Quantum Computing-Enhanced Algorithm Unveils Novel Inhibitors for KRAS Supplementary Materials

S1. Chemistry

S1.1. General Synthetic Procedures

Unless otherwise noted, solvents and reagents were obtained from commercial suppliers and were used without further purification. Structures of the target compounds in this work were assigned by use of NMR and MS spectroscopy. Proton nuclear magnetic resonance (¹H NMR) spectra were recorded on a Bruker AVANCE III HD 400 MHz spectrometer and were referenced in parts per million (ppm, δ) relative to the residual solvent peak in the indicated solvent. In the spectral data reported, the format (δ) chemical shift (multiplicity, coupling constants (J) values in Hz, integration) was used with the following abbreviations of splitting patterns: s= singlet, brs= broad singlet, d= doublet, t= triplet, q= quartet, and m= multiplet. The LC separation was done on a SunFire-C18, 5 μm, 50 × 4.6 mm column at a flow rate of 2 ml /minute. Mobile phase A was water with 0.1% FA and mobile phase B was acetonitrile with 0.1% FA. The gradient started at 10% mobile phase B increase to 30% B for 0.40 min, increase to 95% B within 1.60 min, 95% B for 0.90 min, back to 10% B within 0.01 min. LC column temperature was 40 °C. Mass spectrometric (MS) analyses were performed on a Shimadzu LCMS2020 mass spectrometer using electrospray ionization (ESI), a Sun Fire C¹⁸ 4.6× 50 mm column, eluting with a gradient (2.5 min) of acetonitrile (10% to 95%) in water (both containing 0.1% formic acid). High resolution mass spectrometry was performed on Thermo Scientific Q Exactive HF Orbitrap-FTMS. Prep-HPLC was carried out on a C¹⁸ reversed-phase preparative high-performance liquid chromatography column using a Waters system with a LC-20AP solvent pump, SPD-20A detector and a X bridge C¹⁸ 250 × 19 mm I.D. 10 μm column using MeCN/H₂O gradients). Preparative SFC separation was carried out on chiral SFC (column: DAICEL CHIRALPAK OJ (3.0 mm × 100 mm, 3 μm); mobile phase: [MeOH-DEA]; B%: 5%-40%, 2.7; 4.8min). The purity of all the final compounds was greater than 95% by Agilent 1260 HPLC analysis.

S1.1 Preparation of ISM061-1

Scheme S1 Preparation of ISM061-1

Step 1: Preparation of Compound 1-1

To a solution of 2,4-dichloro-5-ethylpyrimidine (5.00 g, 28.20 mmol) in DMF (50 mL) were added tributyl(1-ethoxyvinyl)stannane (11.22 g, 31.1 mmol) and bis (triphenylphosphine) palladium(II) chloride (0.99 g, 1.41 mmol). After the addition, the mixture was degassed and purged with N2 for 3 mins and then stirred at 85 °C for 4 hrs. After cooling to 20 °C, The reaction solution was poured into water (50 mL) and extracted with EtOAc (50 mL \times 3). The combined organic layers were washed with brine (50 mL \times 2), dried over Na2SO4, filtered and concentrated under reduced pressure to give a residue, which was purified by flash silica gel chromatography (SiO2, ethyl acetate: petroleum ether = 0% - 10%) to afford the title compound (5.00 g, 83% yield) as colorless oil. LCMS(ESI): m/z 213.2 [M+H]+.

Step 2: Preparation of Compound 1-2

To a solution of 2-chloro-4-(1-ethoxyvinyl)-5-ethylpyrimidine (5.00 g, 23.51 mmol) in THF (50 mL) was added HCl (20 mL, 6 M) dropwise. After addition, the mixture was stirred at 25 °C for 2 hrs. The residue was adjusted pH = $9 \sim 10$ with saturated Na2CO3 at 0 °C and extracted with EtOAc (25 mL \times 3). The combined organic layers were washed with brine (40 mL \times 2), dried over Na2SO4, filtered and concentrated under reduced pressure to give a residue, which was purified by flash silica gel chromatography (SiO2, ethyl acetate: petroleum ether = 0% - 10%) to afford the title compound (2.50 g, 58% yield) as colorless oil. LCMS (ESI): m/z 185.3 [M+H]+.

Step 3: Preparation of Compound 1-3

To a solution of 1-(2-chloro-5-ethylpyrimidin-4-yl)ethan-1-one (2.50 g, 13.54 mmol) in MeOH (50 mL) were added 2,6-difluoroaniline (2.10 g, 16.25 mmol) and AcOH (0.78 mL, 13.54 mmol). After addition, the mixture was stirred at 25 °C for 30 min. And then sodium cyanoborohydride (2.13 g, 33.9 mmol) was added at 0 °C. The resulting mixture was stirred at 25 °C for 7 hrs. The reaction solution was poured into water (30 mL) and extracted with DCM (30 mL × 3). The combined organic layers were washed with brine (25 mL × 2), dried over Na2SO4, filtered and concentrated under reduced pressure to give a residue, which was purified by flash silica gel chromatography (SiO2,

ethyl acetate: petroleum ether = 0% - 7%) to afford the title compound (1.00 g, 25% yield) as yellow oil. LCMS (ESI): m/z 298.1 [M+H]+.

Step 4: Preparation of Compound 1-4

To a solution of N-(1-(2-chloro-5-ethylpyrimidin-4-yl)ethyl)-2,6-difluoroaniline (300.0 mg, 1.01 mmol) in 1,4-dioxane (4 mL) were added tert-butyl 3-(hydroxymethyl)azetidine-1-carboxylate (226.0 mg, 1.21 mmol), Cs2CO3 (985.0 mg, 3.02 mmol), CyPF-tBu (84.0 mg, 0.15 mmol) and Pd2(dba)3 (92.0 mg, 0.10 mmol). After addition, the mixture was degassed and purged with N2 for 2 mins and then stirred at 105 °C for 5 hrs under N2. After cooling to 20 °C, the reaction solution was poured into water (50 mL) and extracted with EtOAc (40 mL \times 3). The combined organic layers were washed with brine (20 mL \times 2), dried over Na2SO4, filtered and concentrated under reduced pressure to give a residue, which was purified by flash silica gel chromatography (SiO2, ethyl acetate: petroleum ether = 0% - 22%) to afford the title compound (400 mg, 89.0% yield) as yellow oil. LCMS(ESI): m/z 449.3 [M+H]+.

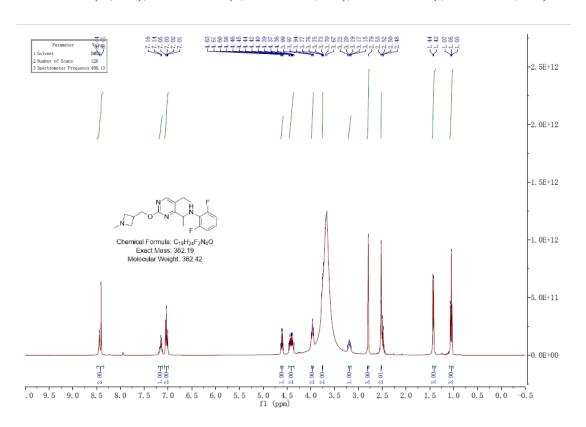
Step 5: Preparation of Compound 1-5

To a solution of tert-butyl 3-(((4-(1-((2,6-difluorophenyl)amino)ethyl)-5-ethylpyrim idin-2-yl)oxy)methyl)azetidine-1-carboxylate (250.0 mg, 0.56 mmol) in DCM (2 mL) was added TFA (0.5 mL, 6.49 mmol) at 0 °C. After addition, the mixture was stirred at 25 °C for 2 hrs. The reaction solution was concentrated under reduced pressure to afford the tittle compound (180.0 mg, 0.52 mmol), which was used in next step directly without further purification. LCMS(ESI): m/z 349.2 [M+H]+.

Step 6: Preparation of ISM061-1

To a solution of N-(1-(2-(azetidin-3-ylmethoxy)-5-ethylpyrimidin-4-yl)ethyl)-2,6-difluoroaniline (130.0 mg, 0.37 mmol) in MeOH (2 mL) were added formaldehyde (0.01 mL, 0.45 mmol) and sodium cyanoborohydride (46.9 mg, 0.75 mmol). After addition, the mixture was stirred at 25 °C for 1 hr. The reaction solution was poured into water (20 mL) and extracted with DCM (25 mL \times 3). The combined organic layers were washed with brine (25 mL \times 2), dried over Na2SO4, filtered and concentrated under reduced pressure to give a residue, which was purified by pre-HPLC (SHIMADZU, LC-20AP, Column: YMC Triart C18, 20 \times 250 mm, 5 μ m; Mobile phase: from 20 % to 31 % ACN [0.1% FA]; Flow rate: 15 mL/min; Retention Time: 15 min of 20 min) to afford the title compound (35.0 mg, 26% yield) as yellow oil. LCMS (ESI): m/z 363.3 [M+H]+; 1H NMR (400 MHz, DMSO-d6) δ 8.44 – 8.41 (m, 2H), 7.18 – 7.12 (m, 1H), 7.05 –7.01 (m, 2H), 4.63 – 4.58 (m,

1H), 4.46 - 4.36 (m, 2H), 3.99 - 3.94 (m, 2H), 3.77 - 3.67 (m, 2H), 3.22 - 3.15 (m, 1H), 2.79 (s, 3H), 2.50 - 2.47 (m, 2H), 1.44 - 1.42 (d, J = 8.0 Hz, 3H), 1.07 - 1.03 (t, J = 8.0 Hz, 3H).



S1.2. Preparation of ISM061-3-2

Scheme S2 Preparation of ISM061-3-2

Step 1: Preparation of Compound 3-1

To a solution of 2,4-dichloro-5-methylpyrimidine (10.00 g, 61.30 mmol) in isopropyl alcohol (200 mL) were added potassium trifluoro(vinyl)borate (9.04 g, 67.50 mmol), TEA (8.55 mL, 61.3 mmol) and Pd(dppf)Cl₂ (2.24 g, 3.07 mmol). The resulting mixture was stirred at 75 °C for 12 hrs under N₂. After cooling to 20 °C, the reaction solution was poured into water (40 mL) and extracted with EtOAc (50 mL \times 3). The combined organic layers were washed with brine (50 mL \times 2), dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified

by column chromatography (SiO₂, ethyl acetate: petroleum ether = 1: 100) to afford **Compound 3-1** (10.00 g, 78% yield) as yellow oil. LCMS (ESI⁺): 155.2 [M+H]⁺.

Step 2: Preparation of Compound 3-2

To a solution of 2-chloro-5-methyl-4-vinylpyrimidine (10.00 g, 64.70 mmol) in THF (200 ml) and water (200 mL) were added potassium osmate dihydrate (2.38 g, 6.47 mmol) and sodium periodate (41.50 g, 194.00 mmol). And then the mixture was stirred at 25 °C for 2 hrs. The reaction mixture was quenched by the addition of ice-water (200 mL) at 0 °C and extracted with EtOAc (200 mL × 3). The combined organic layers were washed with brine (250 mL × 2), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, ethyl acetate: petroleum ether = 1: 100) to afford the **Compound 3-2** (2.50 g, 22% yield) as a gray solid. ¹H NMR (400 MHz, Chloroform-d) δ 10.06 (s, 1H), 8.63 (s, 1H), 2.60 (s, 3H).

