Supporting Information

Multitarget inhibitors/probes that target LRRK2 and AURORA A kinases noncovalently and covalently

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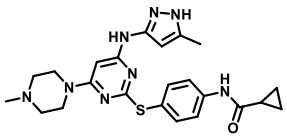
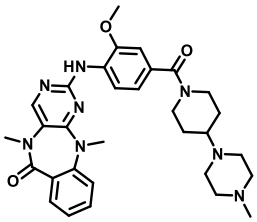
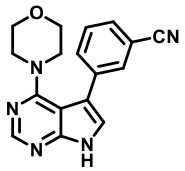
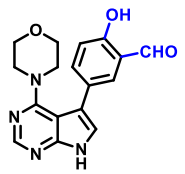
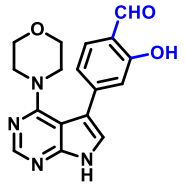
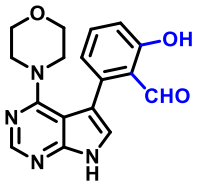
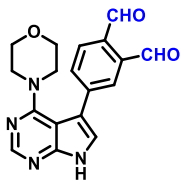
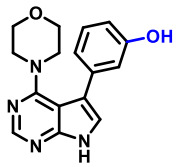
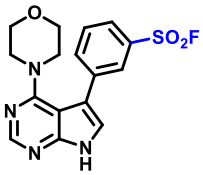
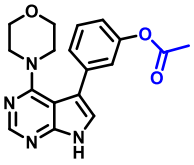
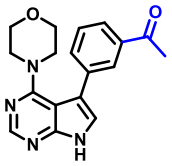
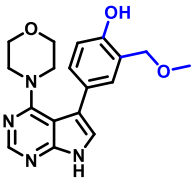
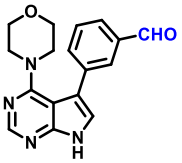
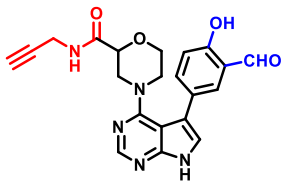
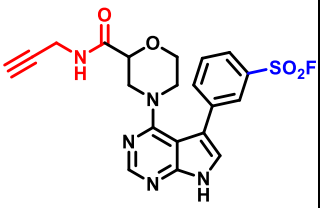
1. Table S1. Summary of key compounds (**A1-A11** and **ABP**s) used in this work

Compound	Chemical Structure	Study goal	Result
VX 680		A reported AURKA inhibitor used as reference compound in biochemical assay and anti-proliferation assay	Has been reported as an AURKA kinase inhibitor with IC_{50} of 0.6 nM and show an IC_{50} of 0.71 nM in our hands
LRRK2-IN-1		A reported LRRK2 inhibitor used as reference compound in biochemical assay and anti-proliferation assay	Has been reported as an LRRK2 kinase inhibitor with IC_{50} of 13 nM/6 nM for $LRRK2^{WT}$/$LRRK2^{G2019S}$ and show an IC_{50} of 5.89 nM/2.89 nM for $LRRK2^{WT}$/$LRRK2^{G2019S}$ in our hands
A1 (PF-06447475)		A reported LRRK2 inhibitor used as reference compound in biochemical assay and anti-proliferation assay	Has been reported as an LRRK2 kinase inhibitor with IC_{50} of 3 nM/6 nM for LRRK2 and show an IC_{50} of 2.36 nM/2.69 nM for $LRRK2^{WT}$/$LRRK2^{G2019S}$, and an IC_{50} of 701.7 nM for AURKA in our hands
A2		To establish the structure-activity relationship among compounds with SA warhead	IC_{50} = 16.0 nM ($LRRK2^{WT}$) IC_{50} = 11.6 nM ($LRRK2^{G2019S}$) IC_{50} = 1.28 nM ($AURKA^{WT}$)
A3		To establish the structure-activity relationship among compounds with SA warhead	IC_{50} = 43.6 nM ($LRRK2^{WT}$) IC_{50} = 18.6 nM ($LRRK2^{G2019S}$)
A4		To establish the structure-activity relationship among compounds with SA warhead	IC_{50} = 28.9 nM ($LRRK2^{WT}$) IC_{50} = 59.4 nM ($LRRK2^{G2019S}$)
A5		To establish the structure-activity relationship among compounds with SA warhead	IC_{50} = 1524 nM ($LRRK2^{WT}$) IC_{50} = 1008 nM ($LRRK2^{G2019S}$)

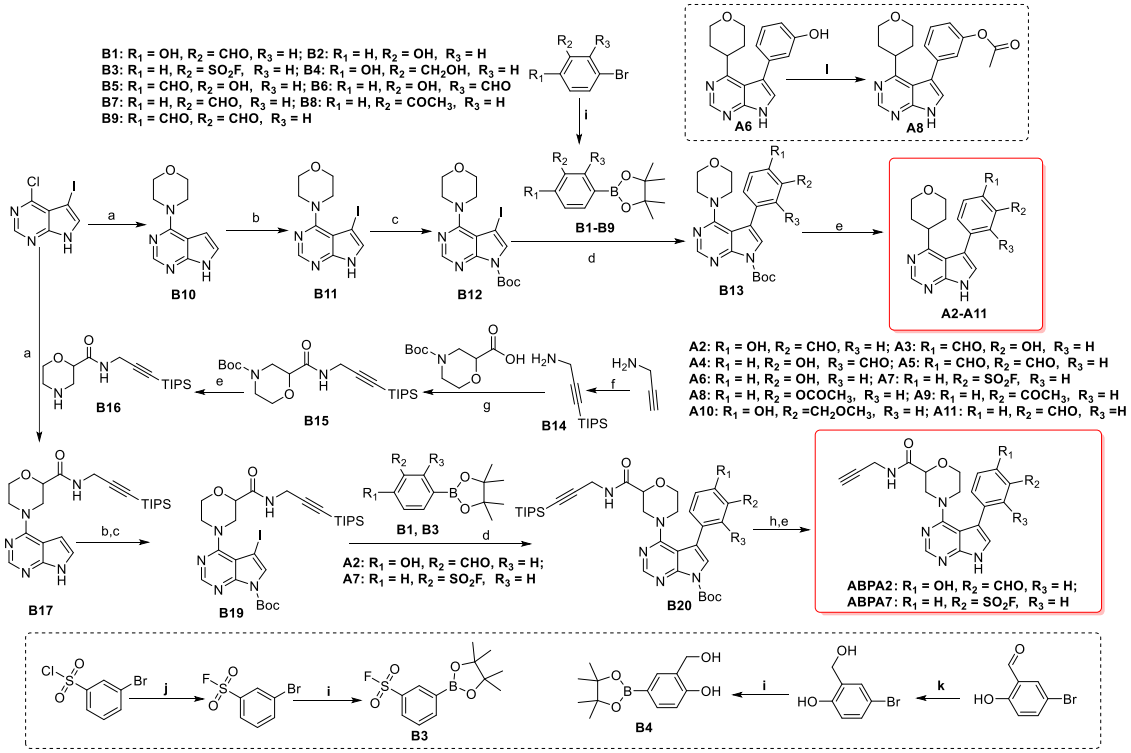
A6		To establish the structure-activity relationship among compounds with SA warhead	IC_{50} = 0.74 nM ($LRRK2^{WT}$) IC_{50} = 0.80 nM ($LRRK2^{G2019S}$)
A7		To establish the structure-activity relationship among compounds with SA warhead	IC_{50} = 931 nM ($LRRK2^{WT}$) IC_{50} = 108 nM ($LRRK2^{G2019S}$) IC_{50} = 718 nM ($AURKA^{WT}$)
A8		To establish the structure-activity relationship among compounds with SA warhead	Not detected
A9		To establish the structure-activity relationship among compounds with SA warhead	IC_{50} = 133 nM ($LRRK2^{WT}$) IC_{50} = 139 nM ($LRRK2^{G2019S}$)
A10		To establish the structure-activity relationship among compounds with SA warhead	IC_{50} = 310 nM ($LRRK2^{WT}$) IC_{50} = 175 nM ($LRRK2^{G2019S}$)
A11		To establish the structure-activity relationship among compounds with SA warhead	IC_{50} = 4.62 nM ($LRRK2^{WT}$) IC_{50} = 5.75 nM ($LRRK2^{G2019S}$)
ABP2		Probe used in chemoproteomic experiments	Successfully labeled off-targets in general in-gel fluorescence experiments; labeled AURKA kinase domain in competition assay and AURKA in pull-down in living cells
ABP7		Probe used in chemoproteomic experiments	Successfully labeled off-targets in general in-gel fluorescence experiments; labeled AURKA kinase domain in competition assay and AURKA in pull-down in living cells

2. Experimental.

2.1 General information

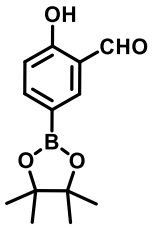
All chemicals were purchased from bide pharm, Sigma-Aldrich or Energy Chemical. All solvents and reagents were used as obtained without further purification, unless otherwise stated. All reactions that required anhydrous conditions were carried out under nitrogen or argon atmosphere using oven-dried glassware. Heating of reactions was accomplished with a silicon oil bath on top of a stirring hotplate equipped with an electronic contact thermometer to maintain the indicated temperature. The reaction process was monitored by analytical thin layer chromatography (TLC) on pre-coated silica plates (Merck 60 F254, 0.25 μm) and spots were visualized by UV (254/365 nm). Flash column chromatography was carried out using 200-300 or 300-400 mesh silica gel. All NMR spectra (^{1}H NMR, ^{13}C NMR and ^{19}C NMR) spectra were on a BRUKER 400-MR spectrometer. Chemical shifts (δ) are reported in ppm ($CDCl_3$ = 7.26 ppm and $(CD_3)_2SO$ = 2.50 ppm for ^{1}H NMR; $CDCl_3$ = 77.0 ppm and $(CD_3)_2SO$ = 39.5 ppm for ^{13}C NMR), The following abbreviations were used for reporting ^{1}H NMR spectra: chemical shift (δ ppm), s = singlet, d = doublet, t = triplet, q = quartet, quin = quintuplet, sext = sextet, sep = septet, dd = doublet of doublets, ddd = doublets of doublets of doublets, td = triplets of doublets, dt = doublets of triplets, br. = broad, app. = apparent, obs. = obscured and m = multiplet. Coupling constants *J*, are measured to the nearest 0.1 Hz. Chemical shifts are quoted to 0.01 ppm. All the measurements were performed at 25 °C. ESI mass spectrometry was measured on an Agilent Mass Spectrometer. High resolution mass spectra (HRMS) were recorded on DIONEX UltiMate 3000 & Bruker Compact TOF mass spectrometer. Purification via preparative HPLC (column, Phenomenex Gemini C18, mobile phase A, 1 ‰ FA in water; mobile phase B, acetonitrile; gradient, 10-50% B).

2.2 Synthesis and characterizations[1]



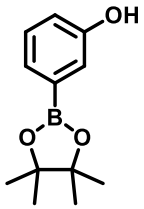
Scheme S1 Synthesis of inhibitors **A2-A11**, probes **ABPA2** and **ABPA7**. Reagents and conditions: (a) DIEA, *n*-BuOH, reflux, 3 h; (b) NIS, DMF, rt; (c) NaH, $(Boc)_2O$, THF; (d) XPhos Pd G2, K_3PO_4, 1,4-dioxane, H_2O, rt; (e) TFA, DCM, rt; (f) *n*-Butyllithium, triisopropylsilyl chloride, DIEA, -78 °C, 1.5 h; (g) HOBT, EDCI, DMF, rt; (h) TBAF, THF, rt; (i) CH_3COOK, $Pd(dppf)Cl_2 \cdot CH_2Cl_2$, 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane); (j) KF, rt; (k) $NaBH_4$, 0˚C; (l) pyridine, rt

2-hydroxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde (B1):[2]



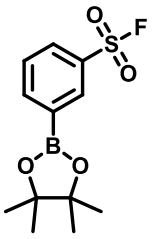
To a solution of 5-bromo-2-hydroxybenzaldehyde (1.50 g, 7.46 mmol) in 1,4-dioxane (20 mL) was added potassium acetate (2.20 g, 22.39 mmol) and Pd(dppf)Cl_2·CH_2Cl_2 (178.7 mg, 0.15 mmol). The solution was stirred for 15 min at room temperature and then 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (2.27 g, 8.95 mmol) was added. The reaction mixture was degassed and purged with nitrogen, then the reaction mixture was stirred at 85 °C for 18 h. Then the reaction was quenched by the addition of water (30 mL). The resulting solution was extracted with ethyl acetate (4×30 mL), dried over anhydrous sodium sulfate and concentrated *in vacuo* to give a residue. Purification via silica gel chromatography (gradient, 0-30% ethyl acetate in petroleum ether) afforded the product as a white solid 936.0 mg, yield: 50.6%. LC-MS: *m/z* 249.1 [M+H$^+$]. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 11.24 (d, J = 1.5 Hz, 1H), 9.94 (d, J = 1.5 Hz, 1H), 8.07 (s, 1H), 7.97 (d, J = 8.4 Hz, 1H), 7.00 (dd, J = 8.4, 1.5 Hz, 1H), 1.37 (s, 12H).

3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) phenol (B2):



To a solution of 3-bromophenol (1.73 g, 10.0 mmol) and 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (3.05 g, 12.0 mmol) in 1,4-dioxane (20 mL) were added Pd(dppf)Cl_2·CH_2Cl_2 (163.3 mg, 0.2 mmol), potassium acetate (2.94 g, 30.0 mmol). The reaction mixture was degassed and purged with nitrogen, then the reaction mixture was stirred at 85 °C for 18 h. After cooling to room temperature, the reaction mixture was diluted with water (100 mL) and extracted with ethyl acetate (3×50 mL). The combined organic layers were washed with saturated aqueous sodium chloride solution (100 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Purification via silica gel chromatography (gradient, 0-30% ethyl acetate in petroleum ether) afforded the product as a colorless solid 1.20 g, yield: 54.5%. LC-MS: *m/z* 221.1 [M+H$^+$]. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.32 (s, 1H), 7.27 - 7.13 (m, 1H), 7.09 (dd, J = 4.5, 2.7 Hz, 2H), 6.94 - 6.76 (m, 1H), 1.28 (s, 12H).

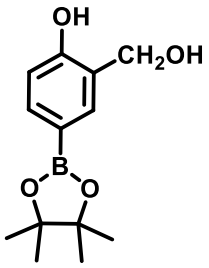
3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) benzenesulfonyl fluoride (B3):[3]



To a solution of 3-bromobenzenesulfonyl chloride (2.0 g, 7.83 mmol) in acetonitrile (8 mL), potassium fluoride (0.91 g, 15.65 mmol) was added. The mixture was stirred under room temperature for 48 h. Upon solvent evaporation, the crude product was partitioned between ethyl acetate and water, and extracted using ethyl acetate (3×30 mL). The combined organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford the corresponding sulfonyl fluoride as a pale oil 1.60 g, yield: 85.5%. LC-MS: *m/z* 240.0 [M+H$^+$]. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.18 (t, J = 1.9 Hz, 1H), 7.99 (d, J = 7.9 Hz, 1H), 7.93 (d, J = 8.1 Hz, 1H), 7.55 (t, J = 8.0 Hz, 1H).

