4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyridazin-7(6H)-one (15)

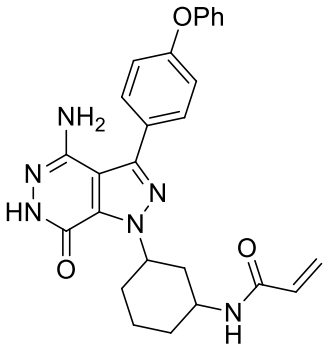
Compound 15 was obtained by prep-HPLC, HPLC purity 98.93%. **^{1}H NMR (400MHz, $CDCl_3$)** δ 9.61-9.57 (m, 1H), 7.57-7.55 (m, 2H), 7.33-7.31 (m, 2H), 7.12-7.02 (m, 5H), 6.29-6.24 (m, 1H), 6.13-6.11 (m, 1H), 6.09-5.95 (m, 1H), 5.62-5.58 (m, 1H), 4.50-4.47 (m, 2H), 4.28-4.08 (m, 4H), 2.96-2.92 (m, 2H), 2.81-2.72 (m, 2H). (ESI) m/z: 469 $[M+H]^+$.



16

N-((1s,4s)-4-(4-amino-7-oxo-3-(4-phenoxyphenyl)-6,7-dihydro-1H-pyrazolo[3,4-d]pyridazin-1-yl)cyclohexyl)acrylamide (**16**)

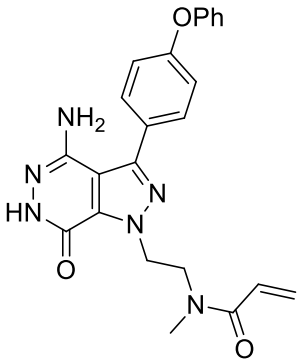
Compound 16 was obtained by prep-HPLC, HPLC purity 96%. **^{1}H NMR(400MHz, MeOD)** δ 7.69-7.67 (m, 2H), 7.42-7.38 (m, 2H), 7.18-7.06 (m, 5H), 6.38-6.34 (m, 1H), 6.25-6.21 (m, 1H), 5.65-5.47 (m, 2H), 2.33-2.30 (m, 2H), 2.05-2.01 (m, 4H), 1.86-1.83 (m, 2H). (ESI) m/z: 471 $[M+H]^+$.



17

N-(3-(4-amino-7-oxo-3-(4-phenoxyphenyl)-6,7-dihydro-1H-pyrazolo[3,4-d]pyridazin-1-yl)cyclohexyl)acrylamide (**17**)

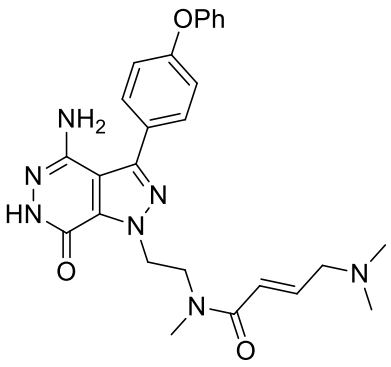
Compound 17 was obtained by prep-HPLC, HPLC purity 99.4%. **^{1}H NMR (400MHz, MeOD)** δ 7.72-7.70 (m, 2H), 7.43-7.41 (m, 2H), 7.21-7.10 (m, 5H), 6.42-6.37 (m, 1H), 6.27-6.23 (m, 1H), 5.69-5.66 (m, 2H), 4.50 (s, 1H), 2.41-2.37 (m, 1H), 2.18-2.08 (m, 3H), 1.92-1.78 (m, 4H). (ESI) m/z: 471 $[M+H]^+$.



18

N-(2-(4-amino-7-oxo-3-(4-phenoxyphenyl)-6,7-dihydro-1H-pyrazolo[3,4-d]pyridazin-1-yl)ethyl)-N-methylacrylamide (18)

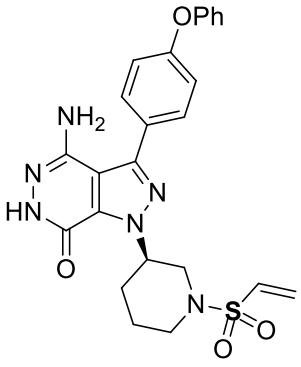
Compound 18 was obtained by prep-HPLC (90 mg, 47%) as white solid, HPLC purity 100%. **^{1}H NMR (400MHz, MeOD)** δ 7.66 (d, *J* = 8.56 Hz, 2H), 7.45-7.33 (m, 2H), 7.22-6.99 (m, 5H), 6.70 (dd, *J* = 10.03, 16.38 Hz, 1H), 6.23-6.04 (m, 2H), 5.68-5.54 (m, 1H), 3.95 (dd, *J* = 4.03, 11.62 Hz, 1H), 3.71 (d, *J* = 12.23 Hz, 1H), 3.27-3.21 (m, 1H), 2.79 (dt, *J* = 2.69, 12.10 Hz, 1H), 2.31-2.11 (m, 2H), 2.00 (d, *J* = 13.94 Hz, 1H), 1.89-1.74 (m, 1H). (ESI) m/z: 493 $[M+H]^+$.



19

(E)-N-(2-(4-amino-7-oxo-3-(4-phenoxyphenyl)-6,7-dihydro-1H-pyrazolo[3,4-d]pyridazin-1-yl)ethyl)-4-(dimethylamino)-N-methylbut-2-enamide (19)

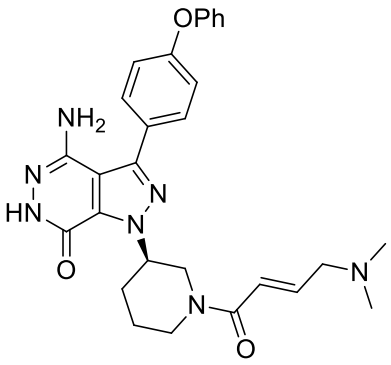
Compound 19 was obtained by prep-HPLC, HPLC purity 99.7%. **^{1}H NMR (400MHz, $CDCl_3$)** δ 11.29 (s,1H), 7.62-7.59 (m, 2H), 7.40-7.38 (m, 2H), 7.15-7.09 (m, 5H), 6.23-6.21 (m, 1H), 6.16-6.16 (m, 1H), 5.48-5.45 (m, 1H), 5.38-5.30 (m, 2H), 4.98-4.95 (m, 2H), 4.00-3.93 (m, 2H), 3.07-2.98 (s, 3H). (ESI) m/z: 431 $[M+H]^+$.



20

(R)-4-amino-3-(4-phenoxyphenyl)-1-(1-(vinylsulfonyl)piperidin-3-yl)-1H-pyrazolo[3,4-d] pyridazin-7(6H)-one (20)

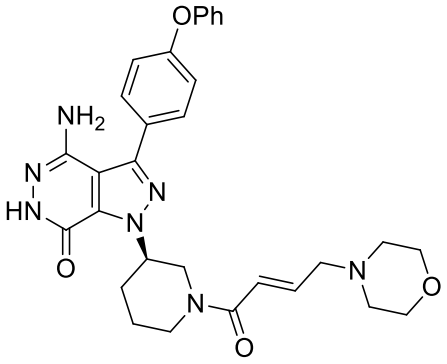
Compound 20 was obtained by prep-HPLC (200 mg, 78%) as white solid, HPLC purity 99.7%. **^{1}H NMR (400MHz, $CDCl_3$)** δ 7.61 (d, *J* = 8.60 Hz, 2H), 7.46-7.34 (m, 2H), 7.20-7.02 (m, 5H), 6.81 (d, *J* = 13.89 Hz, 1H), 6.44 (d, *J* = 14.99 Hz, 1H), 5.57-5.30 (m, 3H), 4.85 (d, *J* = 11.91 Hz, 1H), 4.56 (d, *J* = 12.13 Hz, 1H), 4.28 (d, *J* = 11.47 Hz, 1H), 4.00 (d, *J* = 12.35 Hz, 1H), 3.66 (t, *J* = 11.25 Hz, 1H), 3.54-3.38 (m, 1H), 3.26-2.93 (m, 2H), 2.84 (t, *J* = 12.02 Hz, 1H), 2.38-2.12 (m, 8H), 1.97 (d, *J* = 13.67 Hz, 1H). (ESI) m/z: 514 $[M+H]^+$.



21

(R,E)-4-amino-1-(1-(4-(dimethylamino)but-2-enoyl)piperidin-3-yl)-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyridazin-7(6H)-one (21)

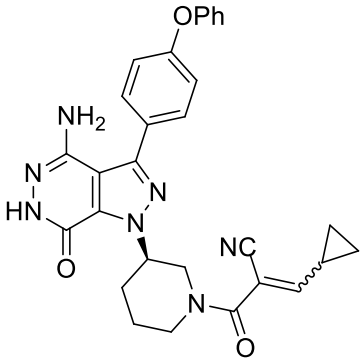
Compound 21 was obtained by prep-HPLC, HPLC purity 99.2%. **^{1}H NMR (400MHz, $CDCl_3$)** δ 10.29 (s, 1H), 7.64-7.62 (m, 2H), 7.42-7.35 (m, 2H), 7.19-6.84 (m, 5H), 6.52-6.52 (m, 1H), 6.49-6.48 (m, 1H), 5.43-5.42 (m, 1H), 4.84-4.83 (m, 2H), 4.59-4.55 (m, 1H), 4.30-4.26 (m, 1H), 4.00-3.98 (m, 1H), 3.73-6.60 (m, 4H), 3.49-3.46 (m, 1H), 3.15-3.10 (m, 2H), 2.88-2.86 (m, 1H), 2.48-1.97 (m, 5H). (ESI) m/z: 556 $[M+H]^+$.



22

(R,E)-4-amino-1-(1-(4-morpholinobut-2-enoyl)piperidin-3-yl)-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyridazin-7(6H)-one (22)

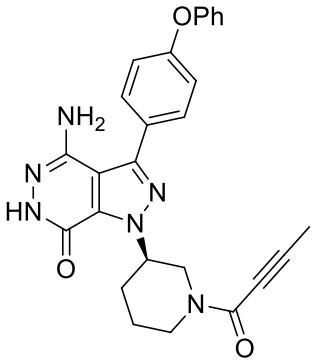
Compound 22 was obtained by prep-HPLC, HPLC purity 100%. **^{1}H NMR (400MHz, $CDCl_3$)** δ 11.19 (s, 1H), 7.64-7.62 (m, 2H), 7.42-7.40 (m, 2H), 7.18-7.09 (m, 5H), 6.74-6.70 (m, 1H), 6.35-6.21 (m, 1H), 5.24-5.20 (m, 2H), 4.97-4.96 (m, 2H), 3.06-2.88 (m, 4H), 2.21-2.13 (m, 5H). (ESI) m/z: 488 $[M+H]^+$.



23

(R)-2-(3-(4-amino-7-oxo-3-(4-phenoxyphenyl)-6,7-dihydro-1H-pyrazolo[3,4-d]pyridazin-1-yl)piperidine-1-carbonyl)-3-cyclopropylacrylonitrile (23)

Compound 23 was obtained by prep-HPLC (35 mg, 62%) as yellow solid, HPLC purity 99.5%. **^{1}H NMR (400MHz, MeOD)** δ 7.67 (d, *J* = 8.80 Hz, 2H), 7.33-7.45 (m, 2H), 7.22-7.03 (m, 5H), 6.39 (d, *J* = 11.00 Hz, 1H), 5.62-5.53 (m, 1H), 4.91 (br. s., 1H), 3.97-3.84 (m, 1H), 2.44-1.64 (m, 5H), 1.40-0.64 (m, 4H). (ESI) m/z: 457 $[M+H]^+$.