Step 3: Preparation of Compound 3-3

To a solution of 2-chloro-5-methylpyrimidine-4-carbaldehyde (500.0 mg, 3.19 mmol) in MeOH (10 mL) were added 2-methoxy-6-(trifluoromethyl)aniline (1.00 g, 5.23 mmol) and AcOH (0.03 mL, 0.52 mmol). After addition, the mixture was stirred at 25 °C for 12 hrs under N₂. Then, sodium cyanoborohydride (973.0 mg, 15.69 mmol) was added at 0 °C. The resulting mixture was stirred at 25 °C for 4 hrs. The reaction solution was poured into water (20 mL) and extracted with DCM (20 mL × 3). The combined organic layers were washed with brine (10 mL × 2), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO₂, ethyl acetate: petroleum ether = 1: 5) to afford **Compound 3-3** (220.0 mg, 8% yield) as a white solid. LCMS (ESI⁺): 332.0 [M+H]⁺. ¹H NMR (400 MHz, Chloroform-d) δ 8.33 (s, 1H), 7.19 - 7.16 (m, 1H), 7.03 - 7.01 (m, 1H), 6.96 - 6.92 (m, 1H), 4.50 - 4.49 (d, J = 4.0 Hz, 2H), 3.91 (s, 3H), 2.23 (s, 3H).

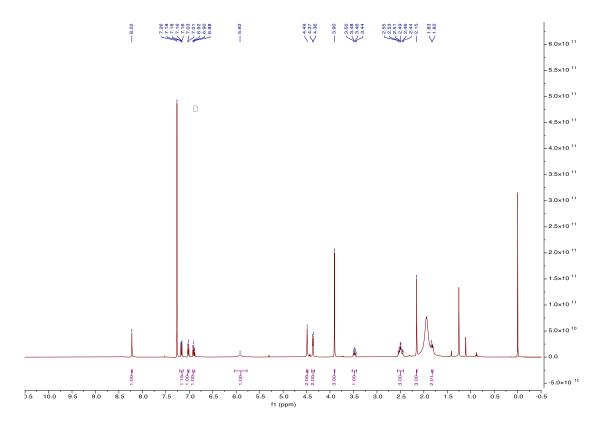
Step 4: Preparation of Compound 3-4

To a solution of **Compound 3-3** (220.0 mg, 0.66 mmol) in 1,4-dioxane (5 mL) were added *tert*-butyl (3-hydroxycyclobutyl)carbamate (124.0 mg, 0.66 mmol), Cs₂CO₃ (648.0 mg, 1.99 mmol), RuPhos (46.4 mg, 0.10 mmol) and Pd₂(dba)₃ (60.7 mg, 0.066 mmol). After addition, the mixture was stirred at 100 °C for 12 hrs under N₂. After cooling to 20 °C, the reaction solution was poured into water (20 mL) and extracted with EtOAc (20 mL × 3). The combined organic layers were washed with brine (20 mL × 2), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue.

The residue was purified by prep-TLC (SiO₂, ethyl acetate: petroleum ether = 1: 3) to afford **Compound 3-4** (40.0 mg, 7% yield) as yellow oil. LCMS (ESI⁺): 497.2 [M+H]⁺.

Step 5: Preparation of ISM061-3-2

To a solution of **Compound 3-4** (35.0 mg, 0.07 mmol) in DCM (2 mL) was added TFA (0.1 mL, 1.30 mmol) dropwise at 0 °C. After addition, the mixture was stirred at 25 °C for 30 min. The reaction mixture was concentrated under reduced pressure to give a residue, which was purified by pre-HPLC (SHIMADZU, LC-20AP, Column: YMC Triart C¹⁸, 20 × 250 mm, 5 μ m; Mobile phase: from 25 % to 55 % ACN [0.1% NH₃HCO₃]; Flow rate: 15 mL/min; Retention Time: 25 min of 35 min) to afford **ISM061-3-2** (4.0 mg, 14% yield) as a white solid. LCMS(ESI⁺): 397.3 [M+H]⁺. ¹H NMR (400 MHz, Chloroform-*d*) δ 8.22 (s, 1H), 7.17 (d, *J* = 8.1 Hz, 1H), 7.02 (d, *J* = 8.0 Hz, 1H), 6.90 (t, *J* = 8.0 Hz, 1H), 6.03 – 5.77 (m, 1H), 4.49 (s, 2H), 4.40 – 4.33 (m, 2H), 3.90 (s, 3H), 3.53 – 3.43 (m, 1H), 2.56 – 2.44 (m, 3H), 2.15 (s, 3H), 1.84 – 1.80 (m, 2H).



S1.3. Preparation of ISM061-6

Scheme S3 Preparation of ISM061-6

Step 1: Preparation of Compound 6-1

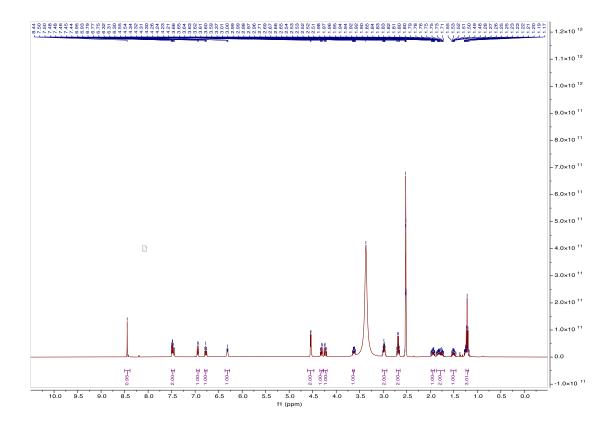
According to the preparation of **Compound 3-3**, **Compound 6-1** was synthesized with the corresponding substrates. LCMS(ESI⁺): 316.1 [M+H]⁺. 1 H NMR (400 MHz, DMSO-d₆) δ 8.62 (s, 1H), 7.50 - 7.43 (m, 2H), 6.92 - 6.90 (d, J = 8.0 Hz, 1H), 6.80 - 6.75 (m, 1H), 6.23 - 6.21 (m, 1H), 4.61 - 4.59 (d, J = 8 Hz, 2H), 2.79 - 2.73 (m, 2H), 1.25 - 1.21 (t, J = 8.0 Hz, 3H).

Step 2: Preparation of Compound 6-2

To a solution of N-((2-chloro-5-ethylpyrimidin-4-yl)methyl)-2-(trifluoromethyl) aniline (100.0 mg, 0.32 mmol) in 1,4-dioxane (2 mL) was added *tert*-butyl 2-(hydroxy methyl)pyrrolidine-1-carboxylate (127.0 mg, 0.63 mmol), Cs₂CO₃ (310.0 mg, 0.95 mmol), CyPF-tBu (35.1 mg, 0.06 mmol) and Pd₂(dba)₃ (29.0 mg, 0.03 mmol). After addition, the mixture was stirred at 100 °C for 12 hrs under N₂. After cooling to 20 °C, the reaction solution was poured into water (20 mL) and extracted with EtOAc (20 mL × 3). The combined organic layers were washed with brine (20 mL × 2), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO₂, ethyl acetate: petroleum ether = 1: 4) to afford the title compound (40.0 mg, 26% yield) as colorless oil. LCMS(ESI⁺): 481.4 [M+H]⁺.

Step 3: Preparation of ISM061-6

To a solution of tert-butyl 2-(((5-ethyl-4-(((2-(trifluoromethyl)phenyl)amino)methyl) pyrimidin-2-yl)oxy)methyl)pyrrolidine-1-carboxylate (35.0 mg, 0.07 mmol) in DCM (5 mL) was added HCl-dioxane (0.5 mL, 2.00 mmol) at 0 °C. After addition, the mixture was stirred at 25 °C for 1 hr. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO₂, DCM: MeOH = 5: 1) to afford the title compound (12.3 mg, 44% yield) as a white solid. LCMS (ESI⁺): 381.3 [M+H]⁺. ¹H NMR (400 MHz, DMSO- d_6) δ 8.44 (s, 1H), 7.51 – 7.44 (m, 2H), 6.94 (d, J = 8.4 Hz, 1H), 6.77 (t, J = 7.5 Hz, 1H), 6.31 (t, J = 4.7 Hz, 1H), 4.54 (d, J = 4.5 Hz, 2H), 4.32 (dd, J = 11.0, 4.7 Hz, 1H), 4.23 (dd, J = 11.1, 8.0 Hz, 1H), 3.66 – 3.61 (m, 1H), 3.02 – 2.92 (m, 2H), 2.68 (q, J = 7.6 Hz, 2H), 1.98 – 1.91 (m, 1H), 1.87 – 1.70 (m, 2H), 1.56 – 1.45 (m, 1H), 1.22 (t, J = 7.5 Hz, 3H).



S1.4. Preparation of ISM061-11

Scheme S4 Preparation of ISM061-11

Step 1: Preparation of Compound 11-1

To a solution of 5-fluoro-2-trifluoromethylbenzoic acid (10.0 g, 48.1 mmol) in N,N-dimethylformamide (100 mL) was added sodium hydride (2.1 g, 53.0 mmol) in four batches under nitrogen protection at 0 °C. The mixture was stirred at 0 °C for 0.5 hr. After the addition of benzyl alcohol (5.7 g, 53.0 mmol) the reaction was stirred at 25 °C for 16 hours. The reaction was quenched with ice water (300 mL). The mixture was extracted with ethyl acetate (100 mL × 3), the combined the organic phases was washed with saturated brine (100 mL × 3), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to afford the title compound (13.8 g, crude

product), which could be used to the next step without further purification. LCMS (ESI): 295.0 [M-H]⁻.

Step 2: Preparation of Compound 11-2

To a solution of **compound 11-1** (13.8 g, 46.6 mmol) in THF (140 mL) was added borane-tetrahydrofuran solution (14 mL, 139.8 mmol) under nitrogen protection dropwise at 0 °C. The mixture was stirred at 25 °C for 16 hours. The reaction was quenched by the addition of methanol (20 mL). After the addition of brine (80 mL), the mixture was extracted with ethyl acetate (100 mL × 3), the combined organic phases was washed with saturated brine (50 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to obtain the title compound (12.1 g, crude product) as a yellow solid, which could be used to the next step without further purification.

Step 3: Preparation of Compound 11-3

To a solution of **compound 11-2** (12.0 g, 42.6 mmol) in dichloromethane (120 mL) was added thionyl chloride (7.6 g, 63.9 mmol) dropwise at 0 °C. The resulting mixture was stirred at 25 °C for 16 hours. The mixture was concentrated under reduced pressure and further purified by silica gel column chromatography (EA:PE=1:10) to obtain the title compound as a yellow oil (9.9 g, total yield of the first three steps: 68.7%).

Step 4: Preparation of Compound 11-4

To a solution of **compound 11-3** (4.0 g, 13.3 mmol) in N,N-dimethylformamide (40 mL) was added acetylacetone (4.0 g, 39.9 mmol), potassium carbonate (5.5 g, 39.9 mmol) and potassium iodide (1.1 g, 6.7 mmol). The mixture was stirred at 80 °C for 2 hours. After the addition of ice water (100 mL) to quench the reaction, the mixture was extracted with ethyl acetate (50 mL × 3), the combined organic phases was washed with saturated brine water (40 mL × 3), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to obtain the title compound (1.3 g, crude product) as a yellow oil, which could be used to the next step without further purification. LCMS (ESI): 365.2 [M+H]⁺.