Next, to a solution of oil from step 1 (1.60 g, 6.70 mmol) and 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (2.04 g, 8.03 mmol) in 1,4-dioxane (20 mL) were added Pd(dppf)Cl_2·CH_2Cl_2 (109.3 mg, 0.13 mmol), potassium acetate (1.97 g, 20.08 mmol). The reaction mixture was degassed and purged with nitrogen, then the reaction mixture was stirred at 85 °C for 18 h. After cooling to room temperature, the reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3×30 mL). The combined organic layers were washed with saturated aqueous sodium chloride solution (50 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Purification via silica gel chromatography (gradient, 0-30% ethyl acetate in petroleum ether) afforded the product as a white solid 1.04 g, Yield: 54.3%. LC-MS: *m/z* 287.0 [M+H$^+$]. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.46 (d, J = 1.5 Hz, 1H), 8.19 (d, J = 7.5 Hz, 1H), 8.10 (dq, J = 8.0, 1.4 Hz, 1H), 7.64 (t, J = 7.7 Hz, 1H), 1.39 (s, 12H). ^{19}F NMR (471 MHz, $CDCl_3$) δ (ppm) 65.86.

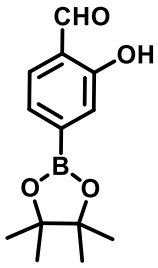
2-(hydroxymethyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (B4):[4]



5-bromo-2-hydroxybenzaldehyde (2.0 g, 10.0 mmol) and 20 mL of tetrahydrofuran was added into a round bottle flask and then 200.0 mg of sodium borohydride was added in 3 portions. This mixture was stirred at 0˚C for 30 min, the reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3×30 mL). The combined organic layers were washed with saturated aqueous sodium chloride solution (50 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo* to afforded the product as a white solid 1.80 g, yield: 89.1%. The crude solid was use for the next step without purified.

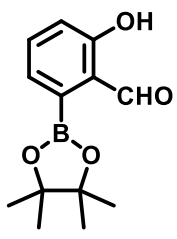
Next, to a solution of 4-bromo-2-(hydroxymethyl) phenol (1.80 g, 7.87 mmol) and 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (2.40 g, 10.64 mmol) in 1,4-dioxane (20 mL) were added Pd(dppf)Cl_2·CH_2Cl_2 (128.7 mg, 0.157 mmol), potassium acetate (2.32 g, 23.64 mmol). The reaction mixture was degassed and purged with nitrogen, then the reaction mixture was stirred at 85 °C for 18 h. After cooling to room temperature, the reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3×30 mL). The combined organic layers were washed with saturated aqueous sodium chloride solution (50 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Purification via silica gel chromatography (gradient, 0-30% ethyl acetate in petroleum ether) afforded the product as a white solid 1.30 g, yield: 58.6%. LC-MS: *m/z* 251.1 [M+H^+]. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.75 (s, 1H), 7.66 (d, J = 22.3 Hz, 1H), 7.56 – 7.30 (m,1H), 6.93 – 6.67 (m, 1H), 5.14 – 4.85 (m, 1H), 4.44 (dt, J = 20.4, 5.5 Hz, 2H), 1.27 (s, 12H).

2-hydroxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde (B5):



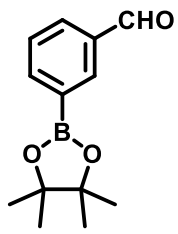
To a solution of 4-bromo-2-hydroxybenzaldehyde (2.01 g, 10.0 mmol) and 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (3.05 g, 12.0 mmol) in 1,4-dioxane (20 mL) were added Pd(dppf)Cl_2·CH_2Cl_2 (163.3 mg, 0.199 mmol), potassium acetate (2.94 g, 30.0 mmol). The reaction mixture was degassed and purged with nitrogen, then the reaction mixture was stirred at 85 °C for 18 h. After cooling to room temperature, the reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3×30 mL). The combined organic layers were washed with saturated aqueous sodium chloride solution (50 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Purification via silica gel chromatography (gradient, 0-30% ethyl acetate in petroleum ether) afforded the product as a white solid 1.60 g, yield: 64.5%. LC-MS: *m/z* 249.1 [M+H^+]. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 10.62 (s, 1H), 10.33 (s, 1H), 7.64 (d, J = 7.6 Hz, 1H), 7.31 (d, J = 0.9 Hz, 1H), 7.21 (dt, J = 7.5, 0.8 Hz, 1H), 1.30 (s, 12H).

2-hydroxy-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde (B6):



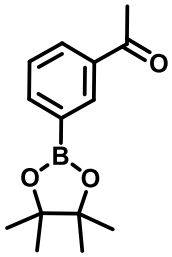
To a solution of 2-bromo-6-hydroxybenzaldehyde (2.01 g, 10.0 mmol) and 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (3.05 g, 12.0 mmol) in 1,4-dioxane (20 mL) were added Pd(dppf)Cl_2·CH_2Cl_2 (163.3 mg, 0.20 mmol), potassium acetate (2.94 g, 30.0 mmol). The reaction mixture was degassed and purged with nitrogen, then the reaction mixture was stirred at 85 °C for 18 h. After cooling to room temperature, the reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3×30 mL). The combined organic layers were washed with saturated aqueous sodium chloride solution (50 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Purification via silica gel chromatography (gradient, 0-30% ethyl acetate in petroleum ether) afforded the product as a yellow solid 700.0 mg, yield: 28.2%. LC-MS: *m/z* 249.1 [M+H^+]. ^{1}H NMR. (400 MHz, DMSO-d_6) δ (ppm) 11.02 (s, 1H), 10.38 (s, 1H), 7.50 (dd, J = 8.4, 7.0 Hz, 1H), 7.02 (ddd, J = 7.1, 3.8, 1.2 Hz, 2H), 1.32 (s, 12H).

3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde (B7):



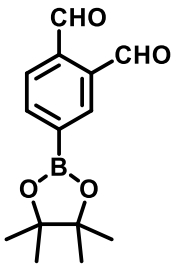
To a solution of 3-bromobenzaldehyde (1.83 g, 10.0 mmol) and 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (3.01 g, 11.87 mmol) in 1,4-dioxane (20 mL) were added $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (161.5 mg, 0.20 mmol), potassium acetate (2.91 g, 29.67 mmol). The reaction mixture was degassed and purged with nitrogen, then the reaction mixture was stirred at 85 °C for 18 h. After cooling to room temperature, the reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3×30 mL). The combined organic layers were washed with saturated aqueous sodium chloride solution (50 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Purification via silica gel chromatography (gradient, 0-30% ethyl acetate in petroleum ether) afforded the product as a white solid 1.80 g, yield:78.4 %. LC-MS: *m/z* 233.1 [M+H$^+$]. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ (ppm) 10.07 (s, 1H), 8.22 (t, J = 1.7 Hz, 1H), 8.03 (dt, J = 7.7, 1.6 Hz, 1H), 8.02 – 7.95 (m, 1H), 7.63 (t, J = 7.5 Hz, 1H), 1.33 (s, 12H).

1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)ethan-1-one (B8):



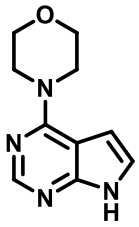
To a solution of 1-(3-bromophenyl) ethan-1-one (1.97 g, 10 mmol) and 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (3.02 g, 11.88 mmol) in 1,4-dioxane (20 mL) were added $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (161.7 mg, 0.20 mmol%), potassium acetate (2.91 g, 29.69 mmol). The reaction mixture was degassed and purged with nitrogen, then the reaction mixture was stirred at 85 °C for 18 h. After cooling to room temperature, the reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3×30 mL). The combined organic layers were washed with saturated aqueous sodium chloride solution (50 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Purification via silica gel chromatography (gradient, 0-30% ethyl acetate in petroleum ether) afforded the product as a white solid 1.60 g, yield: 65.7%, LC-MS: *m/z* 247.1 [M+H$^+$]. ^{1}H NMR. (400 MHz, DMSO-*d*$_6$) δ (ppm) 8.22 (dt, J = 1.8, 0.7 Hz, 1H), 8.09 (dt, J = 7.8, 1.5 Hz, 1H), 7.91 (dt, J = 7.3, 1.3 Hz, 1H), 7.56 (t, J = 7.6 Hz, 1H), 2.60 (s, 3H), 1.32 (s, 12H)

4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) phthalaldehyde (B9):



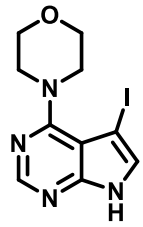
A mixture of 3-bromo-4-methoxyphenol (350.0 mg, 1.64 mmol), B_2pin_2 (500.6 mg, 1.81 mmol), $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (120.0 mg, 0.16 mmol) and potassium acetate (483.0 mg, 4.93 mmol) were dissolved in 20 mL of dioxane. The reaction mixture was degassed and purged with nitrogen, then the reaction mixture was stirred at 100 °C for 7 h. The product formation was confirmed by TLC and LC-MS. Upon solvent evaporation, the crude product was partitioned between ethyl acetate and water, and extracted using ethyl acetate (3×50 mL). The combined organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude product was purified by flash chromatography (hexane/ethyl acetate 0%-5%) to give the target compound as a yellow solid 300.0 mg, yield: 70.2%, LC-MS: *m/z* 261.1 [M+H$^+$]. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ (ppm) 10.52 (s, 1H), 10.48 (s, 1H), 8.26 (d, J = 1.2 Hz, 1H), 8.11 (dd, J = 7.6, 1.2 Hz, 1H), 7.97 (d, J = 7.5 Hz, 1H), 1.34 (s, 12H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ (ppm) 193.90, 193.72, 139.82, 138.71, 136.62, 136.00, 129.52, 85.01, 27.51, 25.30, 25.14.

4-(7*H*-pyrrolo[2,3-*d*] pyrimidin-4-yl) morpholine (B10):



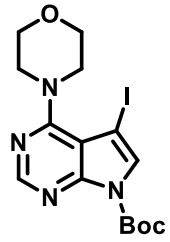
Morpholine (1.14 g, 13.10 mmol) and N, N-diisopropylethylamine (3.43 g, 21.83 mmol) were added to a solution of 4-chloro-5-iodo-7*H*-pyrrolo[2,3-*d*] pyrimidine-7-carboxylate (3.05 g, 8.06 mmol) in n-butanol (20 mL), and the reaction mixture was heated at reflux for 3h, then concentrated under reduced pressure. Aqueous hydrochloric acid (0.1 M, 100 mL) was added and the resulting solid was collected by filtration, washed with water (20 mL), and dried under vacuum to provide the product 4-(7*H*-pyrrolo[2,3-*d*] pyrimidin-4-yl) morpholine as a yellow solid 1.70 g, Yield: 76.3%, LC-MS: *m/z* 205.1 [M+H$^+$]. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 11.73 (s, 1H), 8.17 (d, J = 1.3 Hz,1H), 7.20 (dd, J = 3.6, 2.3 Hz, 1H), 6.89 – 6.46 (m, 1H), 3.83 (dd, J = 5.9, 3.9 Hz, 4H), 3.72 (dd, J = 5.9, 3.9 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm)156.87, 150.69, 150.57, 123.74, 105.02, 100.14, 65.88, 50.04.

4-(5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)morpholine (B11):[5]



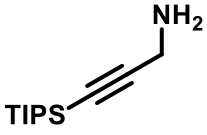
4-(7*H*-pyrrolo[2,3-*d*] pyramidin-4-yl) morpholine (204.0 mg, 1.0 mmol) was dissolved in 5 mL of N, N-dimethylformamide which was stirred for about 20 min. N-iodosuccinimide (337.0 mg, 1.50 mmol) was added to the solution at room temperature and stirred for another 3 h. The mixture was quenched with saturated solution of sodium thiosulfate, and water was added to afford a yellow solid 277.0 mg, yield: 84.0%, LC-MS: *m/z* 331.0 [M+H$^+$]. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 11.73 (s, 1H), 8.16 (d, J = 2.0 Hz, 1H), 7.20 (dd, J = 3.7, 2.3 Hz, 1H), 4.06 – 3.81 (m, 4H), 3.81 – 3.51 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm)156.87, 150.57, 123.74, 105.02, 100.14, 65.88, 56.01, 50.04.

***tert*-butyl 5-iodo-4-morpholino-7*H*-pyrrolo[2,3-*d*] pyrimidine-7-carboxylatestep (B12):**



A solution of 4-(5-iodo-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-yl) morpholine (360.0 mg, 1.10 mmol) in dry tetrahydrofuran (10 mL) was cooled to 0 °C and treated with sodium hydride (60% in oil, 64.0 mg, 1.60 mmol) in three portions. After the reaction mixture had stirred at 0 °C for 1 h, *tert*-butyldicarbonate (357.0 mg, 1.64 mmol) was added dropwise, and the reaction mixture was warmed to room temperature and allowed to stir for 3 h. The reaction was quenched with saturated aqueous sodium chloride solution (20 mL), and the organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*. Silica gel chromatography (petroleum ether/ethyl acetate, v/v, 10:1) afforded the product as a white solid 450.0 mg, yield: 95.9% LC-MS: *m/z* 431.05 [M+H$^+$]. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.50 (s, 1H), 7.90 (s, 1H), 3.83 (t, J = 4.7 Hz, 4H), 3.47 (t, J = 4.6 Hz, 4H), 1.59 (s, 9H).

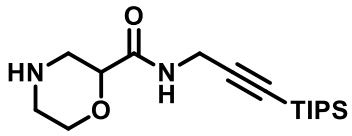
3-(triisopropylsilyl)prop-2-yn-1-amine (B14):[6]



A solution of propargylamine (1.10 g, 20.0 mmol) in tetrahydrofuran (50 mL) was placed in a three-necked, 250 mL round-bottom flask equipped with a magnetic stirring bar and cooled to -78 °C while stirring in an argon atmosphere. *N*-Butyllithium (2.5 M in hexanes, 9 mL) was added slowly through a septum by using a syringe, and the reaction was allowed to proceed for 15 min. The mixture was then warmed to 0 °C, and triisopropylsilyl chloride (4.24 g, 22.0 mmol) was added drop-wise. After stirring for 1.5 h at 0 °C, the reaction mixture was diluted with saturated sodium bicarbonate solution (50 mL) and stirred for 5 min. The solution was diluted with water (100 mL), and extracted with diethyl ether. The combined organic phase was washed with brine, dried with anhydrous sodium sulfate, filtered, and the solvents evaporated. The dark-yellow oily residue was purified by flash chromatography on silica (ethyl acetate), to give amine as an oil 3.50 g, yield: 82.8%. LC-MS: *m/z* 212.2 [M+H$^+$].^{1}H NMR (500 MHz, $CDCl_3$) δ

(ppm) 3.45 (s, 2H), 1.51 (s, 2H), 1.20-1.00 (m, 21H).

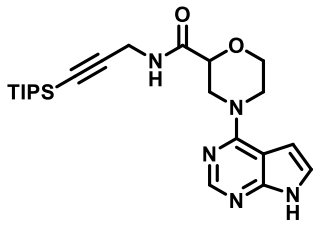
***N*-(3-(triisopropylsilyl) prop-2-yn-1-yl) morpholine-2-carboxamide (B16)**:



Step 1: To a solution of 4-(*tert*-butoxycarbonyl) morpholine-2-carboxylic acid (253.0 mg, 1.10 mmol), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (191.3 mg, 2.0 mmol) and 1-Hydroxybenzotriazole (269.70 mg, 2.0 mmol) in N, N-dimethylformamide was add 3-(triisopropylsilyl) prop-2-yn-1-amine (211.0 mg, 1.0 mmol), and then the mixture was stirred for overnight under room temperature. The reaction mixture was diluted with water (50 mL) and extracted with EA (200 mL). The combined organic phase was washed with brine (200 mL), dried with anhydrous sodium sulfate, filtered, and the solvents evaporated. The dark-yellow oil residue was purified by flash chromatography on silica (ethyl acetate), to give white solid *tert*-butyl 2-((3-(triisopropylsilyl) prop-2-yn-1-yl) carbamoyl) morpholine-4-carboxylate 370.0 mg, yield: 87.3%. LC-MS: *m/z* 425.2 [M+H$^+$].^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 6.67 (s, 1H), 4.33 (s, 1H), 4.11 (t, J = 5.2 Hz, 2H), 3.94 (ddd, J = 32.5, 11.1, 3.3 Hz, 3H), 3.58 (td, J = 11.7, 2.8 Hz, 1H), 2.96 – 2.76 (m, 1H), 2.73 (d, J = 12.6 Hz, 1H) , 1.46 (s, 9H), 1.06 (d, J = 2.8 Hz, 21H).