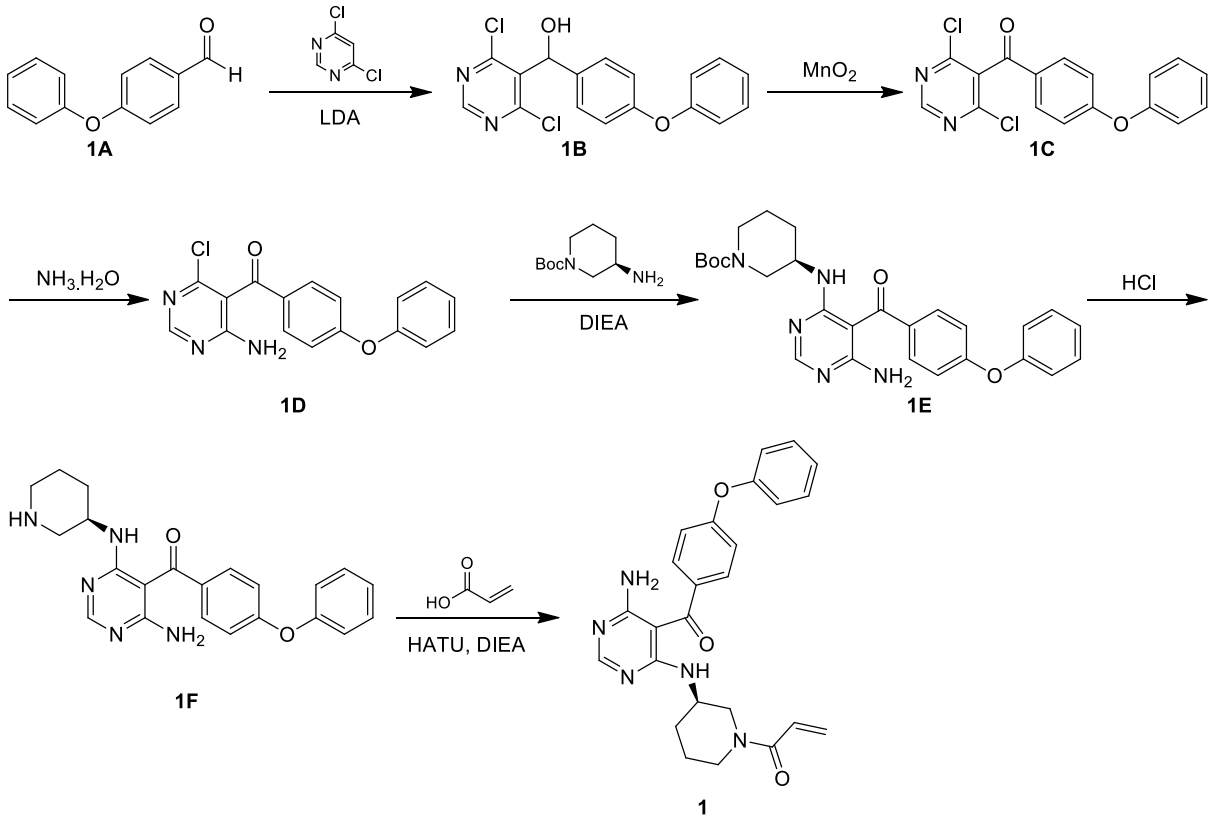


24

(R)-4-amino-1-(1-(but-2-ynoyl)piperidin-3-yl)-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyridazin-7(6H)-one (24)

Compound 24 was obtained by prep-HPLC, HPLC purity 94.31%. **^{1}H NMR (400MHz, $CDCl_3$)** δ 9.28 (s, 1H), 7.64-7.59 (m, 2H), 7.38-7.37 (m, 2H), 7.13-7.07 (m, 5H), 5.54-5.46 (m, 1H), 4.76-4.73 (m, 2H), 4.54-4.31 (m, 3H), 3.91-3.85 (m, 1H), 3.52-3.46 (m, 1H), 3.25-3.00 (m, 1H), 2.28-2.25 (m, 2H), 2.02 (s, 3H). (ESI) m/z: 469 $[M+H]^+$.

Procedures for Preparation of Compound 1:



(4,6-dichloropyrimidin-5-yl)(4-phenoxyphenyl)methanol (**1B**)

To a solution of 4, 6-dichloropyrimidine (2.00 g, 13.42 mmol) in THF (30 mL) was added LDA (1.44 g, 6.75 mL, 13.42 mmol) dropwise at -78°C under N_2 protection. The mixture was stirred for 1.5 hours at -78°C. And then a solution of 4-phenoxybenzaldehyde (2.93 g, 14.77 mmol) in THF (10 mL) was added. And then the reaction mixture was stirred for 1.5 hours at -78°C. The mixture was cooled with an ice-H_2O bath and 50 mL concentrated aq. NH_4Cl was added slowly and extracted with EtOAc (30 mL×2). The combined organic layers were dried over Na_2SO_4 and condensed. The obtained mixture was purified by column chromatography (petroleum ether/EtOAc=5:1) to afford **compound 1B** (700 mg, 15%) as brown oil. (ESI) m/z: 347 $[M+H]^+$.

(4,6-dichloropyrimidin-5-yl)(4-phenoxyphenyl)methanone (1C)

To a mixture of **1B** (700mg, 2.02mmol) in CH_2Cl_2 (30mL) was added MnO_2 (3.51g, 40.32 mmol). The reaction mixture was stirred at 50°C for 16 hours. This mixture was filtered immediately. And the solvent was evaporated. The residue was purified by column chromatography (petroleum ether/EtOAc=24:1) to give **compound 1C** (200 mg, 29%) of pure target as brown oil. (ESI) m/z: 345 $[M+H]^+$.

(4-amino-6-chloropyrimidin-5-yl)(4-phenoxyphenyl)methanone (**1D**)

To a mixture of **1C** (200 mg, 0.579 mmol) in THF (5 mL) was added $NH_3.H_2O$ (406.12 mg, 11.59 mmol). The sealed vial was irradiated in the microwave at 80°C for 0.5 hours. During the reaction, LCMS monitored the reaction. The solvent was removed in *vacuo*. The residue was diluted with water and extracted with EtOAc (10 mL×2). The combined organic layers were dried over $MgSO_4$ and condensed to afford **compound 1D** (150 mg, 79%) as brown oil. (ESI) m/z: 326 $[M+H]^+$.

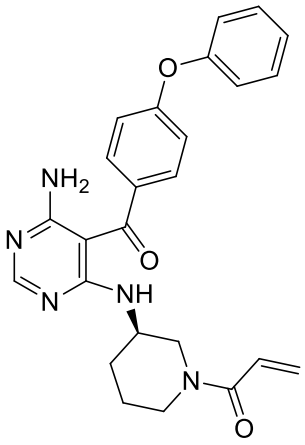
(R)-tert-butyl

3-((6-amino-5-(4-phenoxybenzoyl)pyrimidin-4-yl)amino)piperidine-1-carboxylate (**1E**)

To a solution of **1D** (130 mg, 0.399 mmol) in propan-2-ol (2 mL) was added (R)-tert-butyl 3-aminopiperidine-1-carboxylate (159.85 mg, 0.798 mmol), DIEA (103.16 mg, 0.798 mmol). The sealed vial was irradiated in the microwave at 120°C for 2 hours. During the reaction, LCMS monitored the reaction. The solvent was removed in *vacuo*. The residue was diluted with water and extracted with EtOAc (20 mL×2). The combined organic layers were dried over Na_2SO_4 and condensed to afford **compound 1E** (160 mg, 82%) as brown oil. (ESI) m/z: 490 $[M+H]^+$.

(R)-(4-amino-6-(piperidin-3-ylamino)pyrimidin-5-yl)(4-phenoxyphenyl)methanone (**1F**)

To a solution of **1E** (150 mg, 0.306 mmol) in MeOH (1 mL) was added HCl(MeOH) (3 mL, 4 M). The mixture reacted at 10°C for 0.5 hour. During the reaction, TLC monitored the reaction. The solvent was removed in *vacuo*. The residue was dissolved in water and adjusted pH using sat Na_2CO_3 to basic, extracted with EtOAc (20 mL×2). The combined organic layers were dried over Na_2SO_4 and condensed to afford **compound 1F** (70 mg, 59%) as brown oil. (ESI) m/z: 390 $[M+H]^+$.

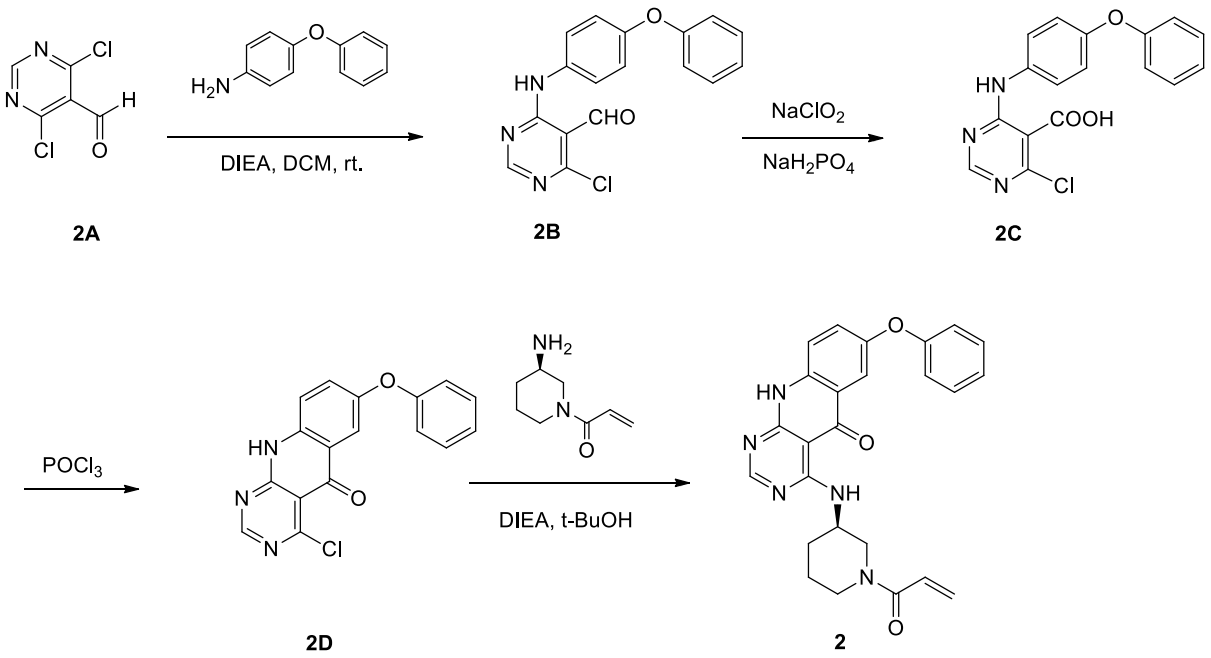


1

(R)-1-(3-((6-amino-5-(4-phenoxybenzoyl)pyrimidin-4-yl)amino)piperidin-1-yl)prop-2-en-1-one (**1**)

To a mixture of **1F** (100 mg, 0.256 mmol) in DCM (1 mL) was added HATU (97.63 mg, 0.256 mmol), acrylic acid (18.50 mg, 0.256 mmol), and DIEA (99.56 mg, 0.770 mmol). The reaction mixture was stirred at 10°C for 16 hours. The solvent was removed in *vacuo*. The residue was diluted with water and extracted with EtOAc (20 mL×2). The combined organic layers were dried over Na_2SO_4 and condensed. The obtained mixture was purified by prep-HPLC to afford **compound 1** (26.5 mg, 24%) as white solid, HPLC purity 100%. **^{1}H NMR (400MHz, MeOD)** δ 8.04 (d, *J* = 11.6 Hz, 1H), 7.68-7.66 (m, 2H), 7.43 (t, *J* = 8.0 Hz, 2H), 7.22 (t, *J* = 8.0 Hz, 1H), 7.08 (d, *J* = 8.0 Hz, 2H), 7.00 (d, *J* = 8.0 Hz, 2H), 6.67-6.58 (m, 1H), 6.12-6.06 (m, 1H), 5.66-5.62 (m, 1H), 4.17-4.08 (m, 1H), 3.83-3.80 (m, 1H), 3.61 (s, 1 H), 3.52-3.40 (m, 2H), 1.89 (s, 1 H), 1.59-1.57 (m, 3H). LC-MS (ESI) m/z: 444 $[M+H]^+$.

Procedures for Preparation of Compound 2:



4-chloro-6-((4-phenoxyphenyl)amino)pyrimidine-5-carbaldehyde (**2B**)

To a solution of 4,6-dichloropyrimidine-5-carbaldehyde (2 g, 11.4 mmol) in 20 mL DCM was added 4-phenoxyaniline (2.1 g, 11.4 mmol) and DIEA (2.94 g, 22.8 mmol) at 0°C under nitrogen. The reaction mixture was stirred at room temperature for 2 hours. The mixture was quenched with water (20 mL) and extracted with DCM (10 mL×2). The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (5%-20% petro ether in ethyl acetate) to give **compound 2B** (2.5 g, 68%). (ESI) m/z: 326 $[M+H]^+$.

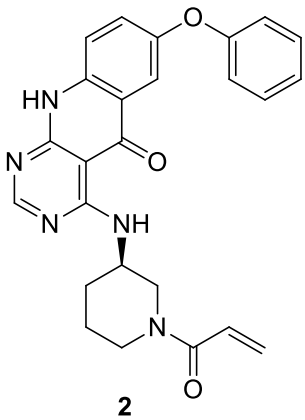
4-chloro-6-((4-phenoxyphenyl)amino)pyrimidine-5-carboxylic acid (2C)

To a mixture of **2B** (1.0 g, 3 mol), 2-methylbut-2-ene (1.05 g, 15 mmol) and NaH_2PO_4 (1.44 g, 12 mmol) in 30 mL t-BuOH and 10 mL H_2O was added $NaClO_2$ (1.08 g, 12 mmol), then the mixture was stirred at room temperature for 16 hours. The mixture was diluted with water, extracted with DCM (10 mL×2), the aqueous phase was acidified by 1N HCl, extracted with 20 mL DCM, dried over Na_2SO_4, filtered and concentrated to give **compound 2C** which was used for the next step. (ESI) m/z: 342 $[M+H]^+$.