Step 5: Preparation of Compound 11-5

A mixture of compound 11-4 (1.2 g,3.3 mmol), sulfuric acid (0.1 mL) and urea(594 mg,9.9 mmol) in a ethanol (10 mL) was stirred at100°C for 16 hours. After the removal of ethanol, saturated sodium bicarbonate aqueous solution (50 mL) was added to the residue. The resulting mixture was extracted with ethyl acetate (30mL × 3). The combined organic phases was washed with saturated brine water (20mL × 2), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford a residue, which was purified by silica gel column chromatography (EA:PE=3:1) to give the title compound as a yellow solid (74 mg). LCMS (ESI): 389.2 [M+H]⁺.

Step 6: Preparation of Compound 11-6

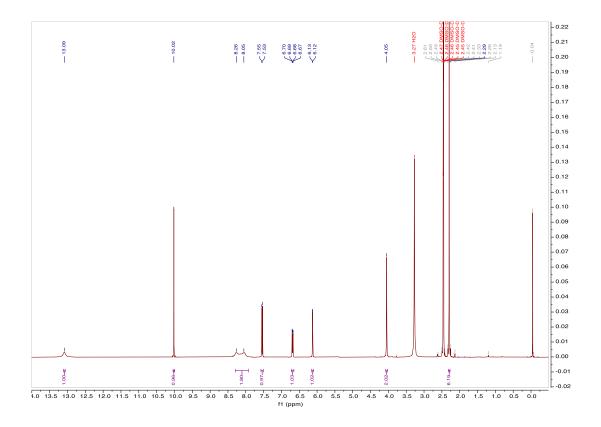
To a solution of **compound 11-5** (361.0 mg, 0.9 mmol) in phosphorus oxychloride (4 mL) was added N,N-dimethylbenzylamine (254.0 mg, 2.1 mmol) at 0 °C. The mixture was stirred at 100 °C for 16 hours After the removal of phosphorus oxychloride under reduced pressure, the residue was dissolved in ethyl acetate (20 mL) and saturated sodium bicarbonate aqueous solution (10 mL). The mixture was extracted with ethyl acetate (20 mL × 3), the combined organic phases was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give the title compound as a white solid (240.0 mg, Y: 63.5%). LCMS (ESI): 407.3 [M+H]⁺.

Step 7: Preparation of Compound 11-7

To a solution of **compound 11-6** (230.0 mg, 566.5 μmol) in dioxane (20 mL) and water (4 mL) was added 4-pyrazoleboronic acid pinacol ester (220.0 mg, 1.1 mmol), cesium carbonate (359.0 mg, 1.1 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloro palladium(II) dichloromethane complex (46.0 mg, 56.7 μmol). The mixture was stirred under nitrogen protection at 100 °C for 2 hours. After the addition of water (20 mL), the mixture was extracted with ethyl acetate (30 mL × 3). The combined organic phases was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to afford a residue, which was further purified by silica gel column chromatography (EA:PE=1:2) to afford the title compound as a yellow solid (160 mg,Y: 64.5%). LCMS (ESI):439.2[M+H]⁺.

Step 8: Preparation of ISM061-11

A mixture of **compound 11-7** (100 mg) and Pd/C (50 mg, 10%) in methanol (10 mL) was stirred under H₂ atmosphere at 25°C for 16 hours. Upon completion, the mixture was filtered, the filtrate was concentrated under reduced pressure to afford a residue, which was further purified by pre-HPLC to afford the title compound as a yellow solid (8 mg, Y: 10%). LCMS (ESI): 349.1 [M+H]⁺. ¹H NMR (400 MHz, DMSO- d_6) δ 13.09 (s, 1H), 10.02 (s, 1H), 8.29 – 7.93 (m, 2H), 7.54 (d, J = 8.6 Hz, 1H), 6.69 (dd, J = 8.6, 2.4 Hz, 1H), 6.13 (d, J = 2.4 Hz, 1H), 4.05 (s, 2H), 2.29 (s, 6H).



S1.5. Preparation of ISM061-12

Scheme S5 Preparation of ISM061-12

Step 1: Preparation of Compound 12-1

To a solution of 1-(5,6,7,8-tetrahydronaphthalen-2-yl)ethan-1-one (10.0 g, 57.5 mmol) in ethyl acetate (100 mL) was added aluminum chloride (19.1 g, 143.8 mmol) and bromine (11.0 g, 69.0 mmol). The resulting mixture was stirred at 85 °C for 16 hours. The reaction was quenched with ice water (100 mL). The resulting mixture was extracted with ethyl acetate (100 mL × 3). The combined organic phases was washed with saturated brine (50 mL × 3), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give the title compound as a yellow oil (5.1 g, Y: 35.2%). LCMS (ESI): 253.0 [M+H]⁺.

Step 2: Preparation of Compound 12-2

To a solution of **compound 12-1** (5.1 g, 20.2 mmol) in dichloromethane (50 mL) was added anhydrous sodium sulfate (2.9 g, 20.2 mmol), m-CPBA (8.7 g, 40.4 mmol) and trifluoromethanesulfonic acid (150.1 mg, 1.0 mmol). The resulting mixture was stirred at 25 °C for 48 hours. After the reaction is complete, quench the reaction solution with saturated sodium sulfite solution (10 mL). The mixture was then extracted with dichloromethane (50 mL × 3). The combined organic phases was washed with saturated brine water (20 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to obtain the title compound as a yellow solid (1.2 g,Y: 22.2%).

Step 3: Preparation of Compound 12-3

To a solution of **compound 12-2** (500 mg, 1.9 mmol) in THF (6 mL) was added water (1 mL) and sodium hydroxide (152 mg, 3.8 mmol). The resulting mixture was stirred at20°C for 4 hours. After the reaction was completed, dilute the reaction solution with water (10 mL). The mixture was then extracted with ethyl acetate (200 mL × 3). The combined organic phases was washed with saturated brine water (10 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give a residue, which was further purified by silica gel column chromatography (EA:PE=1:10) to afford the title compound as a yellow solid (310 mg, Y: 73.5%). LCMS (ESI): 227.1 [M+H]⁺.

Step 4: Preparation of Compound 12-4

To a solution of **compound 12-3** (280 mg, 1.2 mmol) in acetone (18 mL) was added cesium carbonate (782 mg,2.4 mmol) and BnBr (205.2 mg, 1.2 mmol). The resulting mixture was stirred at 70°C for 14 hours. Upon completion, the mixture was concentrated under reduced pressure to give a residue, which was purified by silica gel column chromatography (EA:PE=1:10) to afford the title compound as a yellow oil (200 mg, Y: 51%).

Step 5: Preparation of Compound 12-5

To a solution of 5-bromo-4-methylpyrimidine (2 g,11.6 mmol) in tetrahydrofuran (40 mL) was added LiHMDS (17 mL,17 mmol, 1M in THF) dropwise at -70°C under nitrogen. The resulting mixture was stirred at -70°C for 1 hour. Then diethyl carbonate (1 g, 7 mmol) was added. The mixture was stirred at room temperature for 16 hours. Upon completion, pour the reaction solution into ice water (10 mL), the resulting mixture was extracted with ethyl acetate (50 mL × 3). The combined organic phases was washed with brine (10 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to afford a residue, which was purified by silica gel column

chromatography (EA:PE=5:1) to give the title compound as a yellow oil (1 g, Y: 57%). LCMS (ESI): 245.1 [M+H]⁺.

Step 6: Preparation of Compound 12-6

To a solution of **compound 12-5** (320 mg, 1.3 mmol) in THF (16 mL) was added triethylamine (486 mg, 4.8 mmol), 4-dimethylaminopyridine (37 mg, 0.3 mmol) and p-toluenesulfonyl chloride (664 mg, 3 mmol). The resulting mixture was stirred under nitrogen protection at 20°C for 2 hours. After the addition of ice water (10 mL), the mixture was extracted with dichloromethane (20 mL × 3). The combined organic phases was washed with brine (10 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give a residue, which was further purified by silica gel column chromatography (EA:PE=1:5) to afford the title compound as a yellow oil (203 mg, Y: 80%). LCMS (ESI): 193.1 [M+H]⁺.

Step 7: Preparation of Compound 12-7

To a solution of compound **12-6** (183 mg, 1 mmol) in 1,4-dioxane (8 mL) was added **compound 12-4** (474 mg, 1.5 mmol), Pd₂(dba)₃ (91.5 mg,0.1 mmol), N-methyldicyclohexylamine (585 mg, 3 mmol) and trifluoroborane tri-*tert*-butylphosphine (58 mg, 0.2 mmol). The mixture was stirred at 120°C under N₂ for 3 hours. After the addition of ice water (10 mL), the mixture was extracted with ethyl acetate (20 mL × 3). The combined organic phases was washed with brine (10 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give a residue, which was further purified by silica gel column chromatography (EA:PE=1:2) to afford the title compound as a yellow solid (254 mg, Y: 62%). LCMS (ESI): 429.3 [M+H]⁺.

Step 8: Preparation of Compound 12-8

A mixture of **compound 12-7** (1.3 g,3 mmol) and Pd/C (120 mg) in ethanol(10 mL) was stirred under hydrogen at 20°C for 16 hours. Upon completion, the mixture was filtered, and concentrated under reduced pressure to give a residue, which was further purified by silica gel column chromatography (EA:PE=2:1) to afford the title compound (920 mg, Y: 90%). LCMS (ESI): 341.2 [M+H]⁺.

Step 9: Preparation of Compound 12-9

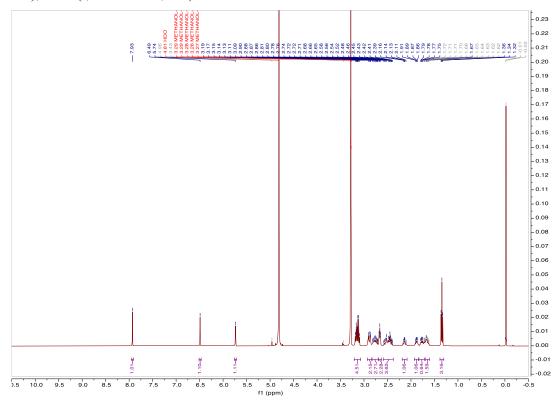
A mixture of **compound 12-8** (920 mg,2.7 mmol) and NaBH₄ (1 g, 27 mmol) in ethanol (10 mL) was stirred at 20°C for 14 hours. After the addition of ice water (10 mL), the mixture was extracted with ethyl acetate (30 mL × 3). The combined organic phases was washed with brine (10 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give a residue, which was further purified by silica gel column chromatography (EA:PE=2:1) to afford the title compound (410 mg, Y: 50%). LCMS (ESI): 299.2 [M+H]⁺.

Step 10: Preparation of Compound 12-10

To a solution of **compound 12-9** (410 mg,1.4 mmol) in dichloromethane (10 mL) was added triethylamine (486 mg,4.8 mmol), 4-dimethylaminopyridine (37 mg,0.3 mmol) and p-toluenesulfonyl chloride (664 mg,3 μmol). The mixture was stirred at 20 °C under N₂ for 2 hours. After the addition of ice water (10 mL), the mixture was extracted with dichloromethane (20 mL × 3). The combined organic phases was washed with brine (10 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give a residue, which was further purified by silica gel column chromatography to afford the title compound (120 mg, Y:14.5%).