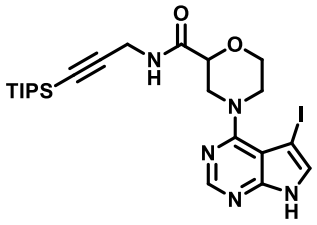
Step 2: A solution of *tert*-butyl 2-((3-(triisopropylsilyl) prop-2-yn-1-yl) carbamoyl) morpholine-4-carboxylate in dichloromethane/trifluoroacetic acid (v/v=1:1) was stirred at room temperature for 2 h. The reaction mixture was washed with water and the organic phase was concentrated under reduced pressure to afford the product as a yellow oil, the crude compound was use for nest step without further purify, yield 100%.

4-(7*H*-pyrrolo[2,3-*d*] pyrimidin-4-yl)-*N*-((triisopropylsilyl)ethynyl) morpholine-2-carboxamide (B17):



N-(3-(triisopropylsilyl) prop-2-yn-1-yl) morpholine-2-carboxamide (280.0 mg, 0.86 mmol) and N, N-diisopropylethylamine (220.0 mg, 1.72 mmol) were added to a solution of 4-chloro-5-iodo-7*H*-pyrrolo[2,3-*d*] pyrimidine (260.0 mg, 0.95 mmol) in *n*-butanol (30 mL), and the reaction mixture was heated at reflux for 6 h, then concentrated under reduced pressure. Aqueous hydrochloric acid (0.1 M, 100 mL) was added and the resulting solid was collected by filtration, washed with water, and dried under vacuum to provide the product as a white solid 270.0 mg, yield: 70.9%. LC-MS: *m/z* 442.2 [M+H$^+$]. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 11.79 (s, 1H), 8.41 (t, J = 5.9 Hz, 1H), 8.19 (s, 1H), 7.24 (dd, J = 3.6, 2.5 Hz, 1H), 6.58 (dd, J = 3.6, 1.8 Hz, 1H), 4.37 (dq, J = 13.3, 2.3 Hz, 1H), 4.10 (dd, J = 9.9, 3.1 Hz, 1H), 4.03 (dt, J = 11.5, 3.0 Hz, 1H), 3.96 (d, J = 5.9 Hz, 2H), 3.71 (td, J = 11.2, 2.9 Hz, 1H), 3.36 (dd, J = 10.6, 3.2 Hz, 1H), 3.17 (dd, J = 13.2, 9.9 Hz, 1H), 1.08 – 0.96 (m, 3+18H). ^{13}C NMR (126 MHz, DMSO-d_6) δ (ppm) 168.85, 156.87, 152.54, 150.92, 122.37, 105.96, 102.93, 100.97, 81.62, 75.17, 65.96, 47.46, 45.49, 29.24, 18.89, 11.10.

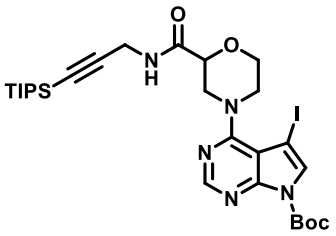
4-(5-iodo-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-yl)-*N*-((triisopropylsilyl)ethynyl) morpholine-2-carboxamide (B18):



To a solution of 4-(7*H*-pyrrolo[2,3-*d*] pyrimidin-4-yl)-*N*-(3-(triisopropylsilyl) prop-2-yn-1-yl) morpholine-2-carboxamide (1.60 g, 3.62 mmol) in tetrahydrofuran (30 mL) was added *N*-Iodosuccinimide (889.0 mg, 3.99 mmol). The resulting mixture was stirred at ambient temperature for 1 h. The mixture was then concentrated and extracted with ethyl acetate (3×50 mL). The combined organic layers were washed with saturated solution of sodium thiosulfate and brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude product was purified by flash chromatography (dichloromethane/methanol 0%-2%) to give the target compound as a yellow solid 770.0 mg, yield: 37.5%. LC-MS: *m/z* 568.1 [M+H$^+$].^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 11.79 (s, 1H), 8.41 (t, J =

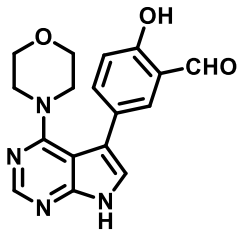
5.9 Hz, 1H), 8.19 (s, 1H), 7.24 (dd, *J* = 3.6, 2.5 Hz, 1H), 4.37 (dq, *J* = 13.3, 2.3 Hz, 1H), 4.10 (dd, *J* = 9.9, 3.1 Hz, 1H), 4.03 (dt, *J* = 11.5, 3.0 Hz, 1H), 3.96 (d, *J* = 5.9 Hz, 2H), 3.71 (td, *J* = 11.2, 2.9 Hz, 1H), 3.36 (dd, *J* = 10.6, 3.2 Hz, 1H), 3.17 (dd, *J* = 13.2, 9.9 Hz, 1H), 1.08 – 0.96 (m, 21H). ^{13}C NMR (126 MHz, DMSO-d_6) δ (ppm) 168.85, 156.87, 152.54, 150.92, 122.37, 107.93, 105.96, 100.97, 81.62, 75.17, 65.96, 47.46, 45.49, 29.24, 18.89, 11.10.

***tert*-butyl 5-iodo-4-(2-(((triisopropylsilyl)ethynyl)carbamoyl)morpholino)-7*H*-pyrrolo[2,3-*d*]pyrimidine-7-carboxylate (B19)**: A solution of 4-(5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)-*N*-(3-(triisopropylsilyl)prop-2-yn-1-yl)morpholine-2-carboxamide (770.0 mg, 1.36 mmol) in dry tetrahydrofuran (20 mL) was cooled to 0 °C and treated with sodium hydride (60% in oil, 64.0 mg, 1.60 mmol) in three portions. After the reaction mixture had stirred at 0 °C for 1 h, *tert*-butyldicarbonate (412.0 mg, 2.04 mmol) was added dropwise, and the reaction mixture was warmed to room temperature and allowed to stir for 3 h. The reaction was quenched with saturated aqueous sodium chloride solution (20mL), and the organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*. Silica gel chromatography (petroleum ether/ethyl acetate, v/v, 10:1) afforded the product as a white solid 850.0 mg, yield: 93.8%. LC-MS: *m/z* 668.2 [M+H$^+$]. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 8.52 (s, 0H), 8.34 (t, *J* = 5.9 Hz,1H), 7.93 (s, 1H), 4.23 (dd, *J* = 10.4, 2.8 Hz, 1H), 4.13 (dt, *J* = 12.9, 2.3 Hz, 1H), 4.09 – 4.01 (m, 1H), 3.94 (d, *J* = 5.8 Hz, 2H), 3.88 (d, *J* = 11.6 Hz, 2H), 3.27 – 3.10 (m, 1H), 2.98 (dd, *J* = 13.0, 10.4 Hz, 1H), 1.60 (s, 9H), 1.03 (d, *J* = 4.2 Hz, 21H). ^{13}C NMR (126 MHz, DMSO-d_6) δ (ppm) 168.56, 160.63, 152.74, 146.42, 130.93, 109.87, 105.91, 85.32, 81.66, 74.94, 65.97, 53.30, 51.22, 29.20, 27.99, 18.90, 11.10.

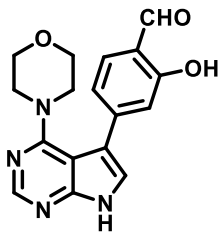


2-hydroxy-5-(4-morpholino-7*H*-pyrrolo[2,3-*d*] pyrimidin-5-yl) benzaldehyde (A2):

A mixture of *tert*-butyl 5-iodo-4-morpholino-7*H*-pyrrolo[2,3-*d*]pyrimidine-7-carboxylate (215.0 mg, 0.50 mmol), 2-hydroxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde (148 mg, 0.6 mmol), XPhos Pd G2 (39.0 mg, 0.05 mmol) and potassium phosphate tribasic (212.3 mg, 1 mmol) was dissolved in 10 mL of dioxane and 1 mL of water and then stirred at 90 °C in a sealed tube for 3 h. The product formation was confirmed by TLC and LC-MS. The crude product was partitioned between ethyl acetate and water, and extracted using ethyl acetate (3×30 mL). The combined organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure give the crude compound as an oil. The crude oil was dissolved in dichloromethane/trifluoroacetic acid (5 mL/3 mL) and stirred at ambient temperature over 1 h. Upon solvent evaporation, the mixture was adjusted to *p*H = 7 and extracted using dichloromethane (3×10 mL). The combined organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude product was purified by flash chromatography (dichloromethane/methanol 0-5%) to give the target compound as a yellow solid 105.0 mg, yield: 64.8%. HRMS (ESI$^+$) calcd for $C_{17}H_{16}N_4O_3$ ([M+H$^+$]): 325.1301, found 325.1295. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.07 (s, 1H), 9.46 (s, 1H), 8.34 (s, 1H), 7.42 (d, *J* = 2.4 Hz, 1H), 7.23 (t, *J* = 7.8 Hz, 1H), 6.98 – 6.88 (m, 2H), 6.70 (d, *J* = 7.9 Hz, 1H), 3.48 (t, *J* = 4.9 Hz, 4H), 3.30 – 3.10 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm) 191.60, 160.44, 159.87, 153.49, 150.67, 136.38, 128.37, 127.19, 122.74, 122.66, 118.01, 115.02, 103.14, 65.88, 50.04. **Noted:** after coupling the target compound and the compound with Boc protection were obtained at the same time, the ratio of target compound and the compound with Boc protection was about 4:6.

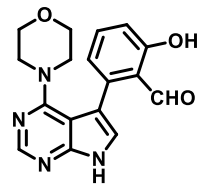


2-hydroxy-4-(4-morpholino-7*H*-pyrrolo[2,3-*d*] pyrimidin-5-yl) benzaldehyde (A3):



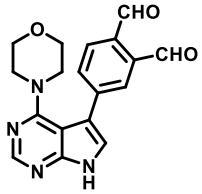
A mixture of *tert*-butyl 5-iodo-4-morpholino-7*H*-pyrrolo[2,3-*d*] pyrimidine-7-carboxylate (215.0 mg, 0.5 mmol), 2-hydroxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde (148.8 mg, 0.6 mmol), XPhos Pd G2 (39.0 mg, 0.05 mmol) and potassium phosphate tribasic (212.3 mg, 1 mmol) was dissolved in 10 mL of dioxane and 1 mL of water and then stirred at 90 °C in a sealed tube for 3 h. The product formation was confirmed by TLC and LC-MS. The crude product was partitioned between ethyl acetate and water, and extracted using ethyl acetate (3×30 mL). The combined organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure give the crude compound as an oil. The crude oil was dissolved in dichloromethane/trifluoroacetic acid (5 mL/3 mL) and stirred at ambient temperature over 1 h. Upon solvent evaporation, the mixture was adjusted to *p*H = 7 and extracted using dichloromethane (3×10 mL). The combined organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude product was purified by flash chromatography (dichloromethane/methanol 0-5%) to give the target compound as a yellow solid 60.0 mg, yield: 37.0%. HRMS (ESI^+) calcd for $C_{17}H_{16}N_4O_3$ ([M+H^+]): 325.1301, found 325.1300. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.34 (d, J = 2.6 Hz, 1H), 10.88 (s, 1H), 10.24 (s, 1H), 8.39 (s, 1H), 7.73 (d, J = 8.1 Hz, 1H), 7.68 (d, J = 2.7 Hz, 1H), 7.19 (dd, J = 7.9, 1.7 Hz, 1H), 7.16 (d, J = 1.6 Hz, 1H), 3.68 – 3.49 (m, 4H), 3.32 – 3.01 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm) 191.79, 161.19, 160.20, 153.98, 150.98, 143.80, 130.21, 124.69, 120.53, 119.61, 116.37, 115.42, 102.53, 65.89, 49.80.

2-hydroxy-6-(4-morpholino-7*H*-pyrrolo[2,3-*d*] pyrimidin-5-yl) benzaldehyde (A4):



A mixture of *tert*-butyl 5-iodo-4-morpholino-7*H*-pyrrolo[2,3-*d*] pyrimidine-7-carboxylate (215.0 mg, 0.5 mmol), 2-hydroxy-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde (148.8 mg, 0.6 mmol), XPhos Pd G2 (39.0 mg, 0.05 mmol) and potassium phosphate tribasic (212.3 mg, 1.0 mmol) was dissolved in 10 mL of dioxane and 1 mL of water and then stirred at 90 °C in a sealed tube for 3 h. The product formation was confirmed by TLC and LC-MS. The crude product was partitioned between ethyl acetate and water, and extracted using ethyl acetate (3×30 mL). The combined organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure give the crude compound as an oil. The crude oil was dissolved in dichloromethane/trifluoroacetic acid (5 mL/3 mL) and stirred at ambient temperature over 1 h. Upon solvent evaporation, the mixture was adjusted to *p*H = 7 and extracted using dichloromethane (3×10 mL). The combined organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude product was purified by flash chromatography (dichloromethane/methanol 5%) to give the target compound as a yellow solid 20.0 mg, yield: 12.3%. HRMS (ESI^+) calcd for $C_{17}H_{16}N_4O_3$ ([M+H^+]): 325.1301, found 325.1294. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.44 (s, 1H), 11.93 (s, 1H), 9.65 (s, 1H), 8.44 (s, 1H), 7.75 – 7.52 (m, 2H), 7.06 (d, J = 7.3 Hz, 1H), 7.00 (d, J = 8.3 Hz, 1H), 3.36 (m, 4H), 3.01 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm) 197.73, 162.08, 160.00, 153.24, 151.03, 140.30, 137.26, 125.39, 122.20, 118.45, 116.33, 111.19, 105.54, 65.57, 49.93.