4-chloro-7-phenoxypyrimido[4,5-b]quinolin-5(10H)-one (**2D**)

A solution of **2C** (500 mg, 1.47 mmol) in $POCl_3$ (5 mL) was heated to 80°C for 3 hours. The mixture was quenched with water (5 mL) and extracted with DCM (5 mL×3,). The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by

column chromatography (30%-50% petro ether in ethyl acetate) to give **compound 2D** (300 mg, 63%) as yellow oil. (ESI) m/z: 324 $[M+H]^+$.

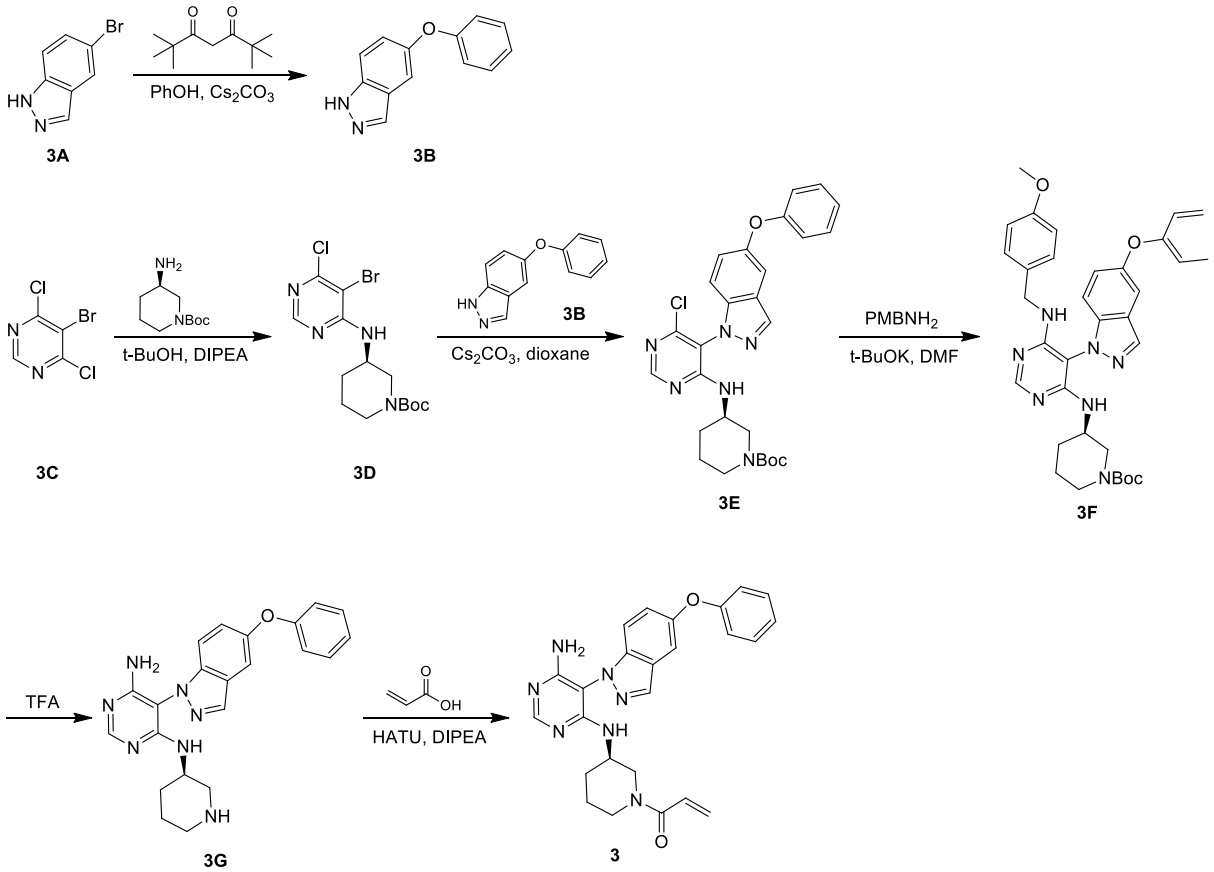


2

(R)-4-((1-acryloylpiperidin-3-yl)amino)-7-phenoxypyrimido[4,5-b]quinolin-5(10H)-one (2)

To a solution of **2D** (50 mg, 0.15 mmol) and DIEA (38.7 mg, 0.3 mmol) in t-BuOH (2 mL) was added (R)-1-(3-aminopiperidin-1-yl)prop-2-en-1-one (23.1 mg, 0.15 mmol) at 0°C under nitrogen. The reaction mixture was stirred at 80°C for 3 hours. The mixture was washed with water (5 mL) and extracted with EtOAc (5 mL×3). The organic phase was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by prep-HPLC to afford **compound 2** (11 mg, 17%) as white solid, HPLC purity 98.8%. **^{1}H NMR (400MHz, MeOD)** δ 8.46 (s, 1H), 7.69-7.68 (m, 2H), 7.59-7.56 (m, 1H), 7.43-7.39 (m, 2H), 7.22-7.20 (m, 1H), 7.07-7.05 (m, 2H), 6.13 (d, *J* = 16.8 Hz, 1H), 5.70 (s, 1H), 4.26 (s, 1H), 3.81-3.57 (m, 3H), 2.13-1.62 (m, 4H). LC-MS (ESI) m/z: 442 $[M+H]^+$.

Procedures for Preparation of Compound 3:



5-phenoxy-1H-indazole (**3B**)

To a solution of 5-bromo-1H-indazole (10 g, 53 mmol) in DMF (50mL) was added phenol (5 g, 53 mmol), CuCl (2.5 g, 26.5 mmol), 2,2,6,6-tetramethylheptane-3,5-dione (5 g, 26.5 mmol) and Cs_2CO_3 (30 g, 106 mmol), the mixture was stirred for 8 hours at 120°C. Water (40 mL) was added, extracted with EtOAc (10 mL×3), the combined organic layers was dried over Na_2SO_4, filtered and concentrated to give the crude. The residue was purified by column chromatography (petroleum ether/EtOAc=10:1-5:1) to give **compound 3B** (0.5 g, 5%). **^{1}H NMR (400MHz, $CDCl_3$)** δ 8.01 (s, 1H), 7.54-7.48 (m, 1H), 7.31 (d, *J* = 7.6 Hz, 3H), 7.19-7.13 (m, 1H), 7.09-7.03 (m, 2H), 6.97 (d, *J* = 8.0 Hz, 2H), 6.89 (d, *J* = 8.0 Hz, 1H). (ESI) m/z: 211 $[M+H]^+$.

(R)-tert-butyl 3-((5-bromo-6-chloropyrimidin-4-yl)amino)piperidine-1-carboxylate (3D)

To the mixture of (R)-tert-butyl 3-aminopiperidine-1-carboxylate (1 g, 5 mmol) in DCM (10 mL) was added DIEA (1.29 g, 10 mmol) and 5-bromo-4,6-dichloropyrimidine (1.13 g, 5 mmol) at 0°C, and the mixture was stirred for 1 hour at room temperature. The solvent was removed. The residue was purified by column chromatography to afford **compound 3D** (1 g, 78%). **^{1}H NMR (400MHz, $CDCl_3$)** δ 8.23-8.21 (m, 1H), 5.69-5.67 (m, 1H), 4.11 (d, *J* = 2.65

Hz, 1H), 3.62-3.53 (m, 1H), 3.53-3.42 (m, 2H), 3.35-3.24 (m, 1H), 1.93-1.82 (m, 1H), 1.82-1.73 (m, 1H), 1.73-1.62 (m, 1H), 1.61-1.50 (m, 1H), 1.39 (br. s., 9H). (ESI) m/z: 393 $[M+H]^+$.

(3R)-tert-butyl 3-((6-chloro-5-(5-phenoxy-1H-indazol-1-yl)pyrimidin-4-yl)amino)piperidine-1-carboxylate (**3E**)

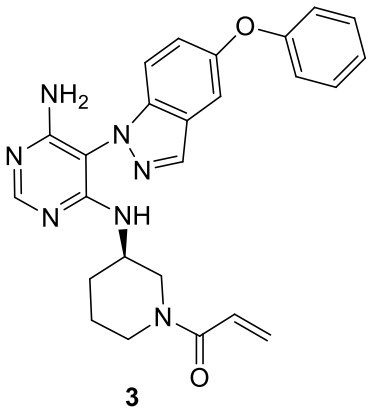
To a solution of **3D** (2 g, 5.26 mmol) in dioxane (15 mL) was added **3B** (1 g, 4.86 mmol), Cs_2CO_3 (3.2 g, 9.72 mmol) and $Pd(dppf)Cl_2$ (0.34 g, 0.48 mmol), the mixture was stirred for 8 hours at 100°C. The mixture was concentrated, the residue was purified by column chromatography to afford **compound 3E** (1 g, 31%). (ESI) m/z: 521 $[M+H]^+$.

(3R)-tert-butyl 3-((6-((4-methoxybenzyl)amino)-5-(5-((E)-penta-1,3-dien-3-yloxy)-1H-indazol-1-yl)pyrimidin-4-yl)amino)piperidine-1-carboxylate (**3F**)

To a solution of **3E** (1.3 g, 2.5 mmol) in DMF (10 mL) was added $PMBNH_2$ (0.69 g, 5 mmol) and t-BuOK (0.56 g, 5 mmol), the mixture was stirred for 10 hours at 120°C. Water (40 mL) was added, extracted with EtOAc (10 mL×3), the combined organic layer was dried over Na_2SO_4, filtered and concentrated to give the crude. The residue was purified by column chromatography (petroleum ether/EtOAc=10:1-5:1) to give **compound 3F** (0.8 g, 53%) as yellow oil. **^{1}H NMR (400MHz, $CDCl_3$)** δ 8.20 (s, 1H), 7.62 (s, 1H), 7.34 (t, *J* = 8.0 Hz, 4H), 7.19 (d, *J* = 8.0 Hz, 3H), 7.12 (d, *J* = 7.6 Hz, 1H), 7.01 (d, *J* = 8.0 Hz, 2H), 6.86-6.76 (m, 3H), 4.39 (d, *J* = 6.0 Hz, 1H), 3.91 (br. s., 2H), 3.80-3.76 (m, 2H), 3.73 (s, 3H), 3.49-3.33 (m, 1H), 3.18-2.97 (m, 1H), 1.84 (br. s., 3H), 1.55-1.45 (m, 2H), 1.40 (br. s., 9H). (ESI) m/z: 612 $[M+H]^+$.

5-(5-phenoxy-1H-indazol-1-yl)-N4-((R)-piperidin-3-yl)pyrimidine-4,6-diamine (**3G**)

A mixture of **3F** (0.4 g, 0.64 mmol) in TFA (5 mL) was stirred for 4 hours at 60°C. The reaction was cooled and concentrated to give **compound 3G** (0.2 g, 78%). (ESI) m/z: 402 $[M+H]^+$.

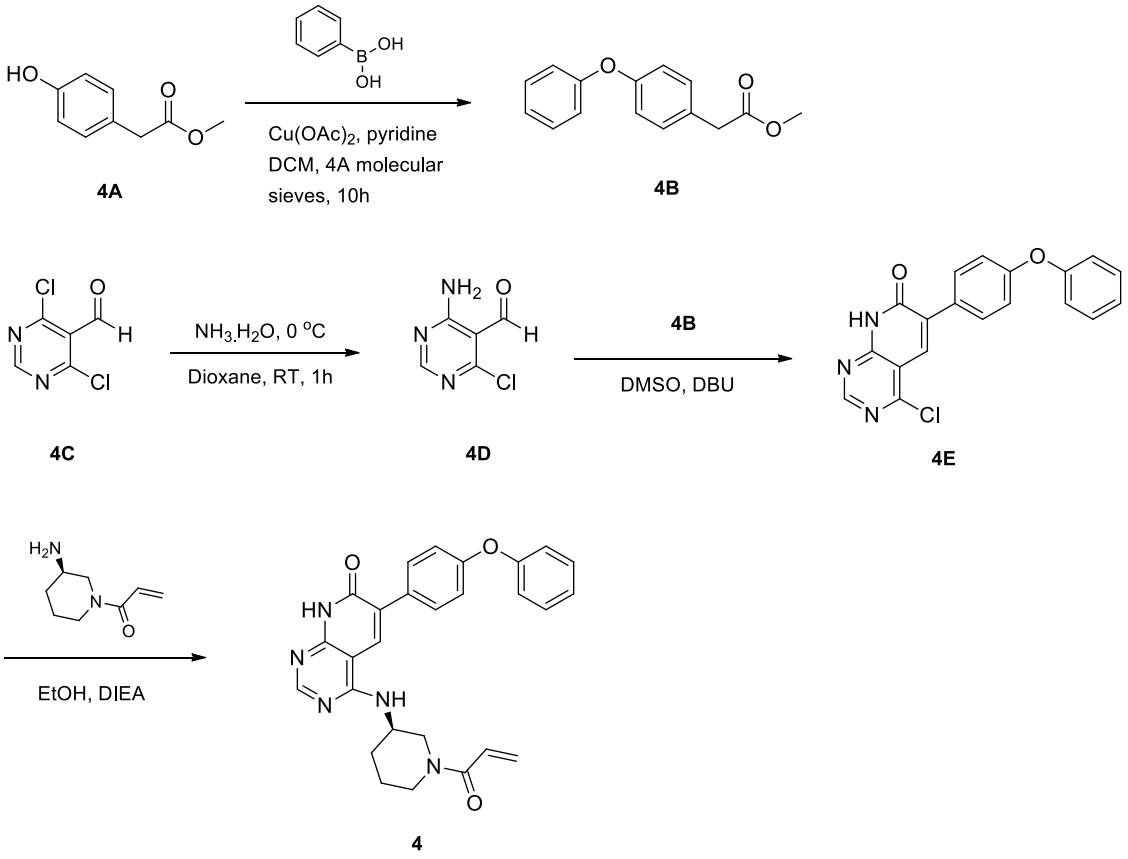


3

1-((3R)-3-((6-amino-5-(5-phenoxy-1H-indazol-1-yl)pyrimidin-4-yl)amino)piperidin-1-yl) prop-2-en-1-one (**3**)