Step 11: Preparation of ISM061-12

A solution of **compound 12-10** (120 mg,0.2 mmol) in ethylamine (5 mL, 1M in THF) was stirred at 25 °C for 16 hours. The mixture was concentrated to give crude produce, which was dissolved in ethanol (2 mL) and water (0.3 mL), and then NaOH (12 mg, 0.3 mmol) was added. The reaction was stirred at 25 °C for 4 hours. The pH is adjusted to 6-7 with 1N hydrochloric acid. The solution is directly purified by HPLC to afford the title compound as a white solid (5.7 mg, Y: 8.9%). LCMS (ESI): 326.2 [M+H]⁺. ¹H NMR (400 MHz, Methanol- d_4) δ 7.93 (s, 1H), 6.49 (s, 1H), 5.74 (s, 1H), 3.21 – 3.07 (m, 4H), 2.93 – 2.84 (m, 2H), 2.75 (td, J = 14.5, 7.0 Hz, 2H), 2.66 (t, J = 6.0 Hz, 2H), 2.59 – 2.38 (m, 3H), 2.19 – 2.08 (m, 1H), 1.93 – 1.84 (m, 1H), 1.83 – 1.71 (m, 1H), 1.69 – 1.60 (m, 2H), 1.34 (t, J = 7.3 Hz, 3H).



S1.6. Preparation of ISM061-13

Scheme S6 Preparation of ISM061-13

Step 1: Preparation of Compound 13-1

To a solution of 3-bromo-4-ethylphenol (1.0 g, 4.4 mmol) in dichloromethane (10 mL) was added boron trifluoride etherate (6.6 ml, 6.6 mmol) and triethylsilane (0.8 g, 6.6 mmol) under nitrogen protection at 0 °C. The mixture was stirred at 25 °C for 16 hours. After the addition of ice water (50 mL), the mixture was extracted with dichloromethane (50 mL × 3). The combined organic phases was washed with brine (10 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give a residue, which was further purified by silica gel column chromatography to afford the title compound as a yellow oil (900 mg, Y: 95.9%).

Step 2: Preparation of Compound 13-2

To a solution of **compound 13-1** (900 mg, 4.2 mmol) in dioxane (9 mL) was added [1,1'-bis(diphenylphosphino)ferrocene]dichloro palladium (II) (160 mg, 0.29 mmol), potassium acetate (1.2 g, 12.6 mmol) and B₂(pin)₂ (1.6 mg, 6.3 mmol). The mixture was stirred at 100 °C for 16 hours. The mixture was then concentrated under reduced pressure to give a residue, which was further purified by silica gel column chromatography (PE:EA=50:1) to afford the title compound as a yellow oil (810 mg, Y: 73.6%). ¹H NMR (400 MHz, DMSO- d_6) δ 7.10 - 7.06 (m, 2H), 6.91 - 6.87 (m, 1H), 3.67 (s, 1H), 2.74 - 2.68 (m, 2H), 1.25 (s, 12H), 1.06 - 1.02 (m, 3H).

Step 3: Preparation of Compound 13-3

To a solution of 2-chloro-3-fluoro-4-iodopyridine (3.5 g, 13.6 mmol) in THF (35 mL) was added LiHMDS (6.8 ml,13.6 mmol) under nitrogen at -70°C. The mixture was stirred at this temperature for another 4 hours. The reaction solution was poured into saturated ammonium chloride (50 mL).

The resulting mixture was extracted with ethyl acetate (20 mL \times 3). The combined organic phases was washed with brine (10 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give a residue, which was further purified by silica gel column chromatography to afford the title compound (820 mg, Y: 23%). ¹H NMR (400 MHz, DMSO- d_6) δ 8.42 (s, 1H), 7.79 (s, 1H).

Step 4: Preparation of Compound 13-4

To a solution of **compound 13-3** (800 mg, 3.1 mmol) in DMF (3 mL) was added triethylamine (327 mg, 3.2 mmol), copper iodide (20 mg, 0.1 mmol), 2-iodobenzoic acid methyl ester (254 mg, 1 mmol) and bis(triphenylphosphine)dichloro palladium (II) (70 mg, 0.1 mmol) under nitrogen. The mixture was stirred at 80°C for 16 hours. The reaction mixture was concentrated under reduced pressure to give a residue, which was purified by silica gel column chromatography (PE:EA=50:1) to afford the title compound as a yellow oil (900 mg, Y: 47%).

Step 5: Preparation of Compound 13-5

To a solution of **compound 13-4** in dioxane (5 mL) was added Cs₂CO₃ (2.73 g, 8.9 mmol), **compound 13-2** (1.12 g, 4.3 mmol) and Pd(PPh₃)Cl₂ (211 mg, 0.3 mmol). The mixture was stirred at 100 °C for 16 hrs. The mixture was then concentrated under reduce pressure to give a residue, which was further purified by silica gel column chromatography to afford the title compound as a yellow oil (500 mg, Y: 42%). LCMS (ESI): 412.2 [M+H]⁺.

Step 6: Preparation of Compound 13-6

To a solution of **compound 13-5** (500 mg, 1.2 mmol) in N,N-dimethylformamide (5 mL), CsF (1.8 g, 12.0 mmol) was added under nitrogen. The resulting mixture was stirred at room temperature for 12 hrs. After the addition of ice water (50 mL), the mixture was extracted with EA (20 mL × 3). The combined organic phases was washed with brine (20 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give a residue, which was further purified by silica gel column chromatography to afford the title compound as a yellow oil (165 mg, Y: 51%). LCMS (ESI): 256.1 [M+H]⁺.

Step 7: Preparation of Compound 13-7

According to a similar procedure of **step 4**, the title compound was obtained as a yellow oil (51.8 mg, Y: 21.8%).

Step 8: Preparation of Compound 13-8

According to a similar procedure with **step 8** in compound **ISM061-12**, the title compound was obtained as a yellow oil (50 mg, Y: 96.4%). LCMS (ESI): 394.2 [M+H]⁺.

Step 9: Preparation of Compound 13-9

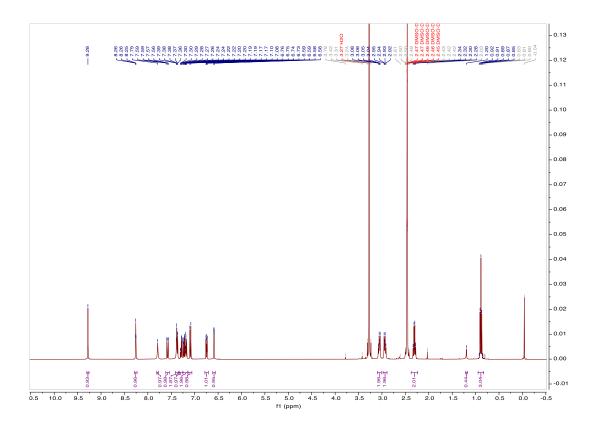
A mixture of **compound 13-8** (50 mg, 0.13 mmol) and NaOH (10 mg, 0.26 mmol) in MeOH (1 mL) and water (2 ml) was stirred at room temperature for 16 hrs. Upon completion, the reaction mixture was poured into ice water (10 mL), the pH was adjusted to 6 with 2 M HCl. The mixture was then extracted with EA (10 mL × 3). The combined organic phases was washed with brine (20 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to afford the crude title compound as a white solid (48.2 mg, Y: 99.2%).

Step 10: Preparation of Compound 13-10

To a solution of compound 13-9 (41 mg, 0.11 mmol) in N,N-dimethylformamide (2 mL) was added ammonium chloride (9 mg, 0.16 mmol), N,N-diisopropylethylamine (43 mg, 0.33 mmol) and 2-(7-azo-biphenyltriazole)-N,N,N',N'-tetramethylurea hexafluorophosphate (61 mg, 0.16 mmol). The resulting mixture was stirred at 25 °C for 16 hours. After the addition of ice water (10 mL), the mixture was extracted with ethyl acetate (10 mL × 3). The combined organic phases was washed with brine (20 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give a residue, which was further purified by silica gel column chromatography to afford the title compound (31.0 mg, Y: 75.2%). LCMS (ESI): 379.2 [M+H]⁺.

Step 11: Preparation of ISM061-13

A solution of **compound 13-10** (31 mg, 0.08 mmol) in hydrobromic acid aqueous solution (3 mL) was stirred at 100 °C for 4 hours. After the addition of ice water (10 mL), the mixture was extracted with ethyl acetate (10 mL × 3). The combined organic phases was washed with brine (20 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give a residue, which was purified by pre-HPLC to afford the title compound as a white solid (12.4 mg, Y: 41.7%). LC-MS (ESI): 365 [M+H]⁺; ¹H NMR (400 MHz, DMSO- d_6) δ 9.28 (s, 1H), 8.28 – 8.23 (m, 1H), 7.79 (s, 1H), 7.58 (dd, J = 10.7, 1.7 Hz, 1H), 7.41 – 7.32 (m, 2H), 7.28 (td, J = 7.4, 1.6 Hz, 1H), 7.25 – 7.15 (m, 2H), 7.09 (d, J = 8.4 Hz, 1H), 6.75 (dd, J = 8.3, 2.7 Hz, 1H), 6.59 (dd, J = 2.7, 0.9 Hz, 1H), 3.06 (dd, J = 9.7, 5.9 Hz, 2H), 2.94 (dd, J = 9.7, 6.0 Hz, 2H), 2.31 (q, J = 7.5 Hz, 2H), 1.20 (s, 0H), 0.89 (t, J = 7.5 Hz, 3H).



S1.7. Preparation of ISM061-14

Scheme S7 Preparation of ISM061-14

Step 1: Preparation of Compound 14-1

To a solution of 2-bromo-4-hydroxybenzaldehyde (1.00 g, 4.98 mmol) and (bromomethyl)benzene (0.7 mL, 5.97 mmol) in MeCN (10 mL) was added K₂CO₃ (2.06 g, 14.93 mmol). After addition, then the mixture was stirred at 80 °C for 3 hrs. After cooling to 20 °C, the reaction solution was poured into water (50 mL) and extracted with EtOAc (25 mL × 3). The combined organic layers were washed with brine (25 mL × 2), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by flash silica gel chromatography (SiO₂, ethyl acetate: petroleum

ether = 0% - 10%) to afford **Compound 14-1** (1.20 g, 83% yield) as a white solid. LCMS (ESI⁺): $291.2 \, [M+H]^+$.

Step 2: Preparation of Compound 14-2

To a solution of 4-(benzyloxy)-2-bromobenzaldehyde (100.0 mg, 0.34 mmol) in DCM (5 mL) was added DAST (0.2 mL, 1.72 mmol) dropwise at 0 °C and then the mixture was stirred at 25 °C for 4 hrs. After cooling to 0 °C, the reaction mixture was quenched by ice-water (20 mL) and extracted with EtOAc (25 mL × 3). The combined organic layers were washed with brine (25 mL × 2), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO₂, ethyl acetate: petroleum ether = 5: 1) to afford **Compound 14-2** (90.0 mg, 84% yield) as colorless oil. ¹H NMR (400 MHz, Methanol-d₄) δ 7.55 - 7.53 (d, J = 8.0 Hz, 1H), 7.43 - 7.29 (m, 5H), 7.27 (s, 1H), 7.09 - 7.06 (m, 1H), 7.03 - 6.75 (t, J = 56 Hz, 1H), 5.09 (s, 2H).