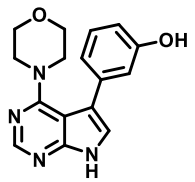
4-(4-morpholino-7*H*-pyrrolo[2,3-*d*] pyrimidin-5-yl) phthalaldehyde (A5):



A mixture of *tert*-butyl 5-iodo-4-morpholino-7*H*-pyrrolo[2,3-*d*] pyrimidine-7-carboxylate (150 mg, 0.35 mmol), 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) phthalaldehyde (100 mg, 0.38 mmol), XPhos Pd G2 (30.0 mg,) and potassium phosphate tribasic (160 mg, 1.0 mmol) was dissolved in 10 mL of dioxane and 1 mL of water and then stirred at 90 °C in a sealed tube for 3 h. The product formation was confirmed by TLC and LC-MS. The crude

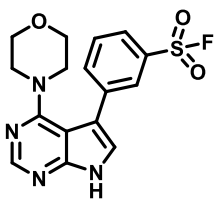
product was partitioned between ethyl acetate and water, and extracted using ethyl acetate (3×30 mL). The combined organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure give the crude compound as an oil. The crude oil was dissolved in dichloromethane/trifluoroacetic acid (5 mL/3 mL) and stirred at ambient temperature over 1 h. Upon solvent evaporation, the mixture was adjusted to *p*H = 7 and extracted using dichloromethane (3×10 mL). The combined organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude product was purified by flash chromatography (dichloromethane/methanol 0-5%) to give the target compound as a yellow solid 7.0 mg, yield: 6.0%. HRMS (ESI$^+$) calcd for $C_{18}H_{16}N_4O_3$ ([M+H$^+$]): 337.1301 found 337.1302. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.36 (s, 1H), 10.35 (s, 1H), 8.39 (s, 1H), 7.97 (d, J = 1.7 Hz, 1H), 7.93 (d, J = 7.9 Hz, 1H), 7.80 – 7.71 (m, 1H), 7.68 (s, 1H), 6.73 (s, 1H), 3.60 - 3.42 (m, 4H), 3.28 – 3.15 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm) 191.75, 160.28, 154.08, 151.05, 142.29, 141.22, 131.63, 129.32, 127.90, 125.59, 124.76, 115.39, 102.61, 95.98, 83.21, 65.60, 49.80.

3-(4-morpholino-7*H*-pyrrolo[2,3-*d*] pyrimidin-5-yl) phenol) (A6):



A mixture of *tert*-butyl 5-iodo-4-morpholino-7*H*-pyrrolo[2,3-*d*]pyrimidine-7-carboxylate (215.0 mg, 0.5 mmol), 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (131.98 mg, 0.6 mmol), XPhos Pd G2 (39.0 mg, 0.05 mmol) and potassium phosphate tribasic (212.3 mg, 1.0 mmol) was dissolved in 10 mL of dioxane and 1 mL of water and then stirred at 90 °C in a sealed tube for 3 h. The product formation was confirmed by TLC and LC-MS. The crude product was partitioned between ethyl acetate and water, and extracted using ethyl acetate (3×30 mL). The combined organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure give the crude compound as an oil. The crude oil was dissolved in dichloromethane/trifluoroacetic acid (5 mL/3 mL) and stirred at ambient temperature over 1 h. Upon solvent evaporation, the mixture was adjusted to *p*H = 7 and extracted using dichloromethane (3×10 mL). The combined organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude product was purified by flash chromatography (dichloromethane/methanol 0-5%) to give the target compound as a white solid 59.8 mg, yield: 40.4%. HRMS (ESI$^+$) calcd for $C_{16}H_{16}N_4O_2$ ([M+H$^+$]): 297.1352, found 297.1340. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.07 (s, 1H), 9.46 (s, 1H), 8.34 (s, 1H), 7.42 (d, J = 2.4 Hz, 1H), 7.23 (t, J = 7.8 Hz, 1H), 6.98 - 6.88 (m, 2H), 6.70 (d, J = 7.9 Hz, 1H), 3.48 (t, J = 4.9 Hz, 4H), 3.30 – 3.10 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm) 160.20, 157.72, 153.47, 150.59, 137.07, 129.93, 122.63, 119.24, 116.54, 115.70, 113.70, 102.90, 65.95, 49.83.

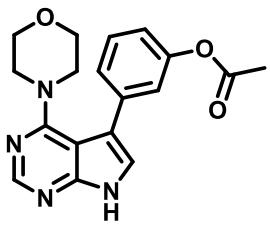
3-(4-morpholino-7*H*-pyrrolo[2,3-*d*] pyrimidin-5-yl) benzenesulfonyl fluoride (A7):



A mixture of *tert*-butyl 5-iodo-4-morpholino-7*H*-pyrrolo[2,3-*d*] pyrimidine-7-carboxylate (215.0 mg, 0.5 mmol), 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) benzenesulfonyl fluoride (176 mg, 0.6 mmol), XPhos Pd G2 (39.0 mg, 0.05 mmol) and potassium phosphate tribasic (212.3 mg, 1.0 mmol) was dissolved in 10 mL of dioxane and 1 mL of water and then stirred at 90 °C in a sealed tube for 3 h. The product formation was confirmed by TLC and LC-MS. The crude product was partitioned between ethyl acetate and water, and extracted using ethyl acetate (3×30 mL). The combined organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure give the crude compound as an oil. The crude oil was dissolved in dichloromethane/trifluoroacetic acid (5 mL/3 mL) and stirred at ambient temperature over 1 h. Upon solvent evaporation, the mixture was adjusted to *p*H = 7 and extracted using dichloromethane (3×10 mL). The combined organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude product was purified by flash chromatography

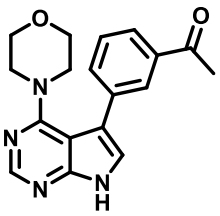
(dichloromethane/methanol 0-5%) to give the target compound as a yellow solid 37.7 mg, yield: 20.8%. HRMS (ESI^+) calcd for $C_{16}H_{15}FN_4O_3S$ ([M+H^+]): 363.0927, found 363.0920. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.43 (d, J = 2.6 Hz, 1H), 8.44 (s, 1H), 8.20 (t, J = 1.9 Hz, 1H), 8.17 (dd, J = 7.8, 1.5 Hz, 1H), 8.08 – 8.01 (m, 1H), 7.88 (t, J = 7.9 Hz, 1H), 7.82 (d, J = 2.7 Hz, 1H), 3.65 - 3.40 (m, 4H), 3.28 – 2.90 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm) 160.42, 153.97, 151.13, 137.70, 135.55,132.22, 131.22, 127.55, 126.02, 125.24, 113.55, 102.89, 65.66, 50.05. ^{19}F NMR (376 MHz, DMSO) δ (ppm) 66.23,66.08.

3-(4-morpholino-7*H*-pyrrolo[2,3-*d*] pyrimidin-5-yl) phenyl acetate (A8):



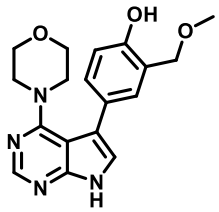
To a solution of 3-(4-morpholino-7*H*-pyrrolo[2,3-*d*] pyrimidin-5-yl) phenol) (50.0 mg, 0.17 mmol) in N, N-dimethylformamide (2 mL) at 0 °C was added pyridine (20.0 mg, 0.26 mmol) followed by acetic anhydride (51.68 mg, 0.51 mmol). The solution was stood for 2 h at room temperature, then evaporated under reduced pressure and the residue partitioned between ethyl acetate (20 mL) and cold 1 M dilute hydrochloric acid (10 mL). The organic layer was washed with further cold dilute hydrochloric acid, water, brine, dried over sodium sulphate, filtered and the filtrate evaporated under reduced pressure to give crude compound and purify by pre-TLC to get target compound as a white solid 5.0 mg, yield: 7.8%. HRMS (ESI^+) calcd for $C_{18}H_{18}N_4O_3$ ([M+H^+]): 339.1457, found: _ (not detected – see Note below). ^1H NMR (500 MHz, $CDCl_3$+Methanol-d_4) δ (ppm) 8.34 (s, 1H), 7.42 (t, J = 7.9 Hz, 1H), 7.38 – 7.29 (m, 1H), 7.21 – 7.16 (m, 2H), 7.02 (ddd, J = 8.1, 2.4, 1.1 Hz, 1H), 3.55 - 3.39 (m, 4 H), 3.30 (t, J = 4.7 Hz, 4H), 2.30 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$+ Methanol-d_4) δ (ppm) 169.83, 160.22, 152.33, 150.76, 150.14, 136.88, 129.51, 125.78, 121.98, 121.76, 119.79, 116.25, 103.27, 66.22, 49.52, 20.94. Noted: this compound is unstable in solvent (dimethyl sulfoxide, dichloromethane/methanol) under room temperature for 24 h. As such, biochemical assays were not further pursued with this compound.

1-(3-(4-morpholino-7*H*-pyrrolo[2,3-*d*] pyrimidin-5-yl) phenyl) ethan-1-one (A9):



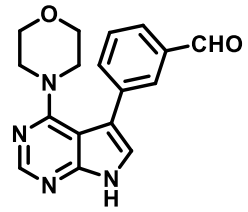
A mixture of *tert*-butyl 5-iodo-4-morpholino-7*H*-pyrrolo[2,3-*d*] pyrimidine-7-carboxylate (215.0 mg, 0.5 mmol), 1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)ethan-1-one (147.58 mg, 0.6 mmol), XPhos Pd G2 (39.0 mg, 0.05 mmol) and potassium phosphate tribasic (212.3 mg, 1.0 mmol) was dissolved in 10 mL of dioxane and 1 mL of water and then stirred at 90 °C in a sealed tube for 3 h. The product formation was confirmed by TLC and LC-MS. The crude product was partitioned between ethyl acetate and water, and extracted using ethyl acetate (3×30 mL). The combined organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure give the crude compound as an oil. The crude oil was dissolved in dichloromethane/trifluoroacetic acid (5 mL/3 mL) and stirred at ambient temperature over 1 h. Upon solvent evaporation, the mixture was adjusted to *p*H = 7 and extracted using dichloromethane (3×10 mL). The combined organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude product was purified by flash chromatography (dichloromethane/methanol 0-5%) to give the target compound as a white solid 127.0 mg, yield: 78.8%. HRMS (ESI^+) calcd for $C_{18}H_{18}N_4O_2$ ([M+H^+]): 323.1508, found 323.1502. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.38 – 11.97 (m, 1H), 8.40 (s, 1H), 8.10 (t, J = 2.0 Hz, 1H), 7.91 (dt, J = 7.7, 1.5 Hz, 1H), 7.88 - 7.80 (m, 1H), 7.66 – 7.58 (m, 2H), 3.46 - 3.38 (m, 4H), 3.20 – 3.13 (m, 4H), 2.66 (s, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm) 198.49, 160.35, 153.72, 150.80, 137.40, 136.15, 132.81, 129.44, 128.16, 126.49, 123.76, 115.39, 102.98, 65.83, 49.94, 27.37.

2-(methoxymethyl)-4-(4-morpholino-7*H*-pyrrolo[2,3-*d*] pyrimidin-5-yl) phenol (A10):



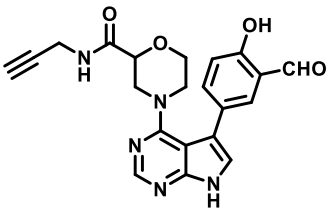
A mixture of *tert*-butyl 5-iodo-4-morpholino-7*H*-pyrrolo[2,3-*d*] pyrimidine-7-carboxylate (215.0 mg, 0.5 mmol), 2-(hydroxymethyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (149.58 mg, 0.6 mmol), XPhos Pd G2 (39.0 mg, 0.05 mmol) and potassium phosphate tribasic (212.3 mg, 1.0 mmol) was dissolved in 10 mL of dioxane and 1 mL of water and then stirred at 90 °C in a sealed tube for 3 h. The product formation was confirmed by TLC and LC-MS. The crude product was partitioned between ethyl acetate and water, and extracted using ethyl acetate (3 × 30 mL). The combined organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure give the crude compound as an oil. The crude oil was dissolved in dichloromethane/trifluoroacetic acid (5 mL/3 mL) and stirred at ambient temperature over 1 h. Upon solvent evaporation, the mixture was adjusted to *p*H = 7 and extracted using dichloromethane (3×10 mL). The combined organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude product was purified by flash chromatography (dichloromethane/methanol 0-5%) to give the target compound as a white solid 37.0 mg, yield: 21.8%. HRMS (ESI^+) calcd for $C_{18}H_{20}N_4O_3$ ([M+H^+]): 341.1614, found 341.1603. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 11.97 (s, 1H), 9.50 (s, 1H), 8.32 (s, 1H), 7.45 – 7.28 (m, 3H), 7.26 (d, J = 8.1 Hz, 1H), 6.87 (d, J = 8.3 Hz, 1H), 4.44 (s, 2H), 3.59 – 3.42 (m, 4H), 3.35 (s, 3H)3.23 - 2.88 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm) 160.37, 153.91, 153.31, 150.45, 129.36, 128.31, 126.60, 124.87, 121.87, 116.48, 115.47, 103.18, 69.32, 65.88, 58.30, 49.94.

3-(4-morpholino-7*H*-pyrrolo[2,3-*d*] pyrimidin-5-yl) benzaldehyde (A11):



A mixture of *tert*-butyl 5-iodo-4-morpholino-7*H*-pyrrolo[2,3-*d*] pyrimidine-7-carboxylate (215.0 mg, 0.5 mmol), 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde (139.20 mg, 0.60 mmol), XPhos Pd G2 (39.0 mg, 0.05 mmol) and potassium phosphate tribasic (212.3 mg, 1.0 mmol) was dissolved in 10 mL of dioxane and 1 mL of water and then stirred at 90 °C in a sealed tube for 3 h. The product formation was confirmed by TLC and LC-MS. The crude product was partitioned between ethyl acetate and water, and extracted using ethyl acetate (3×30 mL). The combined organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure give the crude compound as an oil. The crude oil was dissolved in dichloromethane/trifluoroacetic acid (5 mL/3 mL) and stirred at ambient temperature over 1 h. Upon solvent evaporation, the mixture was adjusted to *p*H = 7 and extracted using dichloromethane (3×10 mL). The combined organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude product was purified by flash chromatography (dichloromethane/methanol 0-5%) to give the target compound as a white solid 120.0 mg, yield: 77.9%. HRMS (ESI^+) calcd for $C_{17}H_{16}N_4O_2$ ([M+H^+]): 309.1352, found 309.1344. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.38 – 12.25 (m, 1H), 10.12 (s, 1H), 8.40 (s, 1H), 8.09 (t, J = 1.8 Hz, 1H), 7.91 (dt, J = 7.6, 1.6 Hz, 1H), 7.86 (dt, J = 7.7, 1.5 Hz, 1H), 7.71 (t, J = 7.6 Hz, 1H), 7.66 (d, J = 2.6 Hz, 1H), 3.45 – 3.40 (m, 4H), 3.22 – 3.11 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm) 193.89, 160.39, 153.77, 150.87, 136.81, 136.57, 134.17, 129.91, 128.98, 128.13, 123.95, 115.00, 102.98, 65.85, 49.93.

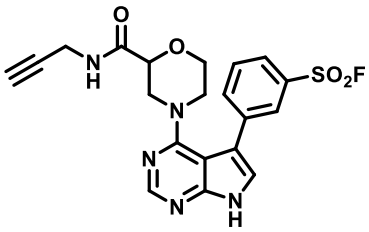
4-(5-(3-formyl-4-hydroxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)-*N*-(prop-2-yn-1-yl)morpholine-2-carboxamide (ABPA2): A mixture of *tert*-butyl 5-iodo-4-(2-((3-(triisopropylsilyl)prop-2-yn-1-yl)carbamoyl)morpholino)-7*H*-pyrrolo[2,3-*d*]pyrimidine-7-carboxylate (200.0 mg, 0.3 mmol), 2-hydroxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde (89.2 mg, 0.36 mmol), XPhos Pd G2 (25.0 mg, 0.03 mmol) and potassium phosphate tribasic (190.8 mg, 0.9 mmol) was dissolved in 10 mL of dioxane and 1 mL of water. The reaction mixture was degassed and purged with nitrogen, then the reaction mixture was stirred at 90 °C



for 3 h. The product formation was confirmed by TLC and LC-MS. The crude product was partitioned between ethyl acetate and water, and extracted using ethyl acetate (3×30 mL). The combined organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure give the crude compound as an oil. The crude oil was dissolved in dichloromethane/trifluoroacetic acid (5 mL/3 mL) and stirred at ambient temperature over 1 h. Upon solvent evaporation, the mixture was adjusted to *p*H = 7 and extracted using ethyl acetate (3×10 mL). The combined organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude product was purified by flash chromatography (dichloromethane/methanol 0%-5%) to give 4-(5-(3-formyl-4-hydroxyphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-yl)-*N*-(3-(triisopropylsilyl) prop-2-yn-1-yl) morpholine-2-carboxamide as a yellow solid 130.0 mg, yield: 77.3%.