To a solution of **3G** (0.3 g, 0.7 mmol) in DCM (10 mL) was added acrylic acid (60 mg, 0.7 mmol), DIEA (540 mg, 4.2 mmol) and HATU (0.27 g, 0.7 mmol), the reaction was stirred at room temperature for 1 hour. The mixture was concentrated. The residue was purified by prep-HPLC to afford **compound 3** (12.9 mg, 4%), HPLC purity 96.77%. **^{1}H NMR (400MHz, MeOD)** δ 7.68 (s., 2H), 7.53-7.34 (m, 3H), 7.16 (d, *J* = 8.0 Hz, 1H), 7.06 (d, *J* = 8.0 Hz, 2H), 6.88-6.61 (m, 1H), 6.25-6.05 (m, 1H), 5.61 (d, *J* = 10.0 Hz, 1H), 4.27-4.15 (m, 1H), 4.10-3.99 (m, 1H), 3.98-3.88 (m, 1H), 3.79-3.69 (m, 1H), 2.19-1.82 (m, 3H), 1.75-1.58 (m, 1H), 1.31 (s, 1H). LC-MS (ESI) m/z: 456 $[M+H]^+$..

Procedures for Preparation of Compound 4:



methyl 2-(4-phenoxyphenyl)acetate (**4B**)

Compound 4A (3.32 g, 20 mmol), $Cu(OAc)_2$ (3.58 g, 20 mmol), phenylboronic acid (4.88 g, 40 mmol), powdered 4A molecular sieves and pyridine (8 mL) were added into DCM (100 mL). The mixture was allowed to room temperature overnight. The reaction mixture was filtered and extracted with EA, the organic lay was isolated, washed with brine, dried and concentrated to give the **compound 4B** (8 g, 68%). **^{1}H NMR (400MHz, $CDCl_3$)** δ 7.38-7.33 (m, 2 H), 7.29-7.27 (m, 1 H), 7.15-7.13 (m, 1 H), 7.05-6.99 (m, 5 H), 3.73 (s, 3 H), 3.64 (s, 2H).

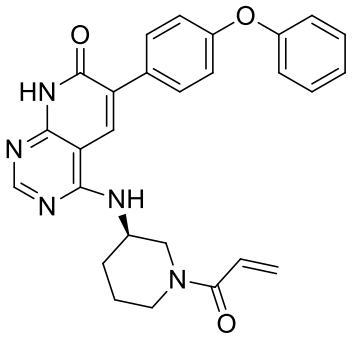
4-amino-6-chloropyrimidine-5-carbaldehyde (**4D**)

To a solution of **4C** (10 g, 56 mmol) in dioxane (200 mL) was added $NH_3.H_2O$ (20 mL) dropwise at 0°C. The mixture was stirred for 1 hour at 20°C. Which was dissolved in EtOAc (20 mL), washed with H_2O (20 mL×2), dried over Na_2SO_4, filtered and concentrated to give **compound 4D** as yellow solid (6 mg, 68%). (ESI) m/z: 158 $[M+H]^+$.

4-chloro-6-(4-phenoxyphenyl)pyrido[2,3-d]pyrimidin-7(8H)-one (**4E**)

To a mixture of **4D** (0.78g, 4.96mmol) and **4B** (1g, 4.13mmoL) in DMSO (10mL) was added DBU (0.78g, 4.96mmol) dropwise at 0°C, after added, the reaction mixture was stirred at

23°C for 16 hours. TLC showed the reaction was completed. Water (40 mL) was added, extracted with EtOAc (10 mL×3), the combined organic layers was dried over Na_2SO_4, filtered and concentrated to give the crude. The residue was purified by column chromatography (petroleum ether/EtOAc=10:1-5:1) to give **compound 4E** (1 g, 71%) as yellow solid. (ESI) m/z: 350 $[M+H]^+$.

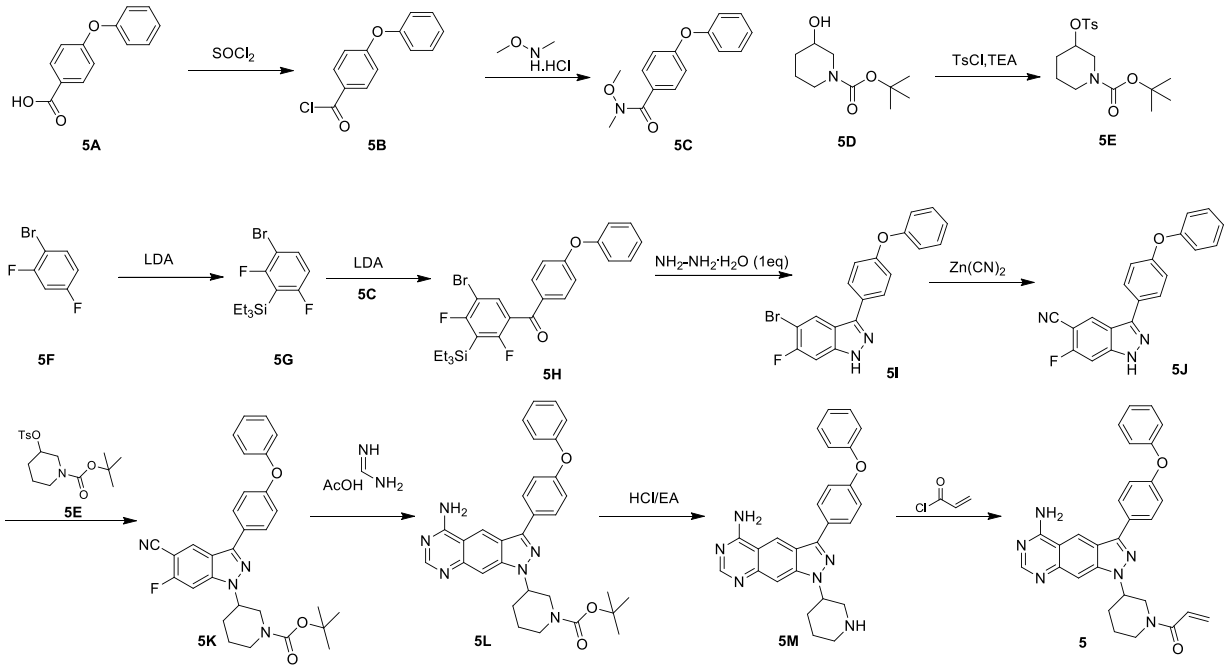


4

(R)-4-((1-acryloylpiperidin-3-yl)amino)-6-(4-phenoxyphenyl)pyrido[2,3-d]pyrimidin-7(8H)-one (**4**)

To a mixture of **4E** (250mg, 0.72 mmol) in EtOH (5 mL) was added (*R*)-1-(3-aminopiperidin-1-yl)prop-2-en-1-one (100 mg, 0.65 mmol) and DIEA (168 mg, 1.29 mmoL). The reaction was stirred at 60°C for 1 hour. It was checked by TLC plate and showed **4E** was almost consumed, which was concentrated to give the crude. The residue was purified by pre-HPLC to afford **compound 4** (30 mg, 20%), HPLC purity 96.97%. **^{1}H NMR (400MHz, DMSO-d_6)** δ 8.43-8.41 (m, 1 H), 8.34-8.30 (m, 1 H), 7.86-7.78 (m, 1 H), 7.44-7.40 m, 2 H), 7.16-7.10 (m, 2 H), 7.08-7.06 (m, 1H), 7.04-6.77 (m, 4 H), 6.74-6.73 (m, 1 H), 6.11-6.07 (m, 1 H), 5.69-5.64 (m, 1 H), 4.58-4.55 (m, 1 H), 4.22-4.00 (m, 3 H), 3.09-2.84 (m, 1 H), 2.06-2.03 (m, 1 H), 1.84-1.81 (m, 1 H), 1.76-1.69 (m, 1 H), 1.64-1.1.61 (m, 1 H), 1.47-1.17 (m, 1 H). LC-MS (ESI) m/z: 468 $[M+H]^+$.

Procedures for Preparation of Compound 5:



4-phenoxybenzoyl chloride (**5B**)

A solution of **compound 5A** (20 g, 93 mmol) and DMF (2 drops) in $SOCl_2$ (60 mL) was stirred at 70°C for 3 hours. The mixture was concentrated to give **compound 5B** (21.7 g, 100%) as pale yellow oil. (ESI) m/z: 233 $[M+H]^+$.

N-methoxy-N-methyl-4-phenoxybenzamide (**5C**)

To a solution of N,O-dimethylhydroxylamine hydrochloride (10 g, 102 mmol) and TEA (39 mL, 279 mmol) in DCM (200 mL) was added **5B** (21.7 g, 93 mmol) in DCM (50 mL) dropwise at 0°C. The reaction mixture was stirred to room temperature for 16 hours. The mixture was washed with water (100 mL) and dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography to give **compound 5C** (20 g, 83%) as pale yellow oil. **^{1}H NMR (400MHz, $CDCl_3$)** δ 7.70 (d, *J* = 8.8 Hz, 2H), 7.38-7.34 (m, 2H), 7.15 (t, *J* = 8.0 Hz, 1H), 7.04 (d, *J* = 8.0 Hz, 2H), 6.97 (d, *J* = 8.8 Hz, 2H), 3.56 (s, 3H), 3.35 (s, 3H).

tert-butyl 3-(tosyloxy)piperidine-1-carboxylate (**5E**)

To a solution of **5D** (10 g, 4.97 mmol), TEA (21 mL, 149 mmol) and DMAP (300 mg) in DCM (200 mL) was added TosCl (14 g, 74 mmol) at 0°C. The reaction mixture was stirred at room temperature for 26 hours. The mixture was washed with water (100 mL) and dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography to give **compound 5E** (17 g, 96%) as white solid. **^{1}H NMR (400MHz,**

$CDCl_3$) δ 7.79 (d, *J* = 8.4 Hz, 2H), 7.33 (d, *J* = 8.4 Hz, 2H), 4.44 (br. s., 1H), 3.54 (d, *J* = 11.6 Hz, 1H), 3.37-3.32 (m, 1H), 3.25 (br. s., 1H), 2.43 (s, 3H), 1.81 (br. s., 1H), 1.77-1.70 (m, 2H), 1.46- 1.41 (m, 11H).

(3-bromo-2,6-difluorophenyl)triethylsilane (**5G**)

To a solution of diisopropylamine (17.6 mL, 124 mmol) in THF (200 mL) was added n-BuLi (50 mL, 124 mmol) at -78°C. The reaction mixture was stirred at -78°C for 30 mins. Then **compound 5F** (20 g, 103 mmol) was added to the mixture dropwise. The reaction mixture was stirred at -78°C for 1 hour, then added Et_3SiCl (21 mL, 124 mmol) dropwise. The reaction mixture was stirred at -78°C for 2 hours. The mixture was quenched with NH_4Cl (aq, 100 mL) extracted with EtOAc (200 mL×3), washed with brine and dried over Na_2SO_4, filtered and concentrated to give **compound 5G** (36.4 g, 100%) as colourless oil. (ESI) m/z: 309 $[M+H]^+$.

(5-bromo-2,4-difluorophenyl)(4-phenoxyphenyl)methanone (**5H**)

To a solution of diisopropylamine (13 mL, 93 mmol) in THF (250 mL) was added n-BuLi (37 mL, 93 mmol) at -78°C. The reaction mixture was stirred at -78°C for 30 mins. Then **5G** (28 g, 93 mmol) was added to the mixture dropwise. The reaction mixture was stirred at -78°C for 1 hour, then added **5C** (24 g, 93 mmol) dropwise. The reaction mixture was stirred at -78°C for 2 hours. The mixture was quenched with NH_4Cl (aq, 100 mL) extracted with EtOAc (200 mL×3), washed with brine and dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography to give **compound 5H** (20 g, 43 %) as yellow oil. **^{1}H NMR (400MHz, $CDCl_3$)** δ 7.81-7.75 (m, 2H), 7.44-7.35 (m, 2H), 7.22 (t, *J* = 7.6 Hz, 1H), 7.09 (d, *J* = 7.6 Hz, 2H), 7.01 (d, *J* = 8.6 Hz, 2H), 1.03-0.87 (m, 15H).