Step 3 & 4: Preparation of Compound 14-4

To a solution of 4-(benzyloxy)-2-bromo-1-(difluoromethyl)benzene (200.0 mg, 0.64 mmol) in 1,4-dioxane (5 mL) were added bis(pinacolato)diboron (194.6 mg, 0.77 mmol), potassium acetate (188.0 mg, 1.92 mmol) and Pd(dppf)Cl₂ (46.7 mg, 0.06 mmol). After addition, the mixture was purged with N₂ for 2 mins and then stirred at 100 °C for 12 hrs under N₂. After cooling to 25 °C, water (1 mL) was added followed by K_2CO_3 (230.2 mg, 1.67 mmol), 2,4-dichloropyrimidine (95.3 mg, 0.64 mmol) and Pd(dppf)Cl₂ (40.6 mg, 0.06 mmol). The mixture was stirred at 100 °C under N₂ for 2 hrs. After cooling to 20 °C, the reaction solution was poured into water (40 mL) and extracted with EtOAc (20 mL × 3). The combined organic layers were washed with brine (20 mL × 2), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by flash silica gel chromatography (SiO₂, ethyl acetate: petroleum ether = 0% - 10%) to afford **Compound 14-4** (150.0 mg, 78% yield) as colorless oil. LCMS (ESI⁺): 347.2 [M+H]⁺. ¹H NMR (400 MHz, Methanol-d₄) δ 8.77 - 8.76 (d, J = 4.0 Hz, 1H), 7.76 - 7.73 (m, 1H), 7.69 - 7.67 (d, J = 4.0 Hz, 1H), 7.48 - 7.45 (m, 2H), 7.40 - 7.29 (m, 6H), 5.20 (s, 2H).

Step 5: Preparation of Compound 14-5

To a solution of 4-(5-(benzyloxy)-2-(difluoromethyl)phenyl)-2-chloropyrimidine (150.0 mg, 0.43 mmol) in MeCN (3 ml) were added *tert*-butyl pent-4-yn-1-ylcarbamate (95.0 mg, 0.52 mmol), copper(I) iodide (16.48 mg, 0.09 mmol), TEA (0.18 ml, 1.30 mmol) and Pd(PPh)₃Cl₂ (30.4 mg, 0.043 mmol). After addition, the mixture was purged with N₂ for 2 mins and then stirred at 85 °C for 12 hrs under N₂. After cooling to 20 °C, the reaction solution was poured into water (20 mL) and extracted with EtOAc (20 mL × 3). The combined organic layers were washed with brine (20 mL × 2), dried

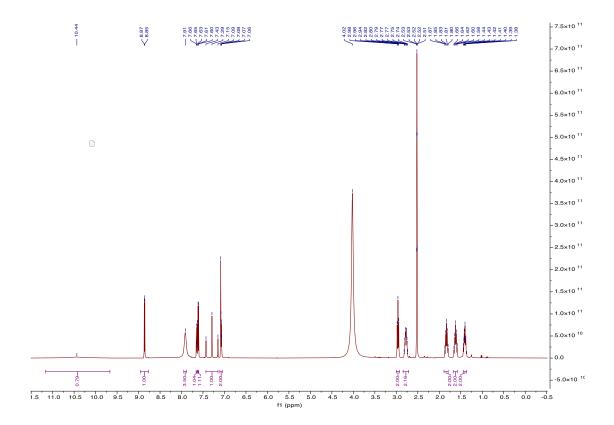
over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by flash silica gel chromatography (SiO₂, ethyl acetate: petroleum ether = 0% - 30%) to afford **Compound 14-5** (100 mg, 47% yield) as yellow oil. LCMS (ESI⁺): Rt=2.092 min; m/z 494.3 [M+H]⁺.

Step 6: Preparation of Compound 14-6

To a solution of *tert*-butyl (5-(4-(5-(benzyloxy)-2-(difluoromethyl)phenyl)pyrimidin-2-yl)pent-4-yn-1-yl)carbamate (100.0 mg, 0.20 mmol) and Pd/C (10.0 mg, 9.40 μmol) in MeOH (5 ml) was degassed and purged with H₂ for 3 times and the reaction was stirred at 25 °C for 1 hr under H₂ atmosphere (15Psi). The reaction mixture was filtered through a celite and the filter cake was rinsed with a small amount of MeOH (20 mL). The combined organic layers were concentrated under reduced pressure to afford **Compound 14-6** (80.0 mg), which was used in next step directly without further purification. LCMS (ESI⁺): 408.3 [M+H]⁺.

Step 7: Preparation of ISM061-14

To a solution of tert-butyl (5-(4-(2-(difluoromethyl)-5-hydroxyphenyl)pyrimidin-2-yl)pentyl)carbamate (100.0 mg, 0.25 mmol) in DCM (5 mL) was added HCl-dioxane (1 mL, 4 M) at 0 °C, and then the mixture was stirred at 25 °C for 30 min. The mixture was concentrated under reduced pressure to give a residue, which was purified by pre-HPLC (SHIMADZU, LC-20AP, Column: YMC Triart C¹⁸, 20 × 250 mm, 5 μ m; Mobile phase: from 5% to 35% ACN [0.1% HCl]; Flow rate: 15 mL/min; Retention Time:17 min of 30 min) to afford the title compound (35.0 mg, 0.10 mmol, 42% yield) as a gray solid. LCMS(ESI⁺): 308.1 [M+H]⁺. ¹H NMR (400 MHz, DMSO- d_6) δ 11.17 – 9.67 (m, 1H), 8.86 (d, J = 5.2 Hz, 1H), 7.91 (s, 2H), 7.64 (d, J = 9.2 Hz, 1H), 7.61 (d, J = 5.2 Hz, 1H), 7.29 (t, J = 55.7 Hz, 1H), 7.11 – 7.05 (m, 2H), 2.96 (t, J = 7.6 Hz, 2H), 2.78 (dq, J = 11.7, 6.0 Hz, 2H), 1.83 (p, J = 7.6 Hz, 2H), 1.63 (q, J = 7.6 Hz, 2H), 1.44 – 1.36 (m, 2H).



S1.8. Preparation of ISM061-15

Scheme S8 Preparation of ISM061-15

Step 1: Preparation of Compound 15-1

To a solution of 2-aminoethan-1-ol (2 g, 32.73 mmol) in PhMe (20 mL) were added DIEA (12.7 g, 98.2 mmol) and isobenzofuran-1,3-dione (4.8 g, 32.73 mmol) at N₂ atmosphere. Then the mixture was heated and stirred at 120 °C for 3 h. After cooling to 25 °C, the reaction mixture was poured into ice water (60 mL) and extracted with EtOAc (40 mL × 3), the combined organic layers were washed with brine (50 mL × 3), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue, which was purified by column chromatography on silica gel eluting with ethyl acetate (from

0% to 50%) in petroleum ether to afford **Compound 15-1** (4 g, 63.9 % yield) as a white solid. LCMS (ESI⁺): 192.3 [M+H]⁺.

Step 2: Preparation of Compound 15-2

To a solution of 1,8-dichloroisoquinoline (1000 mg, 5.05 mmol) in DMF (8 mL) were added TEA (2044 mg, 20.202 mmol), CuI (192 mg, 1.01 mmol), Pd(pph₃)₂Cl₂ (709 mg, 1.01 mmol) and ethynyltrimethylsilane (2480 mg, 25.25 mmol) at N₂ atmosphere. Then the mixture was heated and stirred at 60 °C for 3 h. After cooling to 25 °C, the reaction mixture was poured into ice water (60 mL) and extracted with EtOAc (40 mL × 3), the combined organic layers were washed with brine (50 mL × 3), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue, which was purified by column chromatography on silica gel, eluting with ethyl acetate (from 0% to 30%) in petroleum ether, to afford **Compound 15-2** (1050 mg, 80 % yield) as a yellow oil. LCMS (ESI⁺): 260.3 [M+H]⁺.

Step 3: Preparation of Compound 15-3

To a solution of 2-chloro-5-iodopyrimidine (500 mg,2.08 mmol) in DMF (8 mL) were added TEA (1050 mg, 10.40 mmol), CuI (119 mg, 0.62 mmol), Pd(pph₃)₂Cl₂ (292 mg, 0.42 mmol), Cesium fluoride (632 mg, 4.16 mmol) and 8-chloro-1-((trimethylsilyl)ethynyl)isoquinoline (476 mg, 1.83 mmol) at N₂ atmosphere. Then the mixture was heated and stirred at 60 °C for 12 h. After cooling to 25 °C, the reaction mixture was poured into ice water (40 mL) and extracted with EtOAc (30 mL × 3), the combined organic layers were washed with brine (30 mL × 3), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue, which was purified by column chromatography on silica gel, eluting with ethyl acetate (from 0% to 100%) in petroleum ether, to afford **Compound** 15-3 (400 mg, 64.1% yield) as a yellow solid. LCMS (ESI⁺): 300.1 [M+H]⁺.

Step 4: Preparation of Compound 15-4

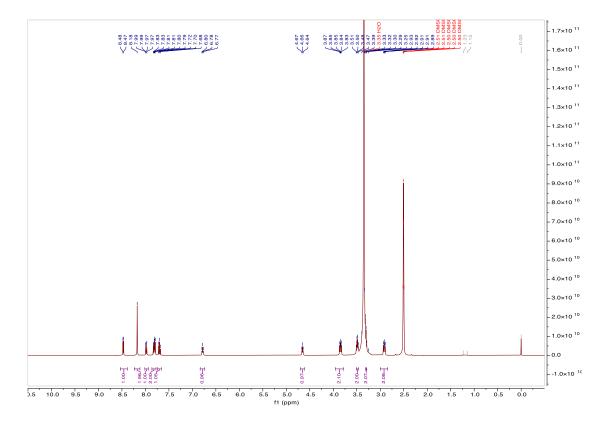
To a solution of 8-chloro-1-((2-chloropyrimidin-5-yl)ethynyl)isoquinoline (400 mg, 1.33 mmol) in dioxane (5 mL) were added Cs₂CO₃ (869 mg, 2.66 mmol), BINAP (166 mg, 0.27 mmol), Pd(dba)₃ (108 mg, 0.13 mmol) and 2-(2-hydroxyethyl)isoindoline-1,3-dione (306 mg, 1.60 mmol) at N₂ atmosphere. Then the mixture was heated and stirred at 100 °C for 2 h. After cooling to 25 °C, the reaction mixture was poured into ice water (40 mL) and extracted with EtOAc (30 mL × 3), the combined organic layers were washed with brine (50 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue, which was purified by column chromatography on silica gel, eluting with ethyl acetate (from 0% to 100%) in petroleum ether, to afford **Compound** 15-4 (400 mg, 66.0% yield) as a yellow solid. LCMS (ESI⁺): 455.0 [M+H]⁺.

Step 5: Preparation of Compound 15-5

To a solution of 2-(2-((5-((8-chloroisoquinolin-1-yl)ethynyl)pyrimidin-2-yl)oxy)ethyl)isoindoline-1,3-dione (400 mg, 0.83 mmol) in MeOH (5 mL) were added Hydrazinium hydroxide solution (2 mL, 1.76 mmol). Then the mixture was heated and stirred at 80 °C for 30 min. After cooling to 25 °C, the reaction mixture was poured into ice water (40 mL) and extracted with EtOAc (30 mL × 3), the combined organic layers were washed with brine (30 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to afford **Compound 15-5** (260 mg, 91.0% yield) as a yellow solid. LCMS (ESI⁺): 325.2 [M+H]⁺.