To a solution of 4-(5-(3-hydroxyphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-yl)-*N*-(3-(triisopropylsilyl) prop-2-yn-1-yl) morpholine-2-carboxamide (130.0 mg, 0.24 mmol) in 5 mL of tetrahydrofuran was added 0.6 mL of tetrabutylammonium fluoride (TBAF) 1.0 M in tetrahydrofuran (0.96 mmol). The reaction mixture was stirred for 0.5 h at room temperature. The crude product was partitioned between ethyl acetate (20 mL) and a dilute solution of NaCl (180 mL), and extracted using dilute NaCl solution (20×180 mL) to remove TBAF completely. The organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude product was purified via preparative HPLC (column, SHIMADZU shim-psck GIS C18, 5 μm; mobile phase A, 1 ‰ FA in water; mobile phase B, acetonitrile; gradient, 10-90% B) to give 4-(5-(3-formyl-4-hydroxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)-*N*-(prop-2-yn-1-yl)morpholine-2-carboxamide as a yellow solid 43.0 mg, yield: 44.1%. HRMS (ESI$^+$) calcd for $C_{21}H_{19}N_5O_4$ ([M+H$^+$]): 406.1515 found 406.1511. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 11.31 (s, 1H), 10.00 (s, 1H), 8.47 (s, 1H), 8.26 (s, 1H), 7.73 (d, J = 2.3 Hz, 1H), 7.68 (dd, J = 8.5, 2.3 Hz, 1H), 7.25 (s, 1H), 7.11 (d, J = 8.5 Hz, 1H), 6.67 (t, J = 5.4 Hz, 1H), 4.25 (dt, J = 13.3, 2.4 Hz, 1H), 4.02 (dd, J = 5.5, 2.6 Hz, 2H), 3.91 – 3.73 (m, 3H), 3.52 (td, J = 11.6, 2.5 Hz, 1H), 2.99 (td, J = 13.5, 12.7, 3.3 Hz, 1H), 2.80 (dd, J = 13.3, 10.7 Hz, 1H), 2.24 (t, J = 2.6 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm) 196.91, 168.66, 166.30, 160.99, 160.43, 152.67, 149.99, 137.84, 133.28, 127.42, 121.82, 120.96, 118.35, 115.93, 104.15, 79.32, 74.96, 72.14, 66.31, 51.80, 49.20, 28.90.

3-(4-(2-(prop-2-yn-1-ylcarbamoyl)morpholino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)benzenesulfonyl fluoride (ABPA7): A mixture of *tert*-butyl 5-iodo-4-(2-((3-(triisopropylsilyl)prop-2-yn-1-yl)carbamoyl)morpholino)-7*H*-pyrrolo[2,3-*d*]pyrimidine-7-carboxylate (200.0 mg, 0.3 mmol), 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) benzenesulfonyl fluoride (102.85 mg, 0.36 mmol), XPhos Pd G2 (25.8 mg, 0.03 mmol) and potassium phosphate tribasic (190.0 mg, 0.9 mmol) was dissolved in 10 mL of dioxane and 1 mL of water.



The reaction mixture was degassed and purged with nitrogen, then the reaction mixture was stirred at 90 °C for 3 h. The product formation was confirmed by TLC and LC-MS. The crude product was partitioned between ethyl acetate and water, and extracted using ethyl acetate (3×30 mL). The combined organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure give the crude compound as an oil. The crude oil was dissolved in dichloromethane/trifluoroacetic acid (5 mL/3 mL) and stirred at ambient temperature over 1 h. Upon solvent evaporation, the mixture was adjusted to *p*H = 7 and extracted using ethyl acetate (3×10 mL). The combined organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude product was purified by flash chromatography (dichloromethane/methanol 0%-5%) to give 3-(4-(2-((3-(triisopropylsilyl) prop-2-yn-1-yl) carbamoyl) morpholino)-7*H*-pyrrolo[2,3-*d*] pyrimidin-5-yl)

benzenesulfonyl fluoride as a yellow 80.0 mg, yield: 44.5%.

To a solution of 3-(4-(2-((3-(triisopropylsilyl) prop-2-yn-1-yl) carbamoyl) morpholino)-7*H*-pyrrolo[2,3-*d*] pyrimidin-5-yl) benzenesulfonyl fluoride (80 mg, 0.13 mmol) in 5 mL of tetrahydrofuran was added 0.6 mL of tetrabutylammonium fluoride (TBAF) 1.0 M in tetrahydrofuran (0.52 mmol, 4 equiv.). The reaction mixture was stirred for 0.5 h at room temperature. The crude product was partitioned between ethyl acetate (20 mL) and a dilute solution of NaCl (180 mL), and extracted using dilute NaCl solution (20×180 mL) to remove TBAF completely. The organic extract was washed with brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude product was purified via preparative HPLC (column, SHIMADZU shim-psck GIS C18, 5 μm; mobile phase A, 1‰ FA in water; mobile phase B, acetonitrile; gradient, 10-90% B) to give 3-(4-(2-(prop-2-yn-1-ylcarbamoyl) morpholino)-7*H*-pyrrolo[2,3-*d*] pyrimidin-5-yl) benzenesulfonyl fluoride as a yellow solid 8.0 mg, yield: 13.5%. HRMS (ESI^+) calcd for $C_{20}H_{18}FN_5O_4S$ ([$M+H^+$]): 444.1142 found 444.1133. ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 10.86 (s, 1H), 8.56 (s, 1H), 8.19 (t, J = 1.9 Hz, 1H), 8.04 – 7.93 (m, 2H), 7.73 (t, J = 7.9 Hz, 1H), 7.37 (s, 1H), 6.63 (t, J = 5.5 Hz, 1H), 4.02 (dt, J = 13.2, 2.5 Hz, 1H), 3.98 (dd, J = 5.4, 2.6 Hz, 2H), 3.87 (dd, J = 11.2, 3.4 Hz, 1H), 3.81 (dd, J = 10.8, 2.7 Hz, 1H), 3.77 – 3.70 (m, 1H), 3.65 (td, J = 11.7, 2.5 Hz, 1H), 3.10 – 2.95 (m, 1H), 2.70 (dd, J = 13.1, 10.8 Hz, 1H), 2.20 (t, J = 2.6 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm) 168.28, 160.22, 153.58, 151.13, 136.97, 134.41,133.35 130.19, 128.24, 126.30, 122.50, 114.94,103.41, 78.98, 74.35, 71.80, 65.82, 52.10, 48.45, 28.53. ^{19}F NMR (471 MHz, $CDCl_3$) δ (ppm) 65.78.

3. Other Experimental Details.

3.1 Cell Culture and anti-proliferative assay

A549 cells and HEK293 were cultured in DMEM (Sigma, D1152) supplemented with 1% Penicillin-Streptomycin and 10% Fetal Bovine Serum and 3.7 g/L sodium bicarbonate. K562 were cultured in RMPI-1640 (Biowest, L0500) supplemented with 1% Penicillin-Streptomycin and 10% Fetal Bovine Serum.

Cells were plated at a density of 5,000 (for K562 and HEK293 cells) cells per well in 96-well cell culture plates for 24 h, then cells were treated with a dilution series of test compounds for 72 hours. Cell viability was determined by CellTiter Glo (Promega, G7572) and the luminescence signal was recorded with Cytation 5 imaging reader (BioTek). The results were converted to cell numbers using a standard curve. Cell viability inhibition (IC_{50}) values were determined by Graph Pad Prism version 8.

3.2 Biochemical Assay

Buffer composition:

40 mM Tris; 20 mM $MgCl_2$; 0.5 mM DTT and 0.10% BSA.

LRRK2 biochemical assay:

The compounds were 50-fold serially diluted from 2 mM stocks for 10 doses in kinase buffer, to obtain 10 different concentrations of 10000, 2500, 625, 156, 39, 10, 2.44, 0.61, 0.15 and 0.04 nM. 1 μL of the compound solution was transferred into each well of a 384-well assay plate. The enzyme master mix was prepared by diluting the stock enzyme (full-length LRRK2, Cat. No. L10-11G from Signalchem; LRRK2-G2019S, Cat. NO. 2341536B from ThermoFisher) until the final kinase concentration was 5 nM. 2 μL of the kinase solution was next transferred into each well of the assay plate, and further incubated with the compound. The incubation time was adjust according to experimental design (1 h in most cases for kinase inhibition assays, and 0 min, 30 min, 1 h, 2 h, 6 h and 12 h for time-dependent kinase inhibition assays). Next, 1 μL of a mixture containing the substrate (2.5 μM) and ATP (50 μM) was added into each reaction well, and incubation was continued at room temperature for 60 min. Separately, a solution containing Sa-XL 665 and TK-antibody-Cryptate in HTRF detection buffer (Cat. No.62TK0PEC; Cisbio Bioassays) was prepared according to vendor's protocol, and 8 μL of this solution was added into each well of the above assay plate, followed by further incubation at room temperature for 40 min. Finally, the assay plate was transferred to a Biotek Microplate reader for reading. The IC_{50} values were calculated using GraphPad Prism by plotting the enzyme activity against log[inhibitor].

AURKA biochemical assay:

The enzymatic activities against AURKA were tested using the AURKA Kinase Enzyme System (Carna, 05-101). First, 2× ATP & Substrate solution and 2× kinase & Metal solution were prepared using assay buffer. Then, Transfer 25 nL compound to 384 assay plate by Echo 655. Next, add 2.5 μL of 2× kinase & Metal solution were mixed and incubated in a polystyrene coated 384 assay plate for 10 minutes at 25 ℃. Next, 2.5 μL of 2× Substrate & ATP solution were added to the well, and incubated at 25 ℃ for 50 minutes. Followed 2× XL665 & Antibody solution were prepared with detection buffer. Finally, 5 μL of Kinase Detection Reagent was added to the well, and incubated for 60 minutes at 25 ℃. The fluorescence signals of 620 nm (Cryptate) and 665 nm (XL665) were read by microtiter-plate reader.

3.3 Cellular inhibition by WB/cell-based LRRK2 inhibition assay

A549 cells were equally dispensed and adhered into 6-well plates with a density of 3×10^5 cells per well for overnight (37 °C, 5% CO_2). The cells were then incubated with compounds at a series of concentrations (0.08, 0.4, 2, 10, 50 μM) or DMSO for 1.5 hours. After incubation, cells were washed by PBS and lysed by RIPA lysis buffer (no 89900, ThermoFisher) containing protease inhibitor (no. S8820, Merck) and phosphatase inhibitors (no. 4906845001, Merck). Protein in cell lysate was quantified by Bradford assay kit (FD.2003, Fdbio Science). Primary

antibodies used in this study include Rab10 antibody (ab237703, Abcam), Phospho-Rab10 (Thr73) antibody (ab230261, Abcam), β-Tubulin antibody (AF7011, Affinity Biosciences). The Millipore immobilon Western chemiluminescence substrate was used for signal development. Blots were imaged in an Amersham Imager 600 (GE Healthcare). For the quantitation of protein, band intensities were measured by using the software ImageJ.

3.4 KINOMEscan®

Kinomescan results were provided by Thermo Fisher Scientific (USA) at a cost, compound **A2** (0.2 μM) was tested against selected 100 representative kinases widely located in the different kinase families. Kinomescan® Data Analysis: First, all tested kinases were divided into three ranges according to the inhibition rate (> 95%, 60-95%, 40-60%, < 40%). All data in each range were set to be unified Value (the size of the circle), and then all preprocessed data (kinase name and corresponding value) were imported into KinMap (www.kinhub.org) for mapping. Selectivity Score (S-score) was calculated by dividing the number of hits at certain percent control (%Ctrl) by the total number of kinases being tested.

3.5 In vitro pure LRRK2 protein labeling

LRRK2 was diluted to 0.1 μM with PBS. LRRK2 (10 μL) was treated with different probes (**ABPA2**, **ABPA7** and Flurorescein-5-EXN-hydroxysuccinimide ester, 1 μL, 20 μM stock in DMSO), followed by addition of $NaCNBH_3$ (1 μL, 750 mM) or PBS (1 μL) to sample treated with probes, then the samples were incubated at room temperature for 12 h. After incubation, the samples were treated with a freshly premixed click chemistry reaction cocktail (100 μM Rhodamine-N_3 from 1 mM stock solution in DMSO, 0.1 mM THPTA from 10 mM stock solution in DMSO, 1 mM TCEP from 100 mM stock solution in deionized water, and 1 mM $CuSO_4$ from 100 mM stock solution in deionized water) and incubated at room temperature for 1 h. The samples were added with 5× loading buffer and heated for 10 min at 95 °C. Sample was separated by SDS-PAGE (8% gel) and then visualized by the GE Healthcare Typhoon 9410 scanner (Cy3 and Cy5 laser channels).

3.6 Protein expression and purification

Genes encoding human AURKA (127-401) were cloned into a modified pRSF-Duet vector, preceded by a His_6-SUMO tag. The fusion proteins were over-expressed in *E. coli* BL21(DE3) cells, which were induced by addition of 0.4 mM isopropyl β-*D*-1-thiogalactopyranoside (IPTG) at an OD_{600} of 0.8 and then grown at 20 ℃ for 16 h. The cells were harvested and lysed in buffer containing 50 mM Tris-HCl (*p*H 8.0), 1 M NaCl, 25 mM imidazole, 0.5 mM β-ME and 1 mM PMSF. The fusion proteins were first purified using a Ni-NTA column. The protein was washed using washing buffer (25 mM imidazole, 1 M NaCl, 25 mM Tris and *p*H 8.0). Then the AURKA protein was eluted with elution buffer (250 mM imidazole,1 M NaCl, 25 mM Tris and *p*H 8.0). The His_6-SUMO tag was cleaved by treatment with ULP1 and the tag-less protein was fractioned by using Ni-NTA column again and a Superdex 75 size-exclusion column (GE Healthcare) pre-equilibrated with a buffer containing 25 mM HEPES (*p*H 8.0), 300 mM NaCl and 1% glycerol. The final purified AURKA protein was concentrated to 25 mg/mL and stored at -80 °C.

3.7 Intact protein mass spectrometry analysis

AURKA domain (10 μM) was incubated with different covalent inhibitors (100 μM) in PBS (total volume was 65 μL) indicated for 12 h. After incubation, the sample was moved to centrifugal filters (10 kDa, Merck) for replacing the solution with deionized water (0.1% formic acid). After collecting the 27~40 μL of sample, the samples were analyzed by LC-MS (Dionex LC coupled to QExactive-Orbitrap, Thermo Fisher Scientific Inc). Deconvolution of multiple charged ions was processed with BioPharma Finder Software (Thermo Fisher Scientific Incm USA).