5-bromo-6-fluoro-3-(4-phenoxyphenyl)-1H-indazole (**5I**)

A solution of **5H** (20 g, 39.7 mmol) and NH_2-NH_2·H_2O (2.34 mL, 39.7 mmol) in ethane-1, 2-diol (80 mL) was stirred at 130°C for 18 hours. The mixture was poured into H_2O (100 mL), extracted with EtOAc (50 mL×3), washed with brine and dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography to give **compound 5I** (1.5 g, 9.8 %) as yellow solid. **^{1}H NMR (400MHz, $CDCl_3$)** δ 8.16 (d, *J* = 5.6 Hz, 1H), 7.86 (d, *J* = 8.4 Hz, 2H), 7.42-7.38 (m, 2H), 7.19-7.11 (m, 4H), 7.00 (d, *J* = 7.6 Hz, 2H).

6-fluoro-3-(4-phenoxyphenyl)-1H-indazole-5-carbonitrile (**5J**)

A mixture of **5I** (1.5 g, 3.91 mmol), $Zn(CN)_2$ (919 mg, 7.83 mmol), $Pd_2(dba)_3$ (1.8 g, 1.95mmol) and DPPF (2.6 g, 4.69 mmol) in DMF (40 mL) was stirred at 100°C for 4 hours. The mixture was poured into H_2O (120 mL), extracted with EtOAc (60 mL×3), washed with brine and dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography to give **compound 5J** (1.8 g, 100 %) as yellow solid. **^{1}H NMR (400MHz, $CDCl_3$)** δ 10.65 (br. s., 1H), 8.31 (d, *J* = 6.0 Hz, 1H), 7.84 (d, *J* = 8.8 Hz, 2H), 7.43-7.32 (m, 2H), 7.23 (d, *J* = 8.8 Hz, 1H), 7.20-7.13 (m, 3H), 7.10 (d, *J* = 7.6 Hz, 2H).

tert-butyl

3-(5-cyano-6-fluoro-3-(4-phenoxyphenyl)-1H-indazol-1-yl)piperidine-1-carboxylate (**5K**)

A mixture of **5J** (1.3 g, 3.95 mmol), **5E** (1.4 g, 3.95 mmol) and Cs_2CO_3 (3.86 g, 11.8 mmol) in DMF (40 mL) was stirred at 90°C for 18 hours. The mixture was poured into H_2O (120 mL), extracted with EtOAc (30 mL×3), washed with brine and dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography to give **compound 5K** (400 mg, 19.7 %) as yellow solid. **^{1}H NMR (400MHz, $CDCl_3$)** δ 8.28 (d, *J* = 6.0 Hz, 1H), 7.83 (d, *J* = 8.6 Hz, 2H), 7.44-7.36 (m, 2H), 7.29 (br. s., 1H), 7.21-7.12 (m, 3H), 7.09 (d, *J* = 7.6 Hz, 2H), 4.42-4.30 (m, 2H), 4.13 (q, *J* = 7.0 Hz, 1H), 2.88 (t, *J* = 11.6 Hz, 1H), 2.49-2.29 (m, 1H), 2.23 (d, *J* = 13.2 Hz, 1H), 1.97 (d, *J* = 12.6 Hz, 1H), 1.79-1.63 (m, 1H), 1.48 (s, 10H).

tert-butyl

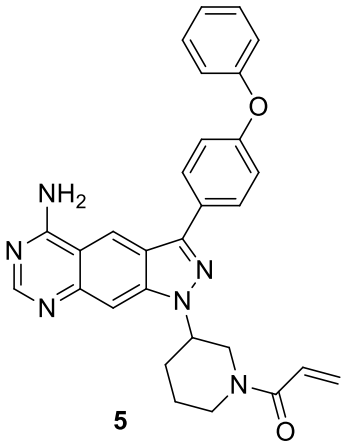
3-(5-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[4,3-g]quinazolin-1-yl)piperidine-1-carboxylate (**5L**)

To a solution of formamidine acetate (210 mg, 2.03 mmol) in DMF (5 mL) was added NaH (162 mg, 4.06 mmol). The reaction mixture was stirred at room temperature for 1 hour, then added **5K** (260 mg, 0.507 mmol). The reaction mixture was stirred at 100°C for 18 hours. The reaction mixture was poured into H_2O (30 mL), extracted with EtOAc (10 mL×3), washed with brine and dried over Na_2SO_4, filtered and concentrated to give **compound 5L** (30 mg, 11 %) as yellow solid. (ESI) m/z: 537 $[M+H]^+$.

3-(4-phenoxyphenyl)-1-(piperidin-3-yl)-1H-pyrazolo[4,3-g]quinazolin-5-amine (**5M**)

A solution of **5L** (30 mg, 0.055 mmol) in 4M HCl/EA (1 mL) was stirred at room temperature for 1 hour. The mixture was concentrated to give **compound 5M** (24 mg, 100 %) as yellow

solid. (ESI) m/z: 437 (M+1).

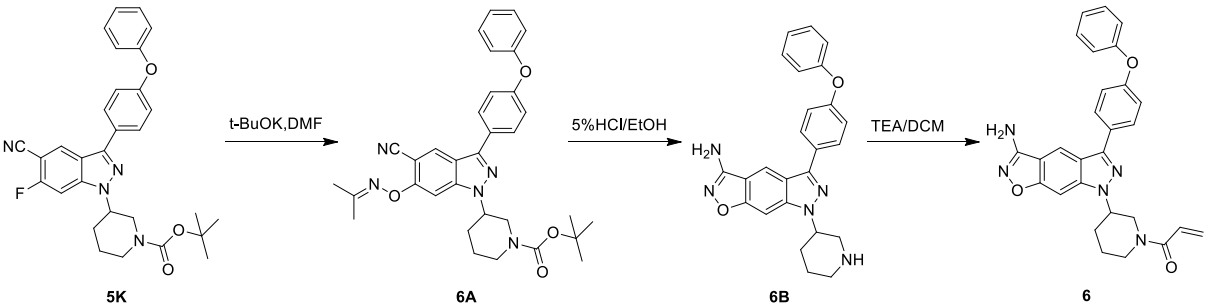


5

1-(3-(5-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[4,3-g]quinazolin-1-yl)piperidin-1-yl)prop-2-en-1-one (**5**)

To a solution of **5M** (24 mg, 0.054 mmol) and Et_3N (0.03 mL, 0.221 mmol) in DCM (5 mL) was added acryloyl chloride (5 mg, 0.054 mmol) drop wise at 0°C under nitrogen atmosphere, the mixture was stirred at room temperature for 30 mins. Water (5 mL) was added and extracted with DCM (5 mL×3), the organic phase was dried over Na_2SO_4, filtered and concentrated, the crude product was purified by prep-HPLC to give **compound 5** (2 mg, 7.4%) as white solid, HPLC purity 92.91%. **^{1}H NMR (400MHz, $CDCl_3$)** δ 9.04 (br. s., 1H), 8.20-8.07 (m, 3H), 77.40-7.37 (m, 2H), 7.22 (d, *J* = 8.0 Hz, 2H), 7.16 (t, *J* = 7.6 Hz, 2H), 7.10 (d, *J* = 7.6 Hz,2H), 6.74- 6.62 (m, 1H), 6.40 (d, *J* = 16.6 Hz, 1H), 5.80 (d, *J* = 10.6 Hz, 1H), 5.01 (br. s., 1H), 4.72 (br. s., 1H), 4.08 (d, *J* = 13.2 Hz, 1H), 3.56 (br. s., 1H), 3.38 (br. s., 1H), 2.34 (d, *J* = 13.2 Hz, 2H), 2.05 (br. s., 1H), 1.40 (br. s., 1H). LC-MS (ESI) m/z: 491 $[M+H]^+$.

Procedures for Preparation of Compound 6:



5K 6A 6B 6

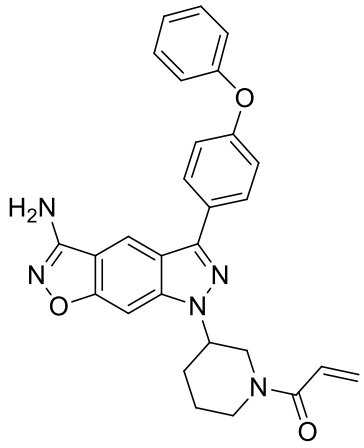
tert-butyl 3-(5-cyano-3-(4-phenoxyphenyl)-6-((propan-2-ylideneamino)oxy)-1H-indazol-1-yl)piperidine-1-carboxylate (**6A**)

A mixture of **5K** (150 mg, 0.292 mmol) and t-BuOK (49 mg, 0.439 mmol) in DMF (5 mL)

was stirred at 0°C for 30 mins, added propan-2-one oxime (32 mg, 0.439 mmol). The reaction mixture was stirred at room temperature for 30 mins. The mixture was poured into H_2O (30 mL), extracted with EtOAc (10 mL×3), washed with brine and dried over Na_2SO_4, filtered and concentrated to give **compound 6A** (100 mg, 60 %) as yellow solid. (ESI) m/z: 566 $[M+H]^+$.

5-(4-phenoxyphenyl)-7-(piperidin-3-yl)-7H-isoxazolo[4,5-f]indazol-3-amine (**6B**)

A solution of **6A** (100 mg, 0.176 mmol) in 5% HCl (7 mL) and EtOH (7 mL) was stirred at 90°C for 18 hours. The mixture was poured into H_2O (30 mL), extracted with EtOAc (10 mL×3), washed with brine and dried over Na_2SO_4, filtered and concentrated to give **compound 6B** (110 mg, 66 %) as yellow solid. (ESI) m/z: 426 $[M+H]^+$.



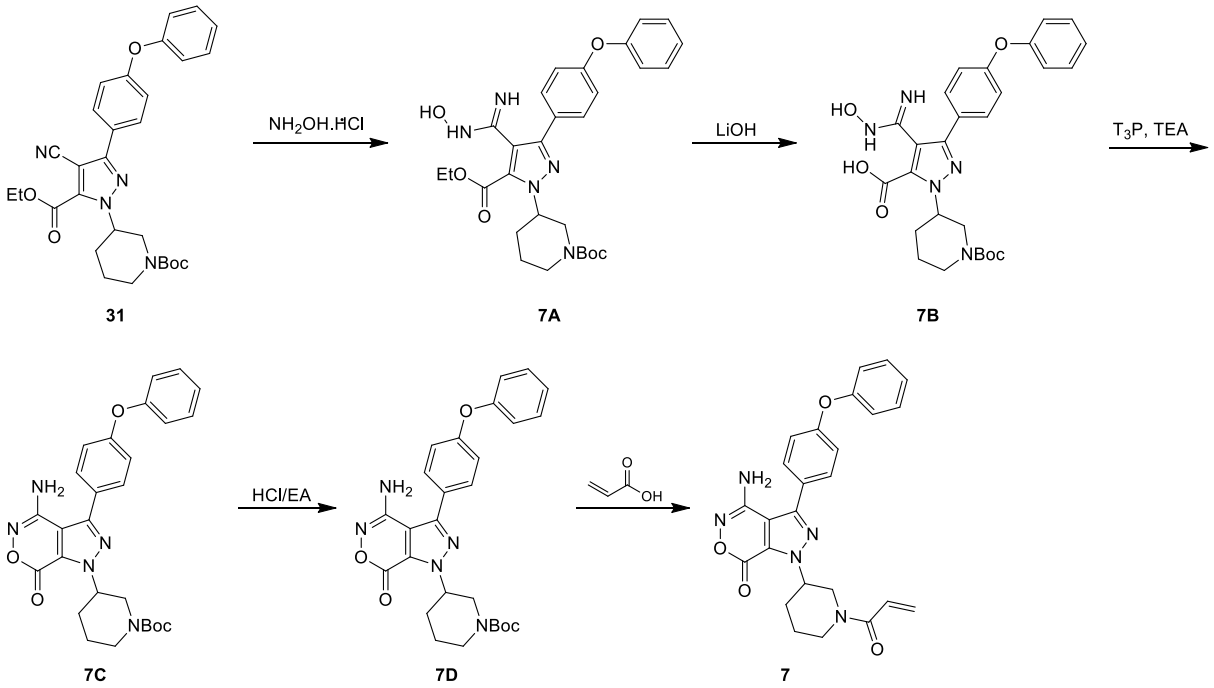
6

1-(3-(3-amino-5-(4-phenoxyphenyl)-7H-isoxazolo[4,5-f]indazol-7-yl)piperidin-1-yl)prop-2-en-1-one (**6**)

To a solution of **6B** (56 mg, 0.073 mmol) and Et_3N (0.03 mL, 0.221 mmol) in DCM (5 mL) was added acryloyl chloride (6.7 mg, 0.073 mmol) drop wise at 0°C under nitrogen atmosphere, the mixture was stirred at room temperature for 30 mins. Water (5 mL) was added and extracted with DCM (5 mL×3), the organic phase was dried over Na_2SO_4, filtered and concentrated, the crude product was purified by prep-HPLC to give **compound 6** (11 mg, 31%) as white solid, HPLC purity 98.66%. **^{1}H NMR (400MHz, $CDCl_3$)** δ 8.07 (br. s., 1H), 7.90 (d, *J* = 8.8 Hz, 2H), 7.40-7.36 (m, 2H), 7.18-7.14 (m, 2H), 7.09 (d, *J* = 8.0 Hz, 2H), 6.70-6.51 (m, 1H), 6.39-6.30 (m, 1H), 5.78- 5.70 (m, 1H), 4.97 (d, *J* = 12.0 Hz, 1H), 4.64 (br. s., 0.5H), 4.22-4.08 (m, 1H), 3.79 (br. s., 1H), 3.32-3.23 (m, 1H), 2.92 (br. s., 0.5H), 2.44 (br. s., 1H), 2.32 (d, *J* = 10 Hz, 1H), 2.06 (d, *J* = 13.6 Hz, 1H), 1.74 (d, *J* = 12.0 Hz, 1H). LC-MS

(ESI) m/z: 480 $[M+H]^+$.