Step 6: Preparation of ISM061-15

To a solution of 2-((5-((8-chloroisoquinolin-1-yl)ethynyl)pyrimidin-2-yl)oxy)ethan-1-amine (150 mg, 0.46 mmol) in MeOH (5 mL) was added Platinum dioxide (21 mg, 0.1 mmol). The reaction mixture was stirred at H₂ at 25 °C for 4 h, the mixture was filtered and the filtrate was concentrated to give the crude product, which was further purified by Prep-HPLC (column: YMC, C¹⁸ (250 × 20mm, 5 µm); mobile phase: [water (0.1%NH4OH) - ACN]; B%: 20ACN% - 50ACN%, 20 min) to afford the title compound (11 mg, 7.2% yield). LCMS (ESI⁺): 329.0 [M+H]⁺; ¹H NMR (400 MHz, DMSO- d_6) δ 8.47 (d, J = 5.5 Hz, 1H), 8.18 (s, 2H), 7.98 (dd, J = 8.1, 1.3 Hz, 1H), 7.86 – 7.77 (m, 2H), 7.70 (t, J = 7.8 Hz, 1H), 6.78 (t, J = 5.8 Hz, 1H), 4.66 (t, J = 5.6 Hz, 1H), 3.96 – 3.78 (m, 2H), 3.51 – 3.47 (m, 2H), 3.31 – 3.29 (m, 2H), 3.00 – 2.84 (m, 2H).



S1.9. Preparation of ISM061-16

Scheme S9 Preparation of ISM061-16

Step 1: Preparation of Compound 16-1

To a solution of 2,4-dichloro-3-methylpyridine (958 mg, 5.91 mmol) in DMF (10 mL) were added Cs₂CO₃ (3210 mg, 9.85 mmol) and 2-fluoro-5-methoxyphenol (700 mg, 4.93 mmol). The resulting mixture was stirred at 100 °C under N₂ atmosphere for 2 hrs. After the addition of water (50 mL), the mixture was extracted with EtOAc (30 mL × 3), the combined organic layers was washed with brine (50 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue, which was purified by column chromatography on silica gel to afford the title compound (680 mg, 51.9% yield) as a yellow oil. LCMS (ESI⁺): 267.9 [M+H]⁺.

Step 2: Preparation of Compound 16-2

To a solution of 2-chloro-4-[(2-fluoro-5-methoxyphenyl)oxy]-3-methylpyridine (300 mg, 1.12 mmol) in DCM (3 mL) were added BBr₃ (280 mg, 1.12 mmol) at 0 °C in N₂ atmosphere. The reaction was stirred at 25 °C for 1 h. The reaction was quenched with ice water. The aqueous layer was extracted with DCM (30 mL × 3). The combined organic layers was washed with brine (30 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue, which was purified by column chromatography on silica gel to afford the title compound (100 mg, 35.2%) as a yellow oil. LCMS (ESI⁺): 254.1 [M+H]⁺.

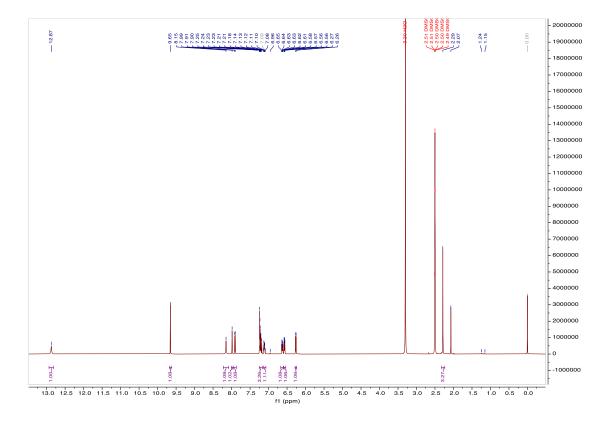
Step 3: Preparation of Compound 15-3

To a solution of 3-[(2-chloro-3-methylpyridin-4-yl)oxy]-4-fluorophenol (90 mg, 0.35 mmol) in 1,4-dioxane (1 mL) were added 1-(3,4,5,6-tetrahydro-2H-pyran-2-yl)indazol-4-amine (115.6 mg, 0.53 mmol), Cs₂CO₃ (231 mg, 0.71 mmol), and XantPhosPdG₄ (34 mg, 0.04 mmol). The reaction was stirred at 120 °C for 10 h in sealed tube. The reaction mixture was poured into ice-water (30 mL) and

extracted with DCM (30 mL × 3). The combined organic layers was washed with brine (30 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue, which was purified by Prep-TLC (SiO₂, DCM: MeOH=10: 1) to afford the title compound (100 mg, 64.9%) as a yellow solid. LCMS (ESI⁺): 435.1 [M+H]⁺.

Step 4: Preparation of ISM061-16

To a solution of **Compound 16-3** (90 mg, 0.21 mmol) in DCM (1 ml) were added HCl (1 mL, 4.00 mmol, 4.0 M in dioxane). After the addition, the reaction mixture was stirred at 25 °C for 1 h. Then the mixture was concentrated to afford a crude product, which was further purified with prep-HPLC (column: YMC, C^{18} (250 × 20mm, 5µm); mobile phase: [water (0.1%NH4OH)- ACN]; B%: 20ACN%-50ACN%, 20min) to afford the title compound (30.28 mg, 41.7%) as a white solid. LCMS (ESI⁺): 351.1 [M+H]⁺. ¹H NMR (400 MHz, DMSO-d6) δ 12.87 (s, 1H), 9.65 (s, 1H), 8.15 (s, 1H), 7.99 (s, 1H), 7.91 (d, J = 5.6 Hz, 1H), 7.24 (s, 1H), 7.23 (s, 1H), 7.23 - 7.18 (m, 1H), 6.66 - 6.60 (m, 1H), 6.57 (dd, J = 6.8, 2.9 Hz, 1H), 6.26 (d, J = 5.6 Hz, 1H), 2.29 (s, 3H).



S1.10. Preparation of ISM061-18 and ISM061-18-2

Scheme S10 Preparation of ISM061-18 and ISM061-18-2

Step 1: Preparation of Compound 18-1

To a solution of 2-chloro-5-iodopyrimidine (1.5 g, 6.24 mmol) in 1,4-dioxane (15 mL) was added C₂H₃BF₃K (1.3 g, 9.36 mmol), CsF (1.9 g, 12.48 mmol) and Pd(dppf)Cl₂ (0.9 g, 1.25 mmol) at 25 °C under N₂ atmosphere. After addition, the reaction mixture was heated and stirred at 80 °C under a nitrogen atmosphere for 12 h. After cooling to 25 °C, the reaction mixture was poured into ice water (150 mL) and extracted with EtOAc (70 mL × 3), the combined organic layers were washed with brine (100 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue, which was purified by column chromatography on silica gel eluting with ethyl acetate (from 0% to 6.4%) in petroleum ether to give the title compound (560 mg, 63.8% yield) as a yellow liquid. LCMS (ESI⁺): 141.3 [M+H]⁺.

Step 2: Preparation of Compound 18-2 and Compound 18-3

To a solution of 2-chloro-5-vinylpyrimidine (540 mg, 3.84 mmol) in 1,4-dioxane-H₂O (6 mL, 5:1) was added K₂CO₃ (1592 mg, 11.52 mmol), (2-fluoro-6-hydroxyphenyl)boronic acid (1198 mg, 7.68 mmol) and Pd(dppf)Cl2 (562 mg, 0.77 mmol) at 25 °C under N₂ atmosphere. After addition, the reaction mixture was heated and stirred at 80 °C under a nitrogen atmosphere for 12 h. After cooling to 25 °C, the reaction mixture was poured into ice water (80 mL) and extracted with EtOAc (40 mL × 3), the combined organic layers were washed with brine (60 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue, which was purified by column chromatography

on silica gel eluting with ethyl acetate (from 0% to 22.3%) in petroleum ether to afford **Compound 18-2** (151 mg, 8.6% yield) as a yellow solid; LCMS (ESI⁺): 217.3 [M+H]⁺; **Compound 18-3** (400 mg, 22.8%) was obtained as a byproduct of this reaction. LCMS (ESI⁺): 217.3 [M+H]⁺;

Step 3: Preparation of Compound 18-4

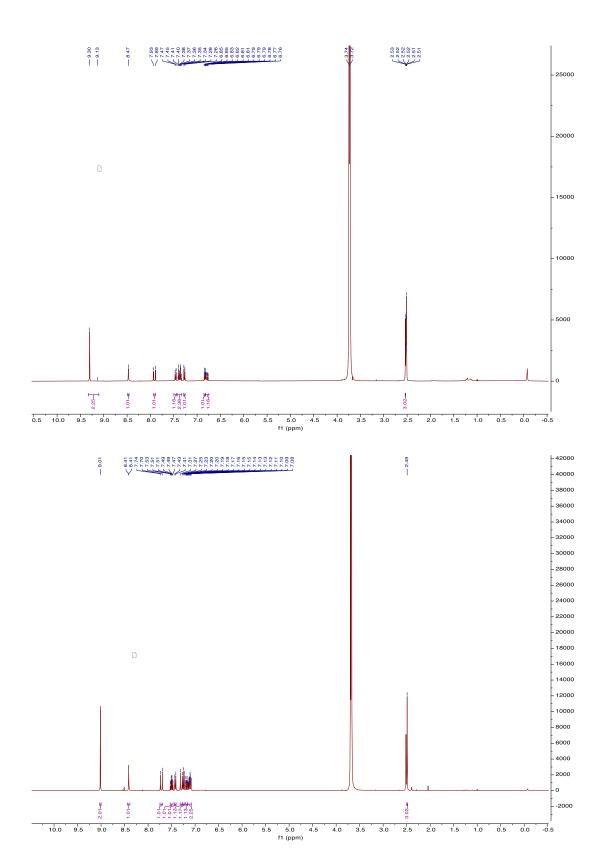
To a solution of **Compound 18-2** (240 mg, 1.11 mmol) in DMF (6 mL) was added KOAc (436 mg, 4.40 mmol), PPh₃ (117 mg, 0.45 mmol), 4-bromo-5-methyl-1-(tetrahydro-2H-pyran-2-yl)-1H-indazole (653 mg, 2.20 mmol) and Pd(OAc)₂ (101 mg, 0.45 mmol) at 25 °C under N₂ atmosphere. After addition, the reaction mixture was heated and stirred at 100 °C under nitrogen atmosphere for 12 h. After cooling to 25 °C, the reaction mixture was poured into ice water (80 mL) and extracted with EtOAc (40 mL × 3), the combined organic layers were washed with brine (60 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue, which was purified by Prep-TLC (SiO₂, PE:EA=3: 1) to give the title compound (150 mg, 15.7% yield) as a yellow solid. LCMS (ESI⁺): 431.1 [M+H]⁺.

Step 4: Preparation of ISM061-18

To a solution of **Compound 18-4** (75 mg, 0.15 mmol) in THF (2 mL) were added HCl (1 mL, 4.00 mmol, 4.0 M in dioxane). After the addition, the reaction mixture was poured into ice water (40 mL) and extracted with DCM (20 mL × 3), the combined organic layers were washed with brine (40 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue, which was purified by Prep-TLC (SiO₂, PE:EA=1: 1) to give ISM023-18 (28.6 mg, 47.4% yield) as a yellow solid. LCMS (ESI⁺): 347.1 [M+H]⁺. ¹H NMR (400 MHz, DMSO- d_6) δ 9.30 (s, 2H), 8.47 (s, 1H), 7.91 (d, J = 16.8 Hz, 1H), 7.46 (d, J = 8.4 Hz, 1H), 7.42 – 7.33 (m, 2H), 7.27 (d, J = 8.6 Hz, 1H), 6.83 (d, J = 8.4 Hz, 1H), 6.79 (ddd, J = 10.9, 8.2, 1.1 Hz, 1H), 2.53 (s, 3H).