3.8 X-ray analysis

To obtain AURKA in complex with an inhibitor, the protein was incubated with a compound at a molar ration of 1:10 at 37 ℃ for 12 h, then clarified by centrifugation at 12000 rpm at 25 ℃ for 2 min. Crystallization was carried out using hanging-drop diffusion method. The hanging drops were prepared by mixing 1 μL of the protein-compound mix solution with 1 μL of the reservoir fluid containing the precipitating agents. If additive solution (improve crystal appearance) was required for crystals, its volume always was 0.2 μL. The crystals of **AURKA-A2** appeared in a buffer containing 1.5 M $(NH_4)_2SO_4$, 0.1 M HEPES (*p*H 7.0) and 4% v/v 1,3-propanediol. Crystals grew to their maximum size in ~ 15 d. The crystals mentioned above all appeared at 293K. Before being flash frozen in liquid nitrogen, the crystals were cryo-protected using the precipitant solution supplemented with glycerol to a final concentration of 27% (v/v). Datasets were collected on the beam-line BL17B and BL19U1 at the Shanghai Synchrotron Radiation Facility. The data were indexed, integrated and scaled using the HKL3000 package. The structural solution was obtained by molecular replacement using PHASER in Phenix and the structure of the kinase domain of AURKA as the search model (PDB ID: 3E5A). Iterative rounds of model building in COOT and refinement in Phenix were carried out. The atomic coordinates and structural factors of **AURKA-A2** have been deposited into the Protein Data Bank under the accession code 8JXM.

3.9 In-gel fluorescence scanning in live K562 cells

K562 cells were treated with DMSO, or 2 μM of a probe (**ABPA2**, **ABPA7** and XO44) at 37 ºC/5% CO_2 for 2 h. For competition experiments, cells were pre-treated with a cell medium containing a competitor at 37 ºC/5% CO_2 for 2 h, before addition of the probe. Following incubation at 37 ºC/5% CO_2 for another 2 h. the labeled cells were collected by centrifugation, washed twice with cold PBS, and lysed in lysis buffer (100 mM HEPES, 150 mM NaCl, 0.1% NP-40 pH 7.5, 2 mM PMSF, 2× EDTA-free complete protease inhibitors). For **ABPA2** labeling, 50 mM sodium cyanoborohydride was added, and the reduction was done by incubating the sample on ice for 60 min with sonication, followed by before lysate clarification with centrifugation. The lysates were centrifuged at 21330 g/4 °C for 30 min. Protein quantification was performed using a Bio-Rad Bradford Protein Assay. The proteomes were next precipitated by adding 5 volumetric times of methanol/chloroform (v/v = 4:1) and 3 volumetric times of deionized water. Upon further centrifugation at 10000 g for 10 min, the precipitated protein pellets were washed with cold methanol and re-dissolved in 0.4% (w/v) SDS/PBS by sonication. The procedures were also used for preparation of pull-down (PD) samples descripted below. The concentrations of the proteomes were normalized to 2.5 mg/mL with PBS. Then a freshly premixed click chemistry reaction cocktail descripted was added to the samples, followed by further incubation at room temperature for 1 h. The samples were added with 5× SDS-loading buffer and heated for 10 min at 95 °C. Around 48 μg (per gel lane) of proteome was separated by SDS-PAGE (12% gel) and visualized on a GE Healthcare Typhoon 9410 scanner (Cy3 and Cy5 laser channels). Fluorescence images were shown in gray scale.

3.10 Large-scale pull-down (PD) in live A549/K562 cells

The proteomes (5 mg/mL, 1.5 mL) were prepared according to procedures descripted above. Following probe labeling, freshly premixed click reagents (final concentration: 100 μM Biotin-PEG3-N_3, 100 μM THPTA, 1 mM TCEP and 1 mM $CuSO_4$) were added, and the samples were incubated at room temperature for 1 h. After click reaction, the proteomes were precipitated and re-dissolved in 0.5 mL of 0.4% (w/v) SDS/PBS by sonication, followed by dilution to 0.2% SDS/PBS with PBS. The same number of proteomes were collected and incubated with high-capacity Neutravidin agarose beads (Thermo #29204, 100 μL for each sample) preequilibrated in PBS, and the resulting mixture was rotated at 4 °C overnight. The beads were washed with 1% NP-40, 0.1% SDS in deionized water (3×10 min, room temperature), 6 M urea in PBS (3x30 min, 4 °C) and cold PBS (3×10 min, room temperature). For Western blotting (WB) experiments, 10% of beads prepared above were added with 2x loading buffer and heated at 95 °C for 30 min. Next, the beads were spun down, and the collected samples were separated on 8% SDS-PAGE

before being transferred onto PVDF membranes. The membranes were blocked with 5% BSA in Tris-buffered saline with 0.1% Tween-20 (TBST) for 1 h at room temperature. The membranes were incubated with anti-LRRK2 (#ab170993, Abcam); anti-LRRK2 (#ab172378, Abcam); anti-LRRK2 (phosphor S935, #ab172382, Abcam); total AURKA (#ab13824, Abcam); anti-Phospho-AURKA antibody (T288, #3079S, Cell Signaling Technology); GAPDH (#sc-166574, Santa Cruz), followed by washing with TBST (3×15 min). Then the indicated membrane was incubated with HRP-*conjugated* anti-rabbit (#7074P2, Cell Signaling Technology) or HRP-*conjugated* anti-mouse (#626520, Invitrogen) for 1 h at room temperature, followed by washing with TBST (3×15 min). The blot was developed by using ECL SelectTM Western Blotting Detection Reagent (#RPN2235, Cytiva) and recorded by using GE ImageQuant LAS 500.

3.11 Cellular phosphorylation assay

7×10^5 K562 cells were seeded into a 12-well plate and pre-incubated with Nocodazole (0.1 μg/mL) in the cell-incubator for 17 h, then the cells were treated with AURKA inhibitor (0-10 μM) followed by further incubation for 3 h. After that, cells were collected, washed with cold PBS twice, then lysed in lysis buffer (RIPA buffer (Thermo Fisher 8990), 1 mM PMSF, 5 mM sodium fluoride, 1 mM sodium orthovandate, 1× EDTA-free cComplete protease inhibitors) on ice for 0.5 h. The cell lysates were centrifuged at 21330 g/4 ℃ for 0.5 h, and the supernatant was collected. The protein concentration was normalized by using Bradford assay. 5× standard SDS loading buffer was added to the sample, followed by heating at 95 °C for 10 min. Equal amounts of proteins were then resolved on 10% SDS-PAGE gels and transferred to PVDF membranes. The membranes were blocked with 5% BSA in TBST (0.1% Tween-20) for 1 h at room temperature. The indicated membrane was incubated with different primary antibodies at room temperature for 1 h. These antibodies included total AURKA (#ab13824, Abcam); anti-Phospho-AURKA antibody (T288, #3079S, Cell Signaling Technology); GAPDH (#sc-166574, Santa Cruz), followed by washing with TBST (3×15 min). Then the indicated membrane was incubated with HRP-*conjugated* anti-rabbit (#7074P2, Cell Signaling Technology) or HRP-*conjugated* anti-mouse (#626520, Invitrogen) for 1 h at room temperature, followed by washing with TBST (3×15 min). The blot was developed by using ECL SelectTM Western Blotting Detection Reagent (#RPN2235, Cytiva) and recorded by using GE ImageQuant LAS 500.

3.12 Cellular washout assay

The cellular washout assay was verified by cell viability assay and detection of the phosphorylation level of AURKA (T288):

Cell viability assay

1.2×10^6 K562 cells per well were equally dispensed into a 24-well plate with a final volume of 1 mL per well and incubated for overnight at 37 °C, 5% CO_2. The cells were then incubated with compounds at the final assay concentration of 20 μM, or DMSO for 8 h (in duplicate). After incubation, the cell mixture of 1 mL per well was divided into 10 aliquots of 100 μL, then 3×100 μL of cells from the 1 mL was added to the 96-well plate (100 μL/well), which were classified as "no-wash" group. For the "wash-out" group, the remaining cells were transferred to a 1.5 mL Eppendorf tube and centrifuged at 1500 rpm for 5 min. Upon removal of supernatant, collected cells were washed twice with 1 mL of drug-free culture medium for 5 min. Finally, cells were resuspended in equal volume of culture medium and 3×100 μL of cells was added to the same 96-well plate (100 μL/well). Cellular viability was measured after 64 h in both "wash-out" and "non-wash" samples by using anti-proliferation assay (CellTiter Glo kit, Promega, G7572). Viability is represented as % viable relative to the DMSO control.

Cellular phosphorylation assay：

7×10^5 K562 cells were seeded into a 12-well plate and pre-incubated with Nocodazole (0.1 μg/mL) in the cell-incubator for 17 h, then the cells were treated with AURKA inhibitors (**A2**: 20 μM; VX 680: 1 μM) and incubated

for further 4 h. For samples under wash-out condition, the cells were collected and washed with warm medium for twice, then the cells were re-suspended in drug-free medium and re-plated in 12-well plate, and incubated at 37 ℃/5% CO_2 for 6 h. After incubation, cells were collected and washed with cold PBS twice, then lysed in lysis buffer (RIPA buffer (Thermo Fisher 8990), 1 mM PMSF, 5 mM sodium fluoride, 1 mM sodium orthovandate, 1× EDTA-free cComplete protease inhibitors) on ice for 0.5 h. The cell lysates were centrifuged at 21330 g/4 ℃ for 0.5 h, and the supernatant was collected. The protein concentration was normalized by using Bradford assay. 5× standard SDS loading buffer was added to the sample, followed by heating at 95 ℃ for 10 min. Equal amounts of proteins were then resolved on 10% SDS-PAGE gels and transferred to PVDF membranes. The membranes were blocked with 5% BSA in TBST (0.1% Tween-20) for 1 h at room temperature. The indicated membrane was incubated with different primary antibodies at room temperature for 1 h. These antibodies included total AURKA (#ab13824, Abcam); anti-Phospho-AURKA antibody (T288, #3079S, Cell Signaling Technology); GAPDH (#sc-166574, Santa Cruz), followed by washing with TBST (3×15 min). Then the indicated membrane was incubated with HRP-*conjugated* anti-rabbit (#7074P2, Cell Signaling Technology) or HRP-*conjugated* anti-mouse (#626520, Invitrogen) for 1 h at room temperature, followed by washing with TBST (3×15 min). The blot was developed by using ECL SelectTM Western Blotting Detection Reagent (#RPN2235, Cytiva) and recorded by using GE ImageQuant LAS 500.

3.13 Molecular docking study

The putative covalent binding modes of LRRK2/AURKA and compound **A2** were obtained by using procedures described below. The X-ray structures of LRRK2/AURKA (PDB ID: 7HLW/3E5A) was downloaded from PDB (www.rcsb.org) and used as the acceptor proteins for docking, respectively. The protein was prepared by using the protein preparation wizard in Maestro release 2018-2 (www.schrodinger.com) with standard settings. This included bond order assignments, protonation state assignment using Epik pH = 7.0 ± 2.0, optimization of the hydrogen bond network, remove water and constrained minimization with the OPLS3 force field. The small molecule file (.mol) was preprocessed by using the "Ligprep", and then the receptor grid was generated after the protein was ready. After that, Ligand Docking was carried out, the desired small molecule was docked into the ATP-binding pocket with the default algorithm, and the conformation with the highest scores was selected as no-covalent docking result. Then, confined the ligand to the enclosing box and chose the reaction type through the SMART (.CDOCK), SMART (.CDOCK): "Arylfluorosulfate warhead forms sulfonamide with the ε-NH_2 of catalytic lysine" was edited according to the instruction of Schrodinger (https://www.schrodinger.com/science-articles/covdock). Finally, "Covalent Docking" was processed to obtain the predicted covalent binding modes of compounds with the SRC/AURKA by using the Ligand Docking core. Results are shown with PyMOL.

4. Results and Discussion.

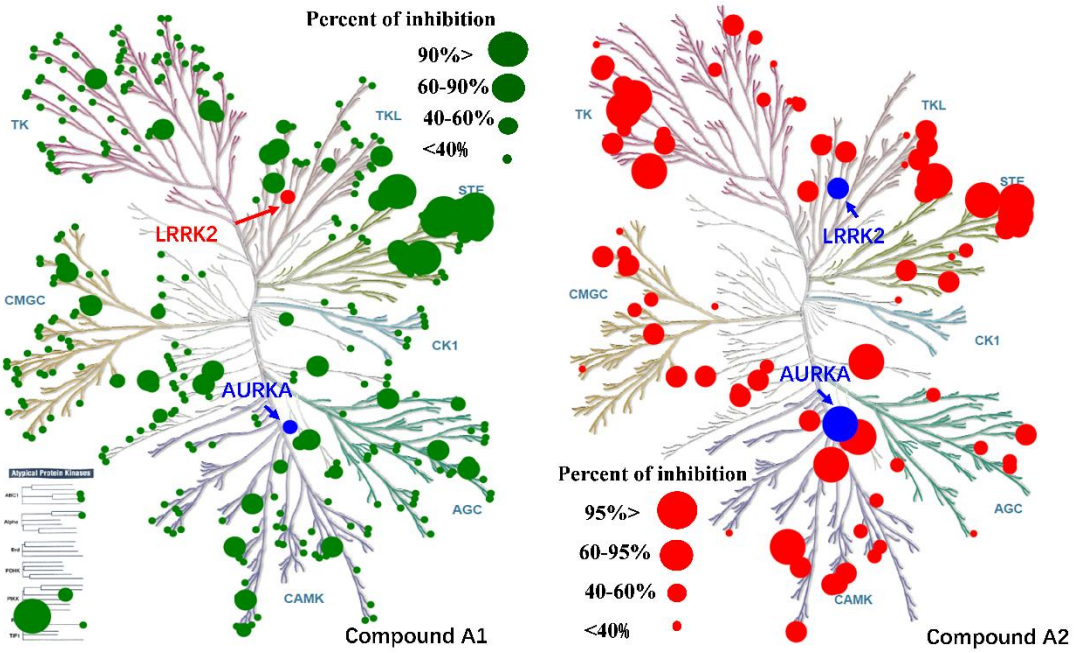


Fig S1. Dendrogram showing KinomeScan™ of compound PF-06447475 (**A1**) at 1000 nM against 460 kinases (left),[1] and compound **A2** at 200 nM against 100 kinases (right).