Procedures for Preparation of Compound 7:



(R)-tert-butyl 3-(5-(ethoxycarbonyl)-4-(N-hydroxycarbamimidoyl)-3-(4-phenoxyphenyl)-1H-pyrazol-1-yl)piperidine-1-carboxylate (**7A**)

A solution of **compound 31** (1 g, 1.94 mol), $NH_2OH.HCl$ (153.2 g, 1.63 mol) and TEA (294.5 mg, 2.91 mmol) in EtOH (20 mL) was stirred at 80°C for 16 hours. The mixture was treated with water (20 mL), and extracted with DCM (30 mL×3), the organic layer was dried over Na_2SO_4, filtered and concentrated to give **compound 7A** (a crude mixture, 490 mg) as white solid. (ESI) m/z: 550 $[M+H]^+$.

(R)-1-(1-(tert-butoxycarbonyl)piperidin-3-yl)-4-(N-hydroxycarbamimidoyl)-3-(4-phenoxyphenyl)-1H-pyrazole-5-carboxylic acid (**7B**)

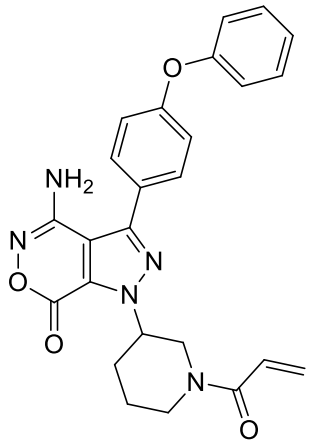
A solution of **7A** (450 mg, 0.82 mmol) and LiOH (103 mg, 2.46 mmol) in THF/EtOH/H_2O (1:1:1, v/v/v, 5mL) was stirred at 14°C for 14 hours. The reaction was detected by TLC and LCMS, the solvent was concentrated to give a crude **compound 7B** (480 mg, crude) as white solid. (ESI) m/z: 522 $[M+H]^+$.

(R)-tert-butyl 3-(4-amino-7-oxo-3-(4-phenoxyphenyl)pyrazolo[4,3-d][1,2]oxazin-1(7H)-yl)piperidine-1-carboxylate (**7C**)

A solution of **7B** (400 mg, 0.768 mmol), T_3P (490 mg, 1.54 mmol) and TEA (233 mg, 2.3 mmol) in DCM (10 mL) was stirred at 14°C for 4 hours. The mixture was treated with water (20 mL) and extracted with DCM (20 mL×3), the organic phase was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography to give **compound 7C** (60 mg, 20%) as white solid. **^{1}H NMR (400MHz, $CDCl_3$)** δ ppm 8.13 (d, *J* = 8.4 Hz, 2H), 7.37 (t, *J* = 7.6 Hz, 2H), 7.14-7.03 (m, 5H), 4.97 (s, 2 H), 4.87 (s, 1H), 4.26 (s, 1H), 4.07 (s, 1 H), 3.43 (t, *J* = 11.6 Hz, 1H), 2.90 (s, 1H), 2.21 (s, 1 H), 1.92 (s, 1H), 1.69 (s, 1H), 1.45 (s,1 H).

(R)-4-amino-3-(4-phenoxyphenyl)-1-(piperidin-3-yl)pyrazolo[4,3-d][1,2]oxazin-7(1H)-one (**7D**)

To a solution of **7C** (56 mg, 0.111 mmol) in DCM (2 mL) was added HCl/EtOAc (5 mL). The reaction mixture was stirred at 14°C for 2 hours. The reaction was detected by TLC and LCMS, the solvent was concentrated to give **compound 7D** (50 mg, 100%) as white solid. (ESI) m/z: 404 $[M+H]^+$.



7

(R)-1-(1-acryloylpiperidin-3-yl)-4-amino-3-(4-phenoxyphenyl)pyrazolo[4,3-d][1,2]oxazin-7(1H)-one (**7**)

To a solution of **7D** (40 mg, 0.099 mmol), HATU (38.2 mg, 0.099 mmol) in DCM (4 mL) was added DIEA (63.8 mg, 0.495 mmol), then the mixture was bubbled with N_2, followed with acrylic acid (7.2 mg, 0.099 mmol), then stirred at 14°C for 2 hours. The mixture was treated with 1N HCl (0.5 mL) and H_2O (3 mL), and extracted with DCM (5 mL×4), the organic phase was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography to give **compound 7** (3 mg, 6.7%) as white solid, HPLC purity 88.6%. **^{1}H**

NMR (400MHz, $CDCl_3$) δ ppm 8.12 (d, *J* = 8.0 Hz, 2H), 7.37 (t, *J* = 8.0 Hz, 2H), 7.14-7.04 (m, 5H), 6.66-6.59 (m, 1H), 6.34 (t, *J* = 7.8 Hz, 1H), 5.74-5.67 (m, 1H), 5.05-4.99 (m, 2H), 4.91-4.89 (m, 1H), 4.59-4.56 (d, *J* = 11.6 Hz, 1H), 4.25-4.22 (d, *J* = 11.2 Hz, 1H), 4.02-3.97 (m, 1H), 3.63-3.58 (m, 1H), 3.22 (s, 1 H), 2.93-2.89 (m, 1H), 2.36-2.27 (m, 2H), 2.02-1.99 (m, 1H), 1.73-1.70 (m, 1H), 1.26 (s, 1 H). LC-MS (ESI) m/z: 458 $[M+H]^+$.

2. *In vitro* Potency

Materials

BTK wild type was acquired from Life technology (Madison, WI). A standard HTRF kit (containing Eu-labeled TK1 antibody, XL665, and biotin conjugated TK1 peptide) were purchased from Cis-Bio International. Plates were read on an Envision multi-label plate reader (PerkinElmer).

Methods

The test compounds were treated in 8 doses, duplicates with 5-fold dilution as the final concentrations were from 10 μM to 0.05 nM.

The enzyme reaction mixture of BTK wild type standard HTRF assay contained 1 nM BTK wild type, 1 μM biotin-TK1 peptide, and 30 μM ATP in a buffer containing 50 mM Hepes (pH 7.5), 10 mM $MgCl_2$, 0.01 mM NaV_3VO_4 at a final volume of 10 μL. The enzyme reaction were carried out at room temperature in black Proxiplate 384-Plus plate (Corning, Costar) within 60 minutes. 5 μL of 0.2 M EDTA were added to quench the reaction and then the detection reagents (5 μL) were added at final concentrations of 2 nM antibody and 62.5 nM XL665. The plates were incubated at room temperature for 60 minutes and then read in the Envision plate reader.

Data analysis

The readouts were transformed into inhibition rate% by the equation of (Min Ratio) / (Max-Min) * 100%. Hence the IC50 data of test compounds were generated by using four parameters curve fitting (Model 205 in XLFIT5, iDBS).

3. DMPK

Rat / mouse PK studies

Male Sprague Dawley rats or female BALB/c nude mouse (SLAC Laboratory Animal Co. Ltd. (Shanghai, China)) were used to evaluate pharmacokinetics (PK) of the compound post single oral gavage or intravenous bolus injection. Formulation of intravenous dosing was prepared in DMSO / 40% HP-beta-CD in saline / saline (5:50:45, v/v/v) as clear solution. Oral formulation for each of the compound was prepared in DMSO / 40% HP-beta-CD in water / water (5:15:80, v/v/v) as clear solution. Blood samples were collected via jugular vein up to 24 hours post dosing, with EDTA-K2 as anticoagulant. The blood samples were processed for plasma by centrifugation at approximately 4°C, 3000g within half an hour of collection. The collected plasma samples were then stored at -80°C until sample analysis. LC-MS/MS was applied for sample analysis. Plasma concentration versus time data were analyzed by non-compartmental approaches using the Phoenix WinNonlin 6.3 software program.

Plasma protein binding

Pooled plasma (pH 7.4 ± 0.1) was applied in the assay. The dialysis membrane (molecular weight cut off 12-14 kDa, Cat# 1101, HT Dialysis LLC) was soaked in ultra pure water at room temperature for approximately 1 hour, seperated into two strips and then soaked in ethanol/water (20:80, v/v) at 2-8°C overnight. Prior to the experiment, the membrane was rinsed with ultra pure water. Test article was prepared at 2 μM as loading solution in blank plasma with DMSO in the final solution at 0.5%. Aliquots of 150 μL of loading solution were loaded in triplicate to the donor side of each dialysis well (Model HTD 96 b, Cat# 1006, HT Dialysis LLC) and dialyzed against an equal volume of dialysis buffer. Then the plate was sealed with cap strips and rotated at approximately 100 rpm in a humidified incubator with 5% CO_2 at 37°C for 4 hours. Finally, each sample was matched with opposite blank buffer or matrix to obtain a final volume of 100 μL with volume ratio of matrix/dialysis buffer (50:50, v/v) in each well and precipated with 300 μL of stop solution. All sample collected plates were shook at 800 rpm for 5 min to mix samples and centrifuged at 20°C, 4000 rpm for 20 min. Aliquots of supernatant (100 μL) in each well were transferred into new 96-well sample plates mixed with 200 μL ultra pure water before subjecting to LC-MS/ MS analysis.

The concentration was calculated using the peak area ratio of analyte and internal standard.

The results were calculated by the following equations:

% Unbound = 100 * FC / TC, % Bound = 100 - % Unbound

% Recovery = 100 * (FC+ TC) / T0

where

TC = Total compound concentration as determined by the calculated concentration on the retenate side of the membrane

FC = Free compound concentration as determined by the calculated concentration on the dialysate side of the membrane

T0 = Total compound concentration as determined before dialysis

CYP inhibition (%)

Compound was incubated at 7 concentrations from 0.05 to 50 μM with human liver microsomes with protein concentration at 0.253 mg/mL. α-Naphthoflavone, Sulfaphenazole, (+)-N-3-benzylnirvanol, Quinidine and Ketoconazole were used as positive control. The mixture of test compound or positive control were pre-warmed with human liver microsomes at 37°C for 10 min. Phenacetin, Diclofenac, S-mephenytoin, Dextromethorphan and Midazolam were prepared in a mixture used as substrate of CYP1A2, 2C9, 2C19, 2D6 and 3A4, respectively.After addition of 5-in-1 substrates solution and cofactor NADPH, the mixture were incubated for another 10 min.The samples were finally quenched with stop solution containing internal standard and centrifuged at 4000 rpm (Centrifuge, 5810R Eppendorf) atroom temperature for 20 minutes. Then supernatant was diluted with purified water with an appropriate ratio, which was shaken at 1000 rpm (Titer plate shaker, Thermo) for 10 minutes to mix well and for LC-MS/MS injection. The formation of the metabolite Acetaminophen, 4'-hydroxy diclofenac, 4'-hydroxy mephenytoin, Dextrorphan and 1'-hydroxy midazolam were determined by LC-MS/MS and IC50 values were calculated. SigmaPlot (V.11) was used to plot the mean CYP Activity (% VC) versus the test compound concentrations with non-linear regression analysis. IC50 values were determined using 3- or 4- parameter logistic equation. IC50 values were reported as “> 100 μM” when % inhibition at highest concentration (100 μM) was less than 50%.

Equation for three parameters	Equation for four parameters

logistic sigmoidal curve	logistic sigmoidal curve
$y = \frac{max}{1+\left(\frac{x}{IC_{50}}\right)^{-hillslope}}$	$y = min + \frac{max-min}{1+\left(\frac{x}{IC_{50}}\right)^{-hillslope}}$

The max represents the maximal enzyme activity and min is the minimal enzyme activity. x represents the concentrations of test compound and y is the enzyme activity at x. Hillslope is the slope factor and IC50 is the concentration to achieve the half maximal inhibition. Four parameters equation will be used when min is between ±10%, otherwise three parameters equation will be used.