Step 5: Preparation of ISM061-18-2

According to a similar procedure of **ISM061-18**, **ISM023-18-2** was synthesized with ISM061-18-3 as the substrate. LCMS (ESI⁺): 347.1 [M+H]⁺. ¹H NMR (400 MHz, DMSO- d_6) δ 9.01 (s, 2H), 8.41 (s, 1H), 7.72 (d, J = 16.7 Hz, 1H), 7.53 – 7.47 (m, 1H), 7.42 (d, J = 8.4 Hz, 1H), 7.29 (d, J = 16.7 Hz, 1H), 7.24 (d, J = 8.5 Hz, 1H), 7.18 (dt, J = 10.1, 2.4 Hz, 1H), 7.16 – 7.08 (m, 2H), 2.49 (s, 3H).



S1.11. Preparation of ISM061-21

Scheme S11 Preparation of ISM061-21

Step 1: Preparation of Compound 21-1

To a solution of 5-bromo-2-chloro-4-methylpyrimidine (1.00 g, 4.82 mmol) in dioxane (10 mL) and water (2 mL) were added LiCl (600 mg, 14.46 mmol) and tributyl(vinyl)stannane (1.50 g, 4.82 mmol) and Pd(pph₃)₂Cl₂ (300 mg, 0.48 mmol) under N₂ atmosphere. Then the mixture was heated and stirred at 100 °C for 2 h. After cooling to 25 °C, the mixture was filtered through a Celite pad. The filtrate was poured into ice water (50 mL) and extracted with EtOAc (40 mL × 3), the combined organic layers were washed with brine (60 mL × 3), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue, which was purified by column chromatography on silica gel eluting with ethyl acetate (from 0% to 18%) in petroleum ether to afford the title compound (291 mg, 39.1 % yield) as a yellow oil. LCMS (ESI⁺): 155.3 [M+H]⁺.

Step 2: Preparation of Compound 21-2

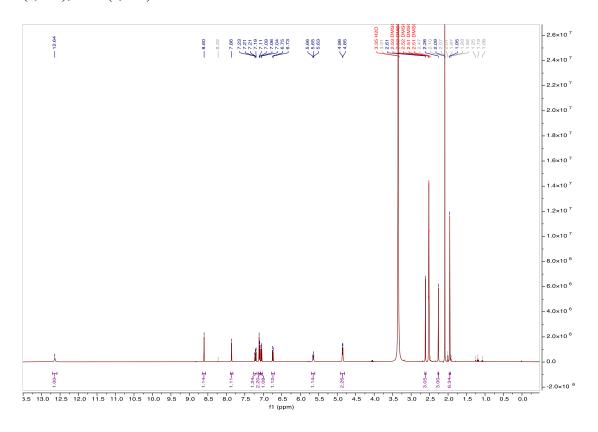
To a solution of Compound 21-1 (270 mg, 1.75 mmol), (2,6-dimethylphenyl)boronic acid (265 mg, 1.75 mmol) and Cs₂CO₃ (1.42 g, 4.37 mmol) in dioxane (10 mL) and Water (2 mL) was added Pd(dppf)Cl₂ (128 mg, 0.17 mmol) at 25 °C. After addition, the mixture was heated and stirred at 100 °C under a nitrogen atmosphere for 2 h. After cooling to 25 °C, the reaction mixture was poured into water (100 mL) and extracted with EtOAc (50 mL × 3). The combined organic layers was washed with brine (60 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue, which was purified by Prep-TLC (SiO₂, PE : EA=3: 1) to afford the title compound (150 mg, 38.3% yield) as a yellow oil. LCMS (ESI⁺): 225.3 [M+H]⁺.

Step 3: Preparation of Compound 21-3

To a solution of 2-(2,6-dimethylphenyl)-4-methyl-5-vinylpyrimidine (130 mg, 0.58 mmol) and NaIO₄ (372 mg, 1.74 mmol) in THF (2.5 mL) and H₂O (2.5 mL) were added KOsO₄ (26.7 mg, 0.058 mmol) at 0 °C. Then the mixture was heated and stirred at 25 °C for 0.5 h. The reaction mixture was poured into ice water (30 mL) and extracted with EtOAc (15 mL × 3), the combined organic layers were washed with brine (30 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue, which was purified by Prep-TLC (SiO₂, PE:EA=5: 1) to afford the title compound (123 mg, 93.8% yield) as a yellow oil. LC-MS (ESI⁺): 227.3 [M+H]⁺.

Step 4: Preparation of ISM061-21

To a solution of **compound 21-3** (10 mg, 0.04 mmol) in DCM (1 mL) were added 5-methyl-1H-indazol-4-amine (7.2 mg, 0.05 mmol), Titanium tetraisopropanolate (37 mg, 0.13 mmol). The reaction mixture was stirred at 25 °C for 12 h. After the addition of NaBH₃CN (5.5 mg, 0.09 mmol), the mixture was further stirred at 25 °C for 2 hr. The reaction mixture was filtered and concentrated under reduced pressure to give a residue, which was purified by Prep-HPLC to afford the title compound (1.14 mg, 7.2% yield). LCMS (ESI⁺): 358.3 [M+H]⁺; ¹H NMR (400 MHz, DMSO- d_6) δ 12.64 (s, 1H), 8.60 (s, 1H), 7.86 (s, 1H), 7.27 – 7.17 (m, 1H), 7.13 – 7.08 (m, 2H), 7.05 (d, J = 8.3 Hz, 1H), 6.74 (d, J = 8.2 Hz, 1H), 5.65 (t, J = 6.3 Hz, 1H), 4.85 (d, J = 6.1 Hz, 2H), 2.61 (s, 3H), 2.26 (s, 3H), 1.95 (s, 6H).



S1.9. Preparation of ISM061-22

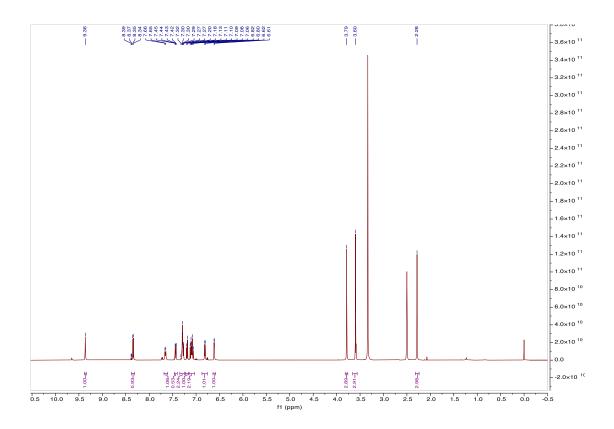
Scheme S8 Preparation of ISM061-22

Step 1: Preparation of Compound 22-1

To a solution of 2-chloropyrimidin-4-amine (130 mg, 1.00 mmol) in dioxane (3 mL) were added H_2O (0.2 mL), (2-methoxy-3-methylphenyl)boronic acid (183 mg, 1.10 mmol), $Pd(dppf)Cl_2$ (73 mg, 0.10 mmol) and K_2CO_3 (347 mg, 2.50 mmol) under N_2 atmosphere. Then the mixture was heated and stirred at 100 °C for 1 h. After cooling to 25 °C, the reaction mixture was poured into ice water (40 mL) and extracted with EtOAc (40 mL × 3), the combined organic layers were washed with brine (30 mL × 3), dried over Na_2SO_4 , filtered and concentrated under reduced pressure to give a residue, which was purified by column chromatography on silica gel eluting with ethyl acetate (from 0% to 50%) in petroleum ether to give the title compound (200 mg, 64.8% yield, purity: 70%) as a yellow oil. LCMS (ESI⁺): 216.1 [M+H]⁺.

Step 2: Preparation of ISM061-22

To a solution of 2-(2-methoxy-3-methylphenyl)pyrimidin-4-amine (161 mg, 0.52 mmol, purity: 70%) in dioxane (4 mL) were added 4-bromo-1-methyl-1H-indole (100 mg, 0.47 mmol), XantphosPdG₄ (77 mg, 0.05 mmol) and Cs₂CO₃ (310 mg, 0.95 mmol) under N₂ atmosphere. Then the mixture was heated and stirred at 110 °C for 3 h. After cooling to 25 °C, the reaction mixture was poured into ice water (40 mL) and extracted with EtOAc (40 mL × 3), the combined organic layers were washed with brine (30 mL × 3), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue, which was purified by column chromatography on silica gel eluting with ethyl acetate (from 0% to 60%) in petroleum ether and Prep-HPLC (column: YMC, C¹⁸ (250 × 20mm, 5µm); mobile phase: [water (0.1%NH₄HCO₃)- ACN]; B%: 30ACN%-60ACN%, 17 min of 25 min) to afford the title compound (70 mg, 42.7% yield) as a white solid. LCMS (ESI⁺): 345.1 [M+H]⁺. ¹H NMR (400 MHz, DMSO- d_6) δ 9.36 (s, 1H), 8.34 (d, J = 5.9 Hz, 1H), 7.65 (d, J = 7.6 Hz, 1H), 7.44 (d, J = 5.9 Hz, 1H), 7.35 – 7.25 (m, 2H), 7.19 (d, J = 8.1 Hz, 1H), 7.09 (dt, J = 11.3, 7.7 Hz, 2H), 6.81 (d, J = 5.9 Hz, 1H), 6.61 (d, J = 3.1 Hz, 1H), 3.79 (s, 3H), 3.60 (s, 3H), 2.28 (s, 3H).



S1.12. Preparation of ISM061-24

Scheme S12 Preparation of ISM061-24

Step 1: Preparation of Compound 24-1

To a solution of methyl 2-ethynylbenzoate (600.0 mg, 3.79 mmol), 2-chloro-3-fluoro-4-iodopyridine (1171.5 mg, 4.55 mmol) in MeCN (3 mL) were added TEA (1.6 mL, 11.38 mmol), CuI (144.5 mg, 0.76 mmol) and Pd(PPh₃)₂Cl₂ (266.2 mg, 0.38 mmol). After addition, the mixture was degassed and purged with N₂ for 2 mins and then stirred at 85 °C for 2 hrs. After cooling to 20 °C, the reaction solution was poured into water (50 mL) and extracted with EtOAc (25 mL × 3). The combined organic layers were washed with brine (25 mL × 2), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by flash silica gel chromatography (SiO₂,

ethyl acetate: petroleum ether = 0% - 6%) to afford **Compound 24-1** (700 mg, 64% yield) as a white solid. LCMS (ESI⁺): 290.2 [M+H]⁺.

Step 2: Preparation of Compound 24-2

To a solution of methyl 2-((2-chloro-3-fluoropyridin-4-yl)ethynyl)benzoate (650.0 mg, 2.24 mmol) in dioxane/water (6 mL, 5: 1) were added o-tolylboronic acid (366.2 mg, 2.69 mmol), Cs₂CO₃ (2193.0 mg, 6.73 mmol) and Pd(dppf)Cl₂ (164.2 mg, 0.22 mmol). After addition, the mixture was degassed and purged with N₂ for 2 mins and then stirred at 100 °C for 3 hrs. After cooling to 20 °C, the reaction solution was poured into water (50 mL) and extracted with EtOAc (25 mL × 3). The combined organic layers were washed with brine (25 mL × 2), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by flash silica gel chromatography (SiO₂, ethyl acetate: petroleum ether = 0% - 10%) to afford **Compound 24-2** (600 mg, 77% yield) as colorless oil. LCMS(ESI⁺): 346.2 [M+H]⁺. ¹H NMR (400 MHz, DMSO-d₆) δ 8.59 - 8.57 (m, 1H), 8.00 - 7.97 (m, 1H), 7.80 - 7.78 (m, 1H), 7.73 - 7.60 (m, 3H), 7.42 - 7.30 (m, 4H), 3.88 (d, J = 4.0 Hz, 3H), 2.20 (s, 3H).