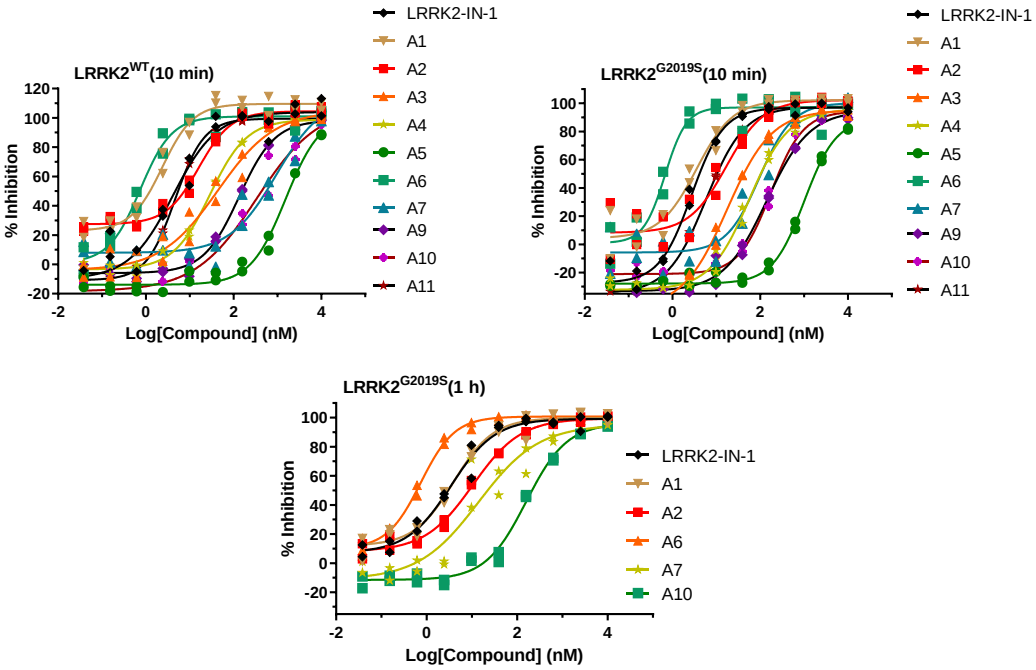


Fig S2. The *in vitro* inhibition potency of compound LRRK2-IN-1, **A1-A11** against LRRK2^{WT} (10 min) LRRK2^{G2019S} (10 min), and compound LRRK2-IN-1, **A1, A2, A6, A7** and **A10** against LRRK2^{G2019S} (1 h)

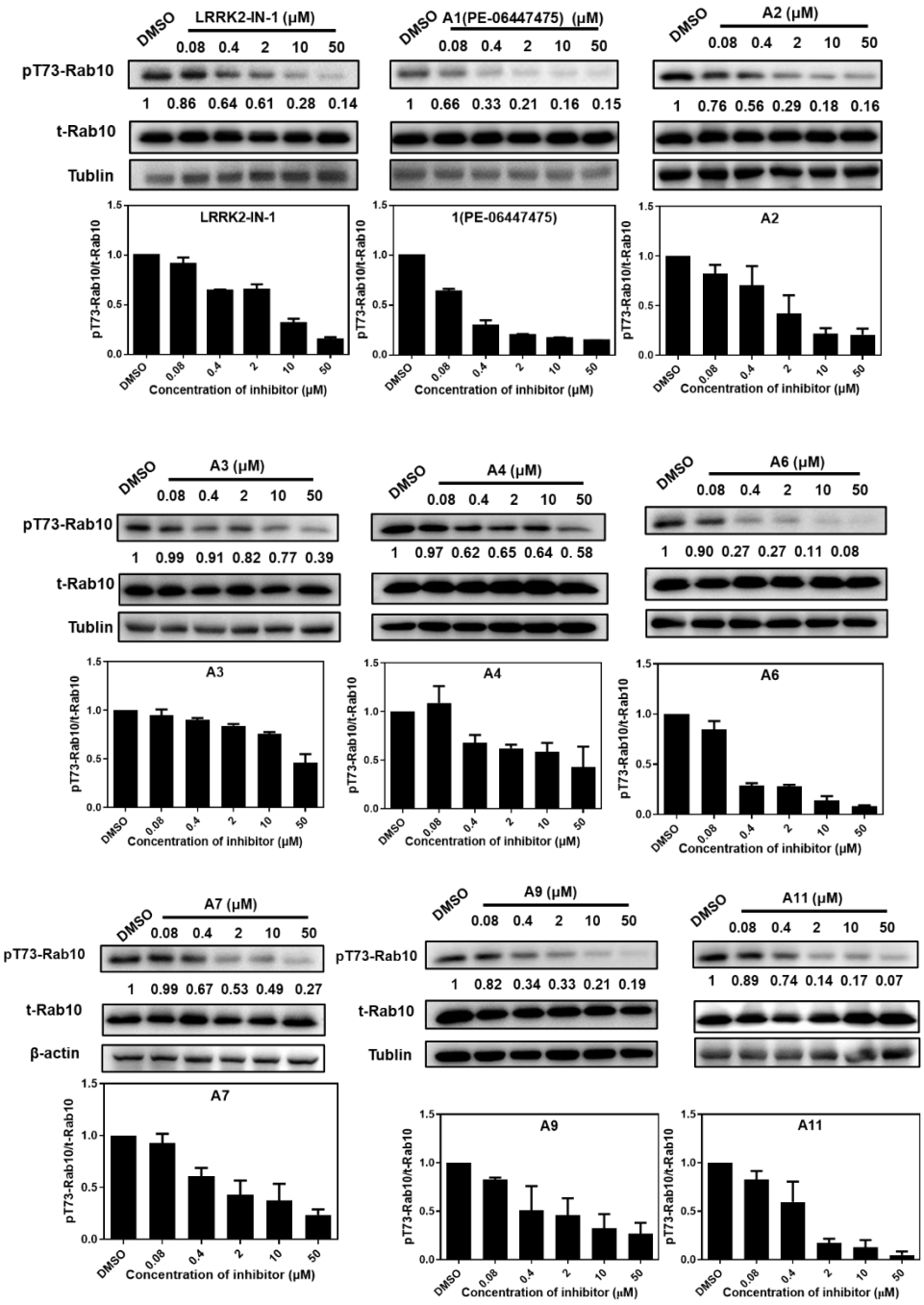


Fig S3. Cellular inhibition in A549 of various compounds. Incubation conditions: 37 ℃ for 1.5 h

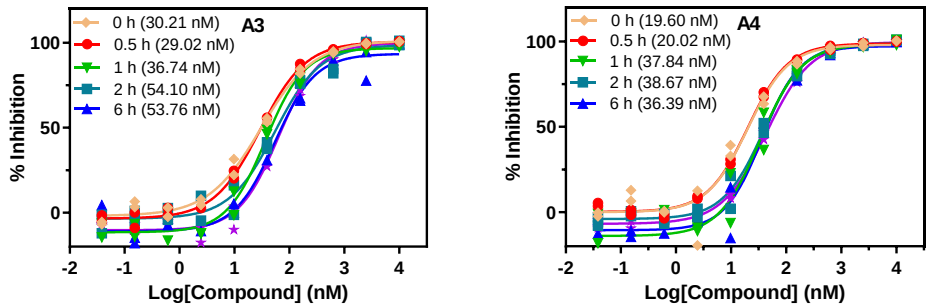


Fig S4. Time-dependent IC_{50} plots and values of **A3** and **A4** against Recombinant Human Protein $LRRK2^{WT}$

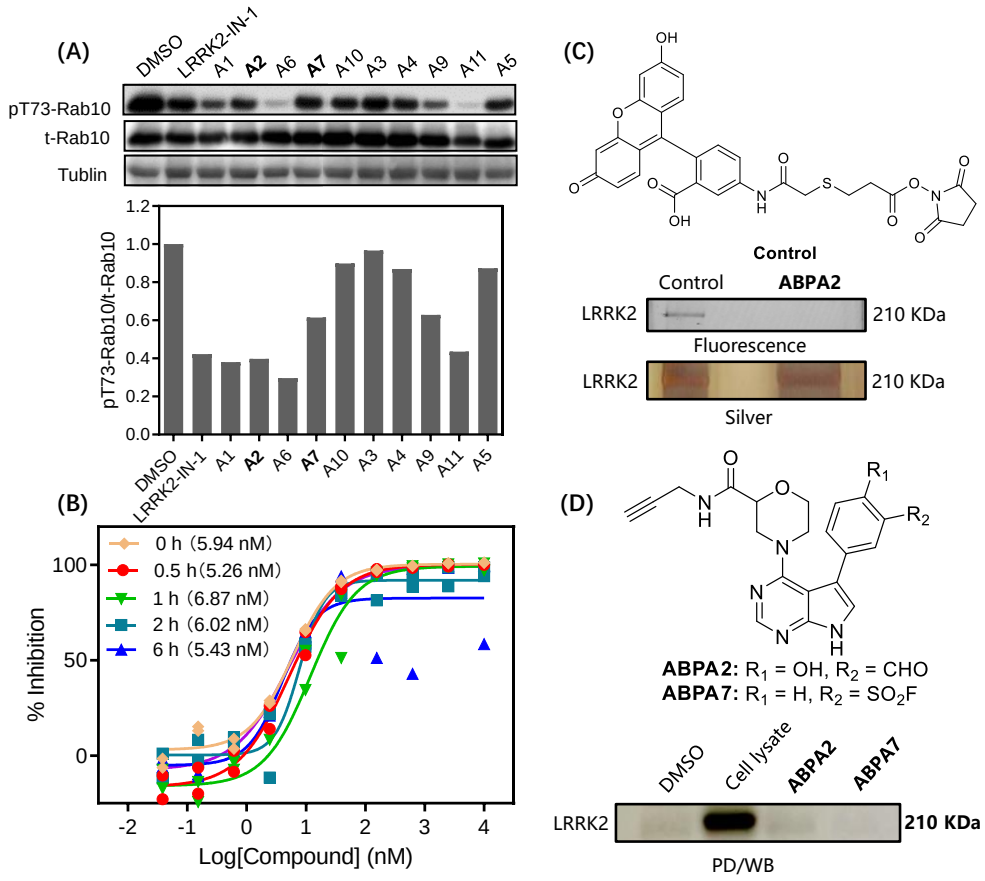


Fig. S5. (A) Cellular inhibition of A1-A11. (B) Time-dependent IC50 plots and values of A2 against recombinant LRRK2WT. (C) Gel-based LRRK2 labelling with control (Fluorescein-5-EX N-hydroxysuccinimide ester) and ABPA2. (D) Pull-down/Western blotting experiments against endogenous LRRK2.

In order to explore the inhibition of selected compounds against endogenous LRRK2, a preliminary screening of phosphorylation level of Rab10 (T73) in compound-treated A549 cells was conducted. Active endogenous LRRK2 is known to phosphorylate Rab10.[7] After treating cells with each compound for 1.5 h (Fig. S5A), **A2**, **A6** and **A11** show similar cellular activity to LRRK2-IN-1 and **A1**, while **A5** and **A10** showed a complete loss in cellular activity. These results are consistent with the *in vitro* results (Table 1 in the maintext). Time-dependent cellular inhibition was also carried out (Fig. S3); surprisingly, amongst the three SA-containing inhibitors, only **A2** maintained comparable LRRK2 cellular inhibition compared to **A1**. We next assessed the potential covalent binding mode of **A2**, by carring out detailed time-dependent *in vitro* IC_{50} determination (Fig. S4 and S5B). Unexpectedly, the IC_{50} of compound **A2** did not show a time-dependent decrease in IC_{50} values (Table S2). Its corresponding activity-based probe, **ABPA2**, also failed to covalently label recombinant LRRK2 (Fig. S5C) and endogenous LRRK2 from live A549 cells (Fig. S5D). Our results thus indicated the newly designed SA-containing **A2** mostly engaged LRRK2 non-covalently.

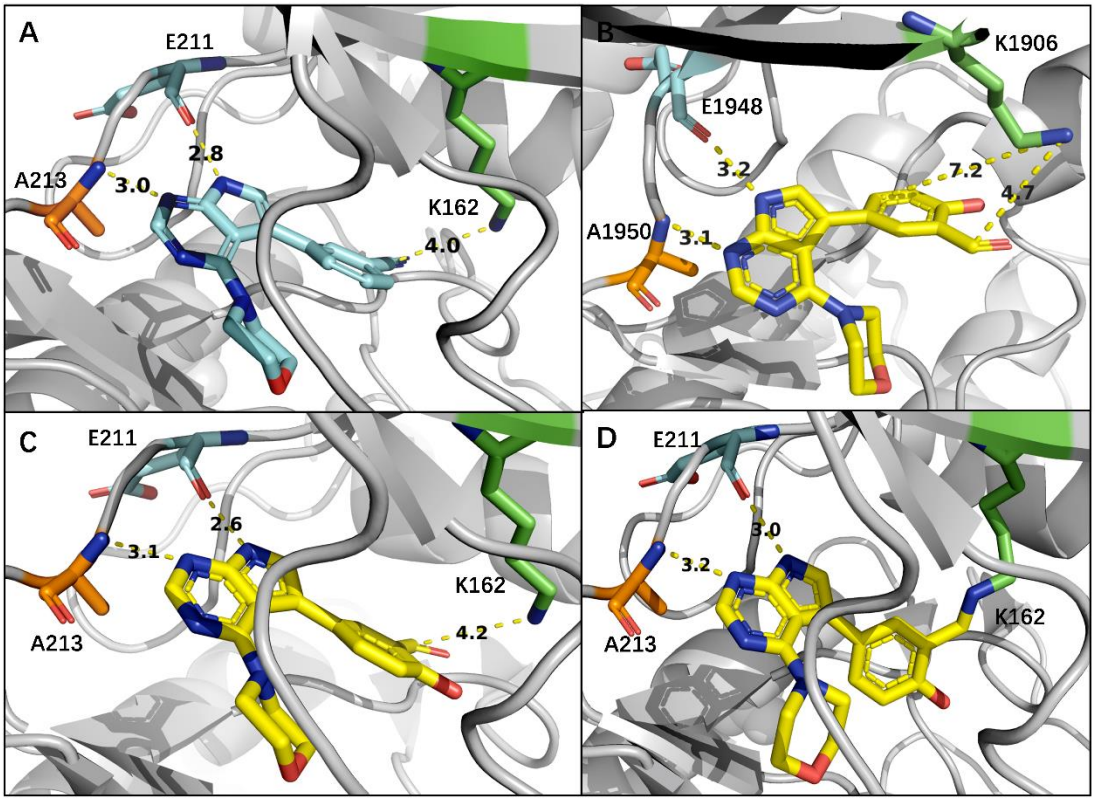


Fig S6. Putative noncovalent/covalent binding mode of compounds in LRRK2 and AURKA domain. (A): **A1** in AURKA domain; (B): **A2** in LRRK2 domain; (C): **A2** in AURKA domain; (D): Putative covalent binding mode of compound **A2** in AURKA domain (AURKA, PDB: 3E5A; LRRK2, PDB: 7LHW)

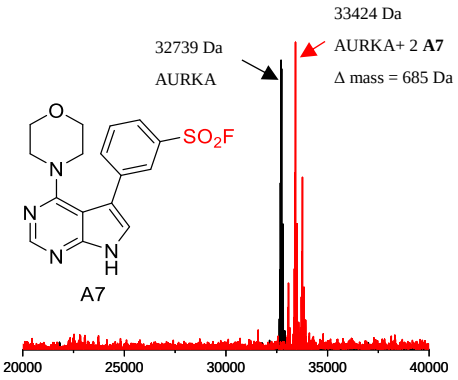


Fig S7. Intact protein MS analysis of **AURKA-A7** complex

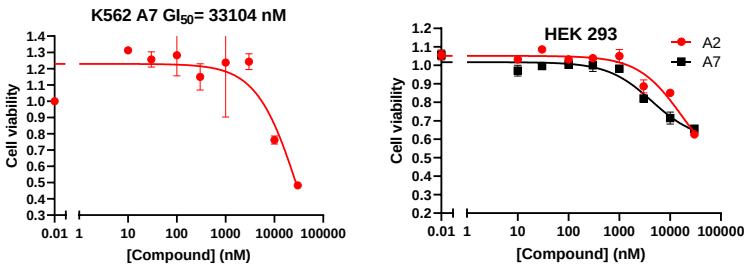


Fig S8. Anti-proliferative activity of **A7** against K562 cells, and compounds **A2** and **A7** against HEK293 cells