4. hERG Channel Test

Protocol of hERG channel test on QPatchHTX

Objective

To evaluate the effects of test compounds on the hERG potassium channels, using the automated patch clamp method (QPatchHTX).

Cell Culture

CHO cells stably expressing hERG potassium channels from Aviva Biosciences were used for this test. The cells were cultured in a humidified and air-controlled (5% CO2) incubator at 37 °C. CHO hERG Culture Medium was summarized below:

Reagent	Supplier	Catalog Number	Volume (mL)
F12 Hams	Invitrogen	31765-092	500
FBS	Corning	35-076-CV	50
G418/Geneticin	Invitrogen	10131-027	1
Hygromycin B	Invitrogen	10687-010	1

Preparation of Cells

The CHO cells which were at least two days after plating and more than 75% confluent would be used for experiments. Before testing, cells were harvested using TrypLE and resuspended in the extracellular solution at the room temperature. Extracellular solution was the solution used to bath the recorded cell (see Part 2.3).

Recording Solutions

For the electrophysiological recordings the following solutions were used (Table 2). The extracellular solution was prepared at least once every one month. The intracellular solution was prepared in batches aliquoted, and stored at −20 °C until used. Composition of External and Internal Solutions was summarized below:

Reagent	External Solution(mM)	Internal Solution(mM)
NaCl	145	-
KCl	4	120
KOH	-	31.25
CaCl2	2	5.374
MgCl2	1	1.75
Glucose	10	-

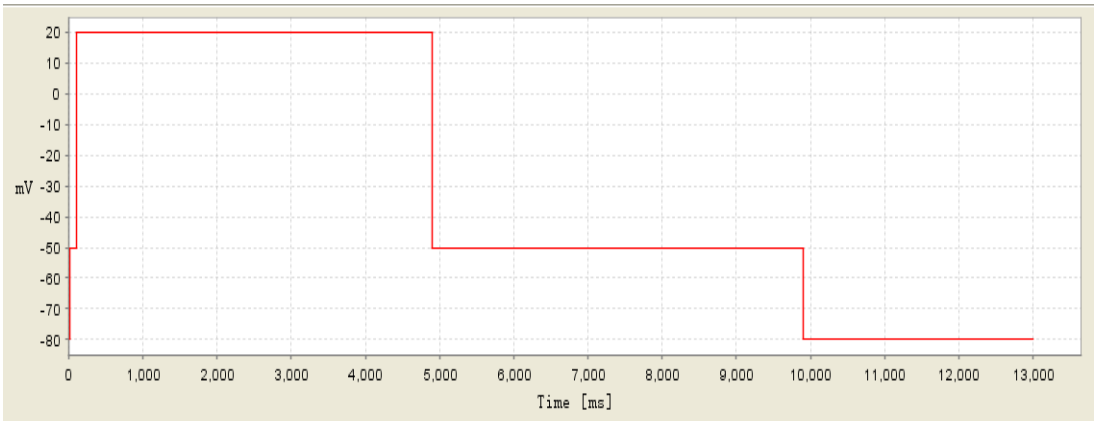
Na2ATP	-	4
HEPES	10	10
EGTA	-	10
pH	7.4 with NaOH	7.2 with KOH
Osmolarity	~295mOsm	~285mOsm

Preparation of Compounds

Test compounds and positive control Amitriptyline were dissolved in 100% DMSO to obtain stock solutions for different test concentrations. Then the stock solutions were further diluted into extracellular solution to achieve final concentrations for testing. Visual check for precipitation was conducted before testing. Final DMSO concentration in extracellular solution was not more than 0.30% for the test compounds and Amitriptyline (positive) control.

Voltage Protocol

Voltage command protocol: From this holding potential of -80 mV, the voltage was first stepped to -50 mV for 80 ms for leak subtraction, and then stepped to +20 mV nfor 4800 ms to open hERG channels. After that, the voltage was stepped back down to -50 mV for 5000 ms, causing a "rebound" or tail current, which was measured and collected for data analysis. Finally, the voltage was stepped back to the holding potential (-80 mV, 3100 ms). This voltage command protocol was repeated every 15000 msec. This command protocol was performed continuously during the test (vehicle control and test compound). Commander voltage protocol for electrophysiological study was shown below:



QPatchHTX Whole-cell Recording

hERG QPatchHTX assay was conducted at room temperature. The whole-cell protocols,

voltage protocols and application protocols were established with QPatch Assay Software 5.6(Sophion Bioscience).

Three additions of 5 µl of the vehicle were applied, followed by 30 runs of voltage protocol for a baseline period. Then the ascending doses of each compound were added with three repetitions (5 µl*3). The exposure of test compound at each concentration was no less than 5 minutes. The recording for the whole process had to pass the quality control or the well was abandoned and the compound was retested, all automatically set by QPatch Assay Software. Four concentrations (0.24 µM, 1.20 µM, 6.00 µM, 30.00 µM) were tested for each compound. Minimum 2 replicates per concentration were obtained.

Data Analysis

Within each well recording, percent of control values were calculated for each test compound concentration current response based on peak current in presence of vehicle control (current response/ peak current) ×100%. The Dose-Response curves were fit to the standard Hill equation using a fixed baseline response (Ib) at 100% of latest vehicle period and full response (Ifr) at 100% of value0.0 as shown below:

$$I(C) = Ib+(Ifr-Ib)*cn/(IC50n+cn)$$

Where c is the compound concentration and n is the Hill Slope.

Curve-fitting and IC50 calculations were performed by QPatch Assay Software. If the inhibition obtained at the lowest concentration tested was over 50%, or at the highest concentration tested was less than 50%, we reported the IC50 as less than lowest concentration, or higher than highest concentration, respectively.

5. *In vivo* Study

General procedure for CIA-induced mouse RA model

Initial Immunization

Emulsion preparation: bovine collagen was dissolved in acetic acid and prepared into bovine collagen solution with a concentration of 8 mg/mL at 4°C overnight. An equal volume of CFA (1 mg/mL) was added to the bovine collagen solution to form a final 4 mg/mL bovine collagen CFA mixture, which was homogenized on wet ice with a high-speed homogenizer until white emulsion was formed.

Immunization: The animals were anesthetized and were injected subcutaneously by emulsion (50 μL) about 2-3 cm away from the root of the tail. Day 0 was the first Day of immunization. After initial immunization, the animals were weighed weekly and recorded until day 21.

Secondary immunization

At day 21, the animals were immunized twice, using the same method as above.

After the second immunization, the animal weight was measured and the animal limb clinical symptom score was performed 3 times per week. Scoring criteria was summarized below:

Scoring	Clinical symptom
0	No erythema or redness
1	Erythema or mild redness near the proximal tarsal bone or the ankle or metatarsal, and erythema on one toe
2	Slight erythema and swelling of the ankle joint and metatarsal, or more than two toes are erythema
3	Moderate erythema and swelling of the ankle, wrist and metatarsal
4	The ankle, wrist, metatarsal and toes are all severely inflamed

Dose

On day 28, when the clinical incidence score of the animals reached 1.7 on average, all the animals were divided into groups, so that the body weight, incidence score and incidence (i.e. the percentage of the number of animal incidence in the total number) of each group were basically consistent. Intragastric administration was started, once a day, with a dose of 10 mL/kg per animal. The experimental design was summarized below:

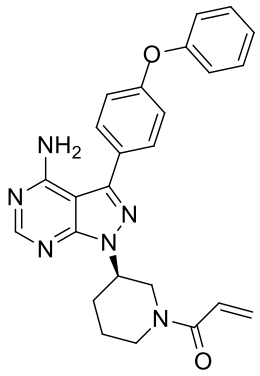
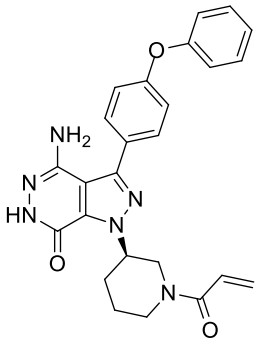
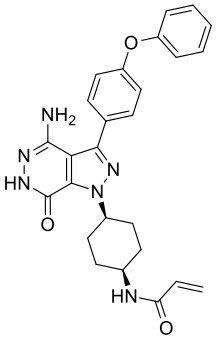
Group	N	Treatment	Dose		Route	Duration time	Frequency
			mL/kg	mg/kg			

1	10	Normal	--	--	--	--	--
2	10	Vehicle*	10	-	p.o.	14 days	QD
3	10	Compound 8	10	3	p.o.	14 days	QD
4	10	Compound 8	10	10	p.o.	14 days	QD
5	10	Ibrutinib	10	10	p.o.	14 days	QD
*Vehicle: DMSO / 40% HP-β-CD in saline / saline = 5:15:80 (v/v/v)							

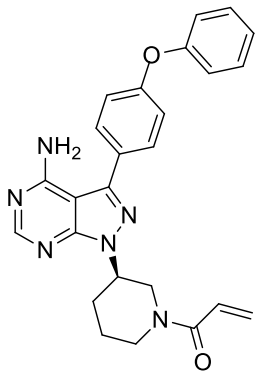
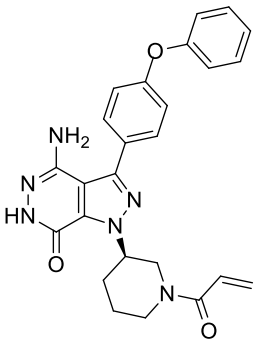
Data statistics

Animal weight, morbidity scores, and morbidity.

6. Compound 8 and Compound 16 *vs* Ibrutinib

Compound ID	**Ibrutinib**	**Compound 8**	**Compound 16**
Structure			
MW, tPSA, ClogP	440.50, 95.88, 4.06	456.50, 112.62, 2.07	470.52, 121.41, 1.85
BTK IC_{50} (nM)	2.0	2.1	1.6
Solubility (μM) (KS, pH=2.5, 6.5, 7.4)	160.1, 53.1, 47.1	35.1, 33.9, 26.5	<1.00, <1.00, <1.00
PPB% (human, rat, mouse)	96.9%, 97.8%, 96.2%	97.5%, 99.7%, 95.3%	98.2%, 99.6%, 95.1%

7. Kinase Selectivity Assay

Compound ID		**Ibrutinib**	**Compound 8**
Structure			
Kinase	**ATP**	**IC_{50} (nM)**	**IC_{50} (nM)**
BLK	5μM	<0.5	<0.5
BMX/ETK	5μM	<0.5	<0.5
EGFR	2μM	44.2	30.1
ERBB4/HER4	5μM	<0.5	<0.5
ITK	5μM	27.5	29.7
TEC	5μM	7.02	3.0
TXK	1μM	1.88	0.9

Materials

Base Reaction buffer; 20 mM Hepes (pH 7.5), 10 mM $MgCl_2$, 1 mM EGTA, 0.02% Brij35, 0.02 mg/ml BSA, 0.1 mM Na_3VO_4, 2 mM DTT, 1% DMSO.

Required cofactors are added individually to each kinase reaction.

Methods

Compounds were tested in 10-dose IC_{50} mode with a 3-fold serial dilution starting at 10μM. Control compound, staurosporine, was tested in 10-dose IC_{50} mode with 4-fold serial dilution starting at 20 μM. Reactions were carried out at 1, 2, or 5 μM ATP.

1. Prepare substrate in freshly prepared Reaction Buffer.
2. Deliver any required cofactors to the substrate solution above.
3. Deliver kinase into the substrate solution and gently mix.
4. Deliver compounds in 100% DMSO into the kinase reaction mixture by Acoustic.

technology (Echo550; nanoliter range), incubate for 20 min at room temp.

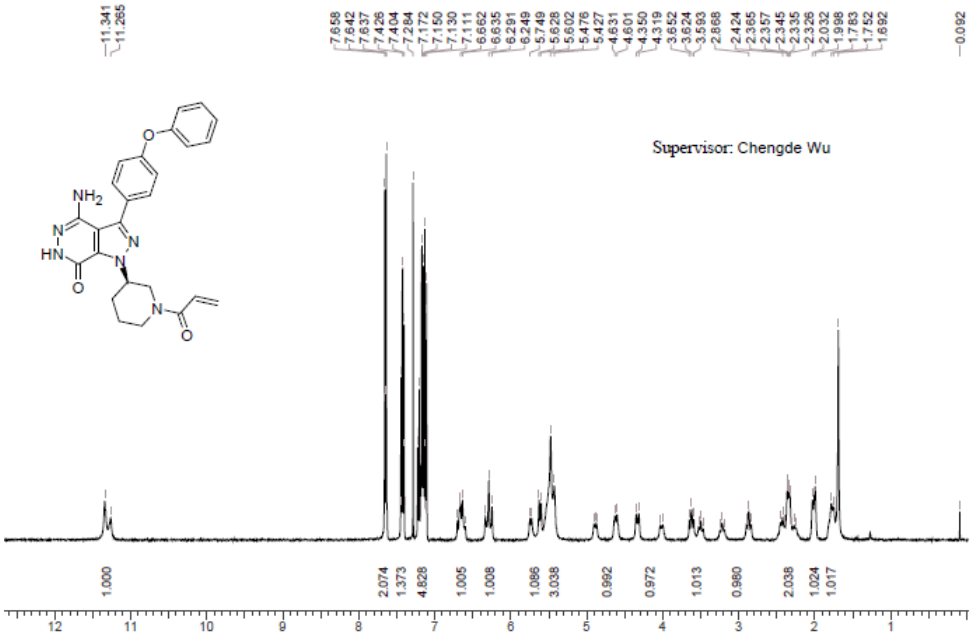
5. Deliver 33P-ATP into the reaction mixture to initiate the reaction.