Step 3: Preparation of Compound 24-3

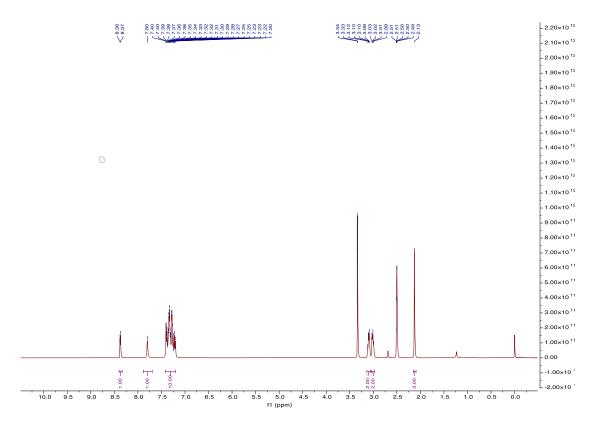
To a solution of methyl 2-((3-fluoro-2-(o-tolyl)pyridin-4-yl)ethynyl)benzoate (500.0 mg, 1.45 mmol) in MeOH (5 mL) was added wet Pd/C (77.0 mg, 10%). After addition, the mixture was degassed and purged with H₂ for 3 times and then the mixture was stirred at 25 °C for 1 hr under H₂ atmosphere (15Psi). The reaction mixture was filtered through a celite and the filter cake was rinsed with a small amount of MeOH (50 mL). The combined organic layers were concentrated under reduced pressure to afford **Compound 24-3** (400 mg), which was used in next step directly without further purification. LCMS (ESI⁺): 350.1 [M+H]⁺.

Step 4: Preparation of Compound 24-4

To a solution of methyl 2-(2-(3-fluoro-2-(o-tolyl)pyridin-4-yl)ethyl)benzoate (100.0 mg, 0.29 mmol) in MeOH (3 mL) and water (3 mL) was added 2 N NaOH (0.7 mL, 1.43 mmol) and then the reaction was stirred at 25 °C for 2 hrs. The reaction mixture was poured into 20 mL of ice-water carefully and the aqueous phase was washed with EtOAc (20 mL \times 2) and adjust pH = 9 with 1 N HCl. The resulting solution was extracted with EtOAc (20 mL \times 2). The combined organic layers were washed with brine (20 mL \times 2), dried over Na₂SO₄, filtered and concentrated under reduced pressure to afford **Compound 24-4** (90 mg), which was used in next step directly without further purification. LCMS(ESI⁺): 336.3 [M+H]⁺.

Step 5: Preparation of ISM061-24

To a solution of 2-(2-(3-fluoro-2-(o-tolyl)pyridin-4-yl)ethyl)benzoic acid (70.0 mg, 0.21 mmol) in DMF (2 mL) were added ammonium chloride (11.2 mg, 0.21 mmol), HATU (87.3 mg, 0.23 mmol) and DIEA (0.1 mL, 0.42 mmol) and then the reaction mixture was stirred at 25 °C for 2 hrs. The reaction solution was poured into water (50 mL) and extracted with EtOAc (25 mL × 3). The combined organic layers were washed with brine (20 mL × 2), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO₂, ethyl acetate: petroleum ether = 1: 1) to afford **ISM061-24** (30 mg, 43% yield) as a white solid. LCMS(ESI⁺): 335.3 [M+H]⁺. ¹H NMR (400 MHz, DMSO- d_6) δ 8.37 (d, J = 4.9 Hz, 1H), 7.80 (s, 1H), 7.41 – 7.20 (m, 10H), 3.14 – 3.06 (m, 2H), 3.04 – 2.97 (m, 2H), 2.13 (s, 3H).



S1.13. Preparation of ISM061-24-2

Scheme S13 Preparation of ISM061-24-2

Step 1: Preparation of Compound 24-5

To a solution of 2-bromobenzonitrile (2 g, 10.99 mmol) in 1,4-dioxane-H₂O (24 mL,5:1) was added 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane (2.5 g, 16.48 mmol), K₂CO₃ (3 g, 21.98 mmol) and

Pd(dppf)Cl2 (804 mg, 1.10 mmol) at 25 °C under N₂ atmosphere. After addition, the reaction mixture was heated and stirred at 100 °C under a nitrogen atmosphere for 12 h. After cooling to 25 °C, the reaction mixture was poured into ice water (200 mL) and extracted with EtOAc (100 mL × 3), the combined organic layers were washed with brine (150 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue, which was purified by column chromatography on silica gel eluting with ethyl acetate (from 0% to 6.8%) in petroleum ether to give the title compound (1.2 g, 84.5% yield) as a yellow liquid.

Step 2: Preparation of Compound 24-6

To a solution of 2-vinylbenzonitrile (500 mg, 3.87 mmol) in DMF (5 mL) was added KOAc (759 mg, 7.74 mmol), PPh₃ (203 mg, 0.77 mmol), 4-bromo-2-chloro-3-fluoropyridine (896 mg, 4.26 mmol) and Pd(OAc)₂ (175 mg, 0.77 mmol) at 25 °C under N₂ atmosphere. After addition, the reaction mixture was heated and stirred at 100 °C under a nitrogen atmosphere for 4 h. After cooling to 25 °C, the reaction mixture was poured into ice water (80 mL) and extracted with EtOAc (40 mL × 3), the combined organic layers were washed with brine (80 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue, which was purified by Prep-TLC (SiO₂, PE:EA=4: 1) to give the title compound (320 mg, 32.0% yield) as a white solid. LCMS (ESI⁺): .259.0 [M+H]⁺.

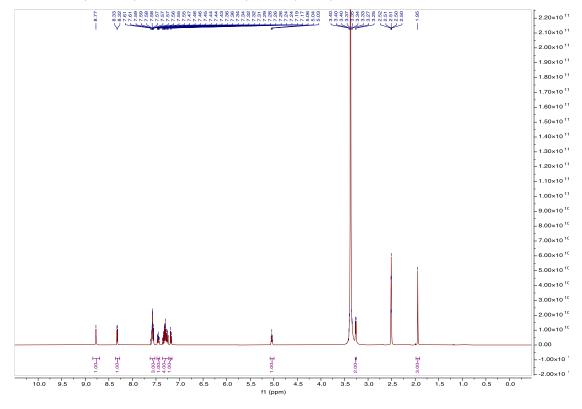
Step 3: Preparation of Compound 24-7

To a solution of **Compound 24-6** (250 mg, 0.97 mmol), o-tolylboronic acid (197 mg, 1.75 mmol) and K₂CO₃ (266 mg, 1.92 mmol) in dioxane (5 mL) and water (1 mL) was added Pd(dppf)Cl₂ (70 mg, 0.09 mmol) at 25 °C. After addition, the mixture was heated and stirred at 100 °C under a nitrogen atmosphere for 1 h. After cooling to 25 °C, the reaction mixture was poured into water (100 mL) and extracted with EtOAc (50 mL × 3), washed with brine (60 mL). The organic layer was dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue, which was purified by Prep-TLC (SiO₂, PE:EA=3: 1) to afford the title compound (300 mg, 98.7% yield) as a yellow soild. LCMS (ESI⁺): 315.2 [M+H]⁺.

Step 4: Preparation of ISM061-24-2

To a solution of **Compound 24-7** (250 mg, 0.79 mmol) and K₂CO₃ (330 mg, 2.38 mmol) in DMSO (5 mL) and Water (3 mL) was added H₂O₂ (5 mL, 30%wt in water) dropwise at 0 °C. After addition, the mixture was heated and stirred at 25 °C for 2 h. The reaction mixture was poured into water (50 mL) and extracted with EtOAc (30 mL × 3). The combined organic layers was washed with brine (30 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue, which was purified by column chromatography on silica gel eluting with ethyl acetate (from 0% to 100%) in petroleum ether to give the title compound (240 mg, 90.8% yield) as a yellow soild. LCMS (ESI⁺):

333.3 [M+H]⁺. ¹H NMR (400 MHz, DMSO- d_6) δ 8.77 (s, 1H), 8.32 (d, J = 4.8 Hz, 1H), 7.62 – 7.52 (m, 3H), 7.45 (ddd, J = 8.0, 5.8, 2.3 Hz, 1H), 7.36 – 7.24 (m, 4H), 7.20 – 7.16 (m, 1H), 5.04 (t, J = 5.5 Hz, 1H), 3.26 (d, J = 5.5 Hz, 2H), 1.95 (s, 3H).



S3. Surface Plasmon Resonance Affinity Measurements

In all experiments, the Biacore 8K system was used. For preliminary compound screening, N-terminal biotinylated KRAS G12D protein (synthesized by VIVA Biotech (Shanghai) Ltd, purity \geq 95%) was captured on a Sensor Chip SA (GE Healthcare) at a density of about 2000RU. Protein immobilization was done using 1x HBS-EP+, 2 mM TCEP, 2% DMSO as running buffer. Protein was injected for 70 s at a flow rate of 5 μ L/min. The protein concentration was 5 μ g/mL. The samples for initial screening of the compounds were prepared by serial two-fold dilutions from 200 μ M to 0.39 μ M in 1x HBS-EP+, 2mM TCEP, 2% DMSO. Samples were injected for 60 s at a flow rate of 30 μ L/min and the dissociation time was 180 s. Biacore 8K software was used for data analysis.

In order to validate the setup, the reference ligands MRTX1133 and BI-2852 where synthesized and tested. It was found that these two compounds exhibit a "slow off" dissociation feature (Figure S1). Their binding parameters (including the dissociation constant K_d) are listed in Table 2.

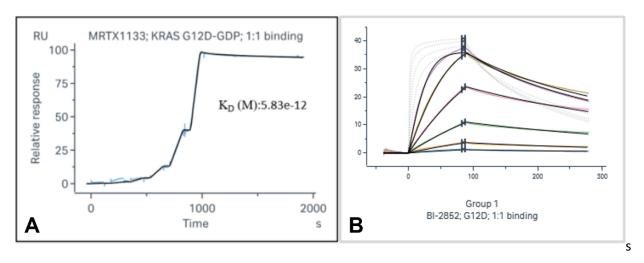


Figure S1. SPR experiments for the reference compounds. A) Response curve of MRTX1133 in single cycle mode. B) Response curve of BI-2852 in multiple cycle mode. MRTX1133 was too active to allow determination of K_d by the multiple cycling mode.

For 5 out of 12 newly generated and synthesized compounds, reasonable (micromolar) affinity was found (Table 3) along with the "fast off" kinetic behavior (Figure S2).

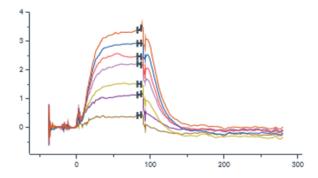


Figure S2. SPR response curves for multiple cycle mode experiments for ISM061-18-2.