6. Incubate for 2 hours at room temperature.

7. Detect kinase activity by P81 filter-binding method.

8. Spectra of Compound 8

^{1}H NMR



Acquisition Time (sec)	2.9884
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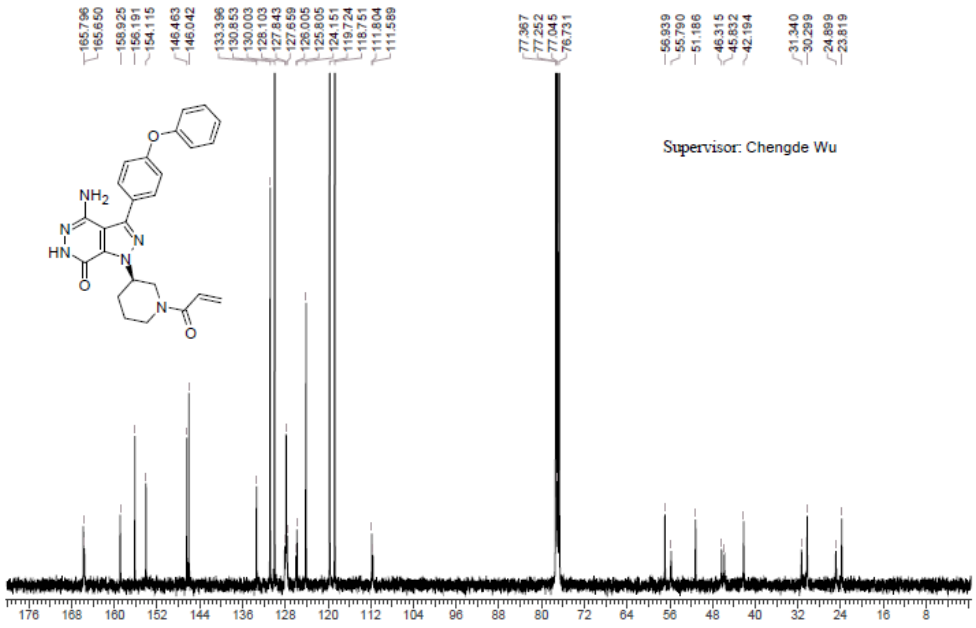
Date	24 Oct 2019 11:57:48
Frequency (MHz)	400.1300
Nucleus	1H
Number of Transients	8
Origin	spect
Original Points Count	24576
Owner	nmrsu
Points Count	32768
Pulse Sequence	zg30
Receiver Gain	181.00
SW(cyclical) (Hz)	8223.68
Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	2470.9683
Spectrum Type	standard
Sweep Width (Hz)	8223.43
Temperature (degree C)	27.000

^{13}C NMR



Acquisition Time (sec)	1.2976
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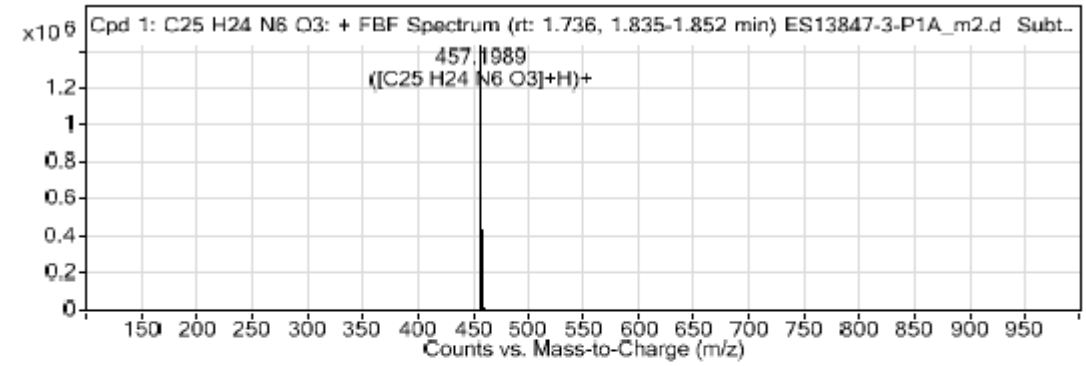
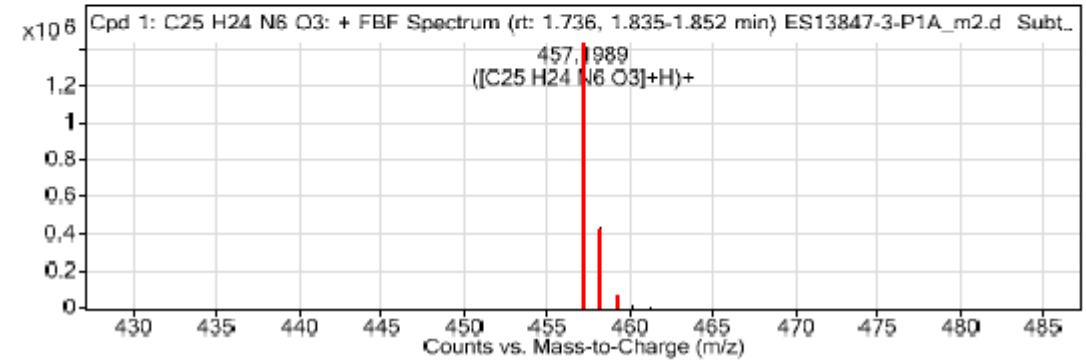
Frequency (MHz)	100.6128
Nucleus	13C
Number of Transients	3000
Origin	spect
Original Points Count	32768
Owner	nmrsu
Points Count	32768
Pulse Sequence	zgpg30
Receiver Gain	172.43
SW(cyclical) (Hz)	25252.53
Solvent	CHLOROFORM-d
Spectrum Offset (Hz)	11067.4014
Spectrum Type	standard
Sweep Width (Hz)	25251.75
Temperature (degree C)	24.993

HRMS

MS Spectrum



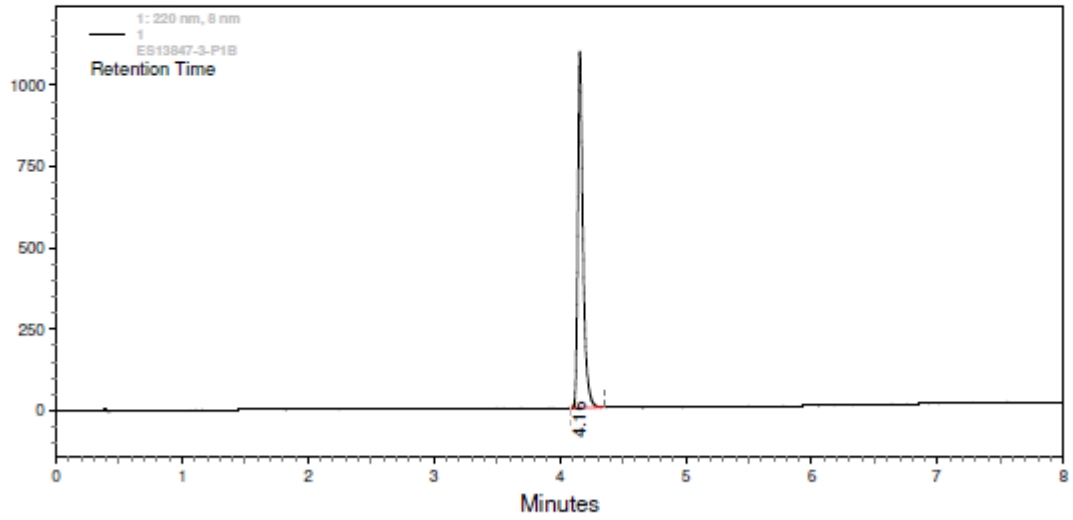
MS Zoomed Spectrum



HPLC

HPLC REPORT

Compound ID	: 1
Filename/Sample ID	: D:\data\2019\1910\191024\ES13847-3-P1B
Method Name	: D:\method\10-80AB_8min.met
Location&Inj.vol	: 61 Injection vol.: 0.5
Instrument & Column	: HPLC-BJ Ultimate 3.0*50mm 3um
Run Time	: 24/10/2019 18:22:31
Print Time	: 24/10/2019 18:32:58



1: 220 nm, 8 nm

Retention Time	*Height*	*Area*	*Area Percent*
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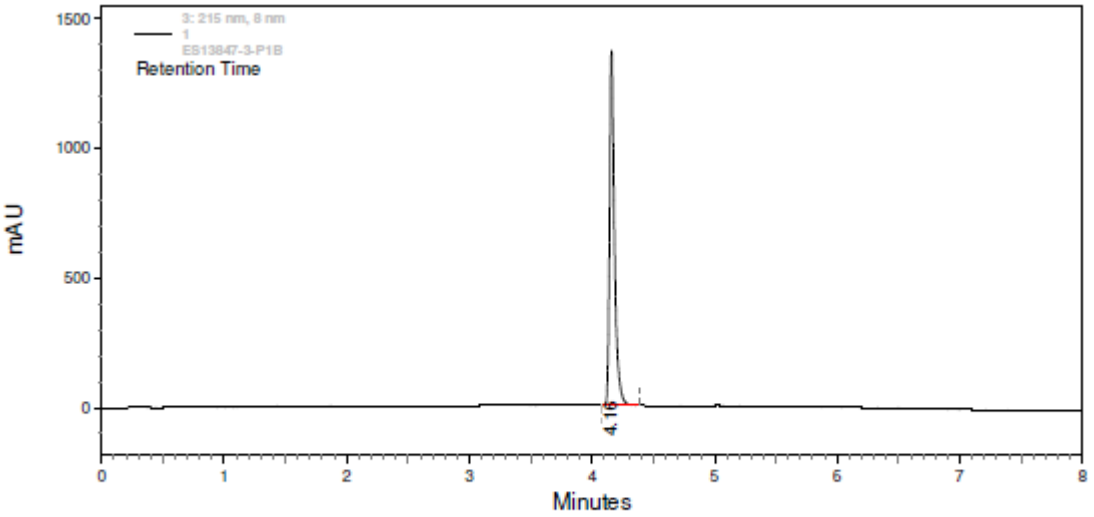

3: 215 nm, 8 nm

Retention Time	*Height*	*Area*	*Area Percent*
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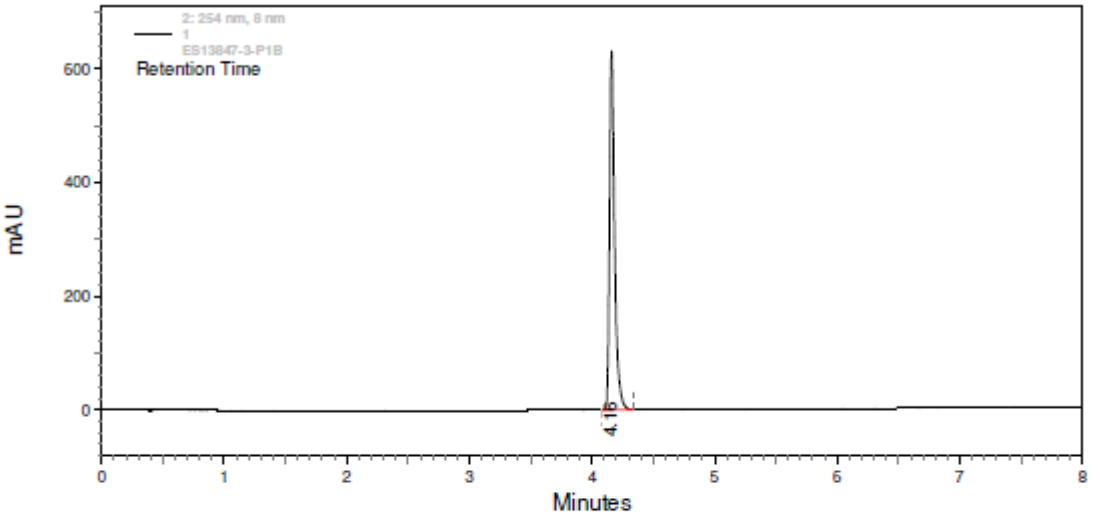

2: 254 nm, 8 nm

Retention Time	*Height*	*Area*	*Area Percent*
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