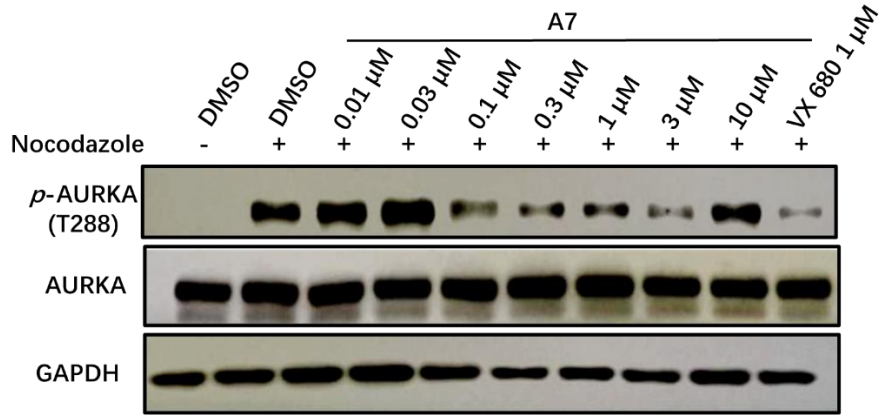


Fig S9. Cellular phosphorylation assay (**A7**)

Table S2. Time-dependent kinase inhibition assays of **A2**, **A3** and **A4** against LRRK2 (IC_{50} /nM)

	0 h	0.5 h	1 h	2 h	6 h
LRRK2-IN-1	4.58±1.14	5.15±0.31	6.15±1.15	6.98±0.43	8.01±0.43
A2	5.94±0.35	5.26±0.68	6.87±6.16	6.02±2.24	5.43±0.55
A3	30.21±5.00	29.02±2.41	36.74±2.2	54.10±2.65	53.76±22.10
A4	19.60±2.64	20.02±0.11	37.84±12.02	38.67±7.87	36.39±4.22

Table S3. Kinome Scan™ results of **A2** (200 nM), and PF-06447475 (1000 nM; extracted from reference 1)

	PF-06447475 **(A1, 1 μM)**[1]	**A2 (0.2 μM)**
KINOMEscan Gene Symbol	**Inhibition (%)**	**Inhibition (%)**
AAK1	86	75
ABL1(H396P)-nonphosphorylated	66	87
ABL1(T315I)-nonphosphorylated	27	73
ACVR1	0	66
ACVR2A	23	11
ACVRL1	0	34
AMPK-alpha1	29	102
AURKA	43	100
AURKB	74	103
AURKC	57	96
BLK	28	102
BMPR1B	69	91
BRAF	20	10
BRAF(V600E)	31	10
BRK	28	62
BTK	0	93
CAMKK2	22	44
CASK	34	51
CDK11(Inactive)	/	87
CDK11	52	80
CHEK1	6	60
CHEK2	0	53
CLK1	37	53
CLK4	62	88

CSNK2A2	55	8
DAPK1	11	7
DYRK1B	30	18
EGFR (G719C)	16	13
EGFR (L858R, T790M)	0	67
EPHA3	34	-2
ERBB2	27	12
FER	16	99
FGFR4	26	74
FGR	0	98
FLT3 (D835Y)	35	81
FRK	12	49
FYN	14	82
GRK7	63	25
HIPK2	35	68
HIPK3	53	30
ICK	4	31
IRAK1	77	96
IRAK4	31	85
ITK	0	43
JAK1(JH1domain-catalytic)	0	13
JAK 2(JH1domain-catalytic)	29	14
JAK JH1 JH2	/	10
JAK JH1 JH2 (V617F)	/	11
JAK3 (JH1domain-catalytic)	97.3	56
KIT	0	54
LIMK1	7	92
LRRK2	57	95
LRRK2 FL	/	92
LRRK2 (G2019S)	25	96
LRRK2 (G2019S) FL	/	97
LRRK2 (I2020T)	/	86
LRRK2 (R1441C)	/	91
MAP3K2	79	98
MAP4K5	47	101
MAPK15 (ERK7)	/	74
MARK2	57	95
MAST1	27	31
MEK1	87	97
MEK2	81	84
MEK5	86	69
MINK	54	75
MLCK	7	22
MST1R	5	27
MST4	93	101
NUAK2	/	79
p38-delta	30	14
PAK7	34	12

PHKG2	60	22
PKN2	65	51
PLK4	67	99
PRKCE	88	34
PRKCH	59	47
RET (V804M)	42	26
RIPK2	16	5
RPS6KA4(Kin.Dom.2-C-terminal)	0	74
RSK1(Kin.Dom.1-N-terminal)	0	84
RSK2(Kin.Dom.1-N-terminal)	54	91
SGK	35	93
SRC	0	40
SRC N1	/	65
SRPK1	75	30
STK16	67	49
STK33	40	98
TAOK1	93.6	42
TAOK3	64	17
TGFBR1	21	91
TNIK	55	93
TNK1	68	59
ULK1	35	57
ULK2	31	60
ULK3	75	96
WEE1	0	4
YES	5	80
YSK1	72	101
YSK4	96.2	86

/: no result.

Table S4. Selectivity Scores for PF-06447475 from KinomeScan™ results[1]

Compound	Selectivity Scores Type	Number of Hits	Number of kinase	Screening Concentration (nM)	Selectivity Score
PF-06447475	S(30)	50	386	1000	0.13

Table S5. X-Ray data collection and refinement statistics for X-ray structures of **AURKA-A2** complex

	AURKA-A2 (8JMX)
Data collection	
Space group	P6122
Cell dimensions	
a, *b*, *c* (Å)	85.263, 85.263, 168.357
α, β, γ (°)	90.00, 90.00, 120.00
Resolution (Å)	44.70-2.95(3.05-2.95)[a]
R_{merge}	0.358(1.028)
$I/\sigma(I)$	24.0(1.8)
$CC_{1/2}$	0.999(0. 925)
Completeness (%)	100.0(100.0)
Redundancy	24.0(26.0)
Refinement	
Resolution (Å)	44.7-2.95
No. reflections	7161
R_{work} / R_{free}	0.2139/0.2983
No. atoms	
Protein	1933
Ligand	23
Water	0
B factors	
Protein	87.60
Ligand	69.90
Water	0
R.m.s.deviations	
Bond lengths (Å)	0.008
Bond angles (°)	1.19

[a]Values in parentheses are for highest-resolution shell

5. Reference:

1 J. L. Henderson, B. L. Kormos, M. M. Hayward, K. J. Coffman, J. Jasti, R. G. Kurumbail, T. T. Wager, P. R. Verhoest, G. S. Noell, Y. Chen, E. Needle, Z. Berger, S. J. Steyn, C. Houle, W. D. Hirst and P. Galatsis, *J. Med. Chem.*, 2015, **58**, 419-432.

2 V. Pandarus, G. Gingras, F. Béland, R. Ciriminna and M. Pagliaro, *BEILSTEIN J ORG CHEM*, 2014, **10**, 897-901.

3 (a) J. A. H. Inkster, K. Liu, S. Ait-Mohand, P. Schaffer, B. Guérin, T. J. Ruth and T. Storr, *CHEM-EUR J*, 2012, **18**, 11079-11087; (b) T. S.-B. Lou and M. C. Willis, *Tetrahedron*, 2020, **76**, 130782.

4 Y. J. Jang, O. G. Tsay, D. P. Murale, J. A. Jeong, A. Segev and D. G. Churchill, *Chem. Commun.*, 2014, **50**, 7531-7534.

5 J. H. Cho, L. C. Bassit, F. Amblard and R. F. Schinazi, *Nucleosides, Nucleotides & Nucleic Acids*, 2020, **39**, 671-687.

6 V. Vaněk, J. Pícha, B. Fabre, M. Buděšínský, M. Lepšík and J. Jiráček, *Eur. J. Org. Chem.*, 2015, **2015**, 3689-3701.

7. T. Kuwahara and T. Iwatsubo, Front Neuros. SWitz., 2020, **14**, 227.

6. NMR Spectra

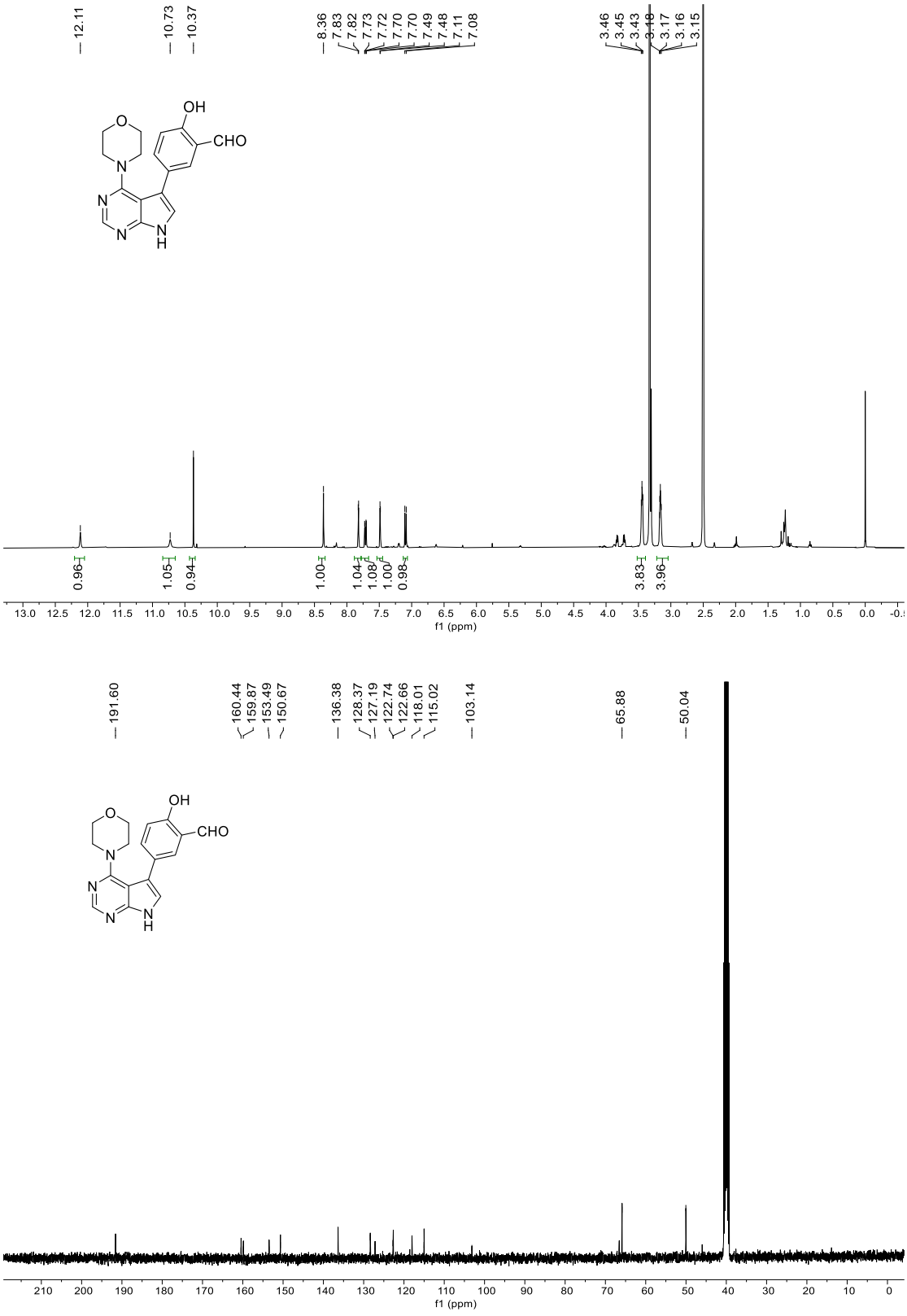


Fig. S9. The ^{1}H-NMR spectrum and ^{13}C-NMR spectrum of compound **A2**

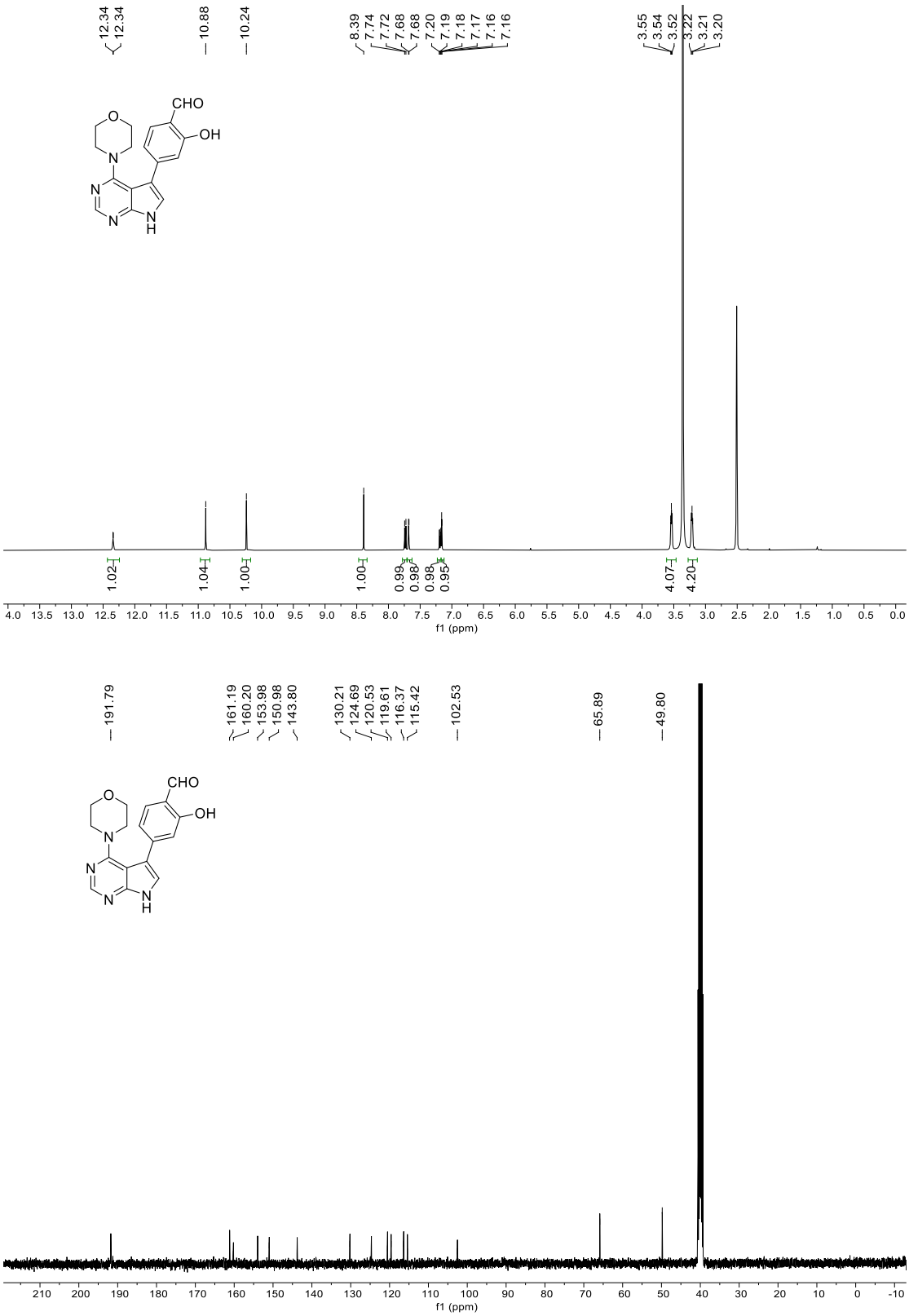


Fig. S10. The ^{1}H-NMR spectrum and ^{13}C-NMR spectrum of compound **A3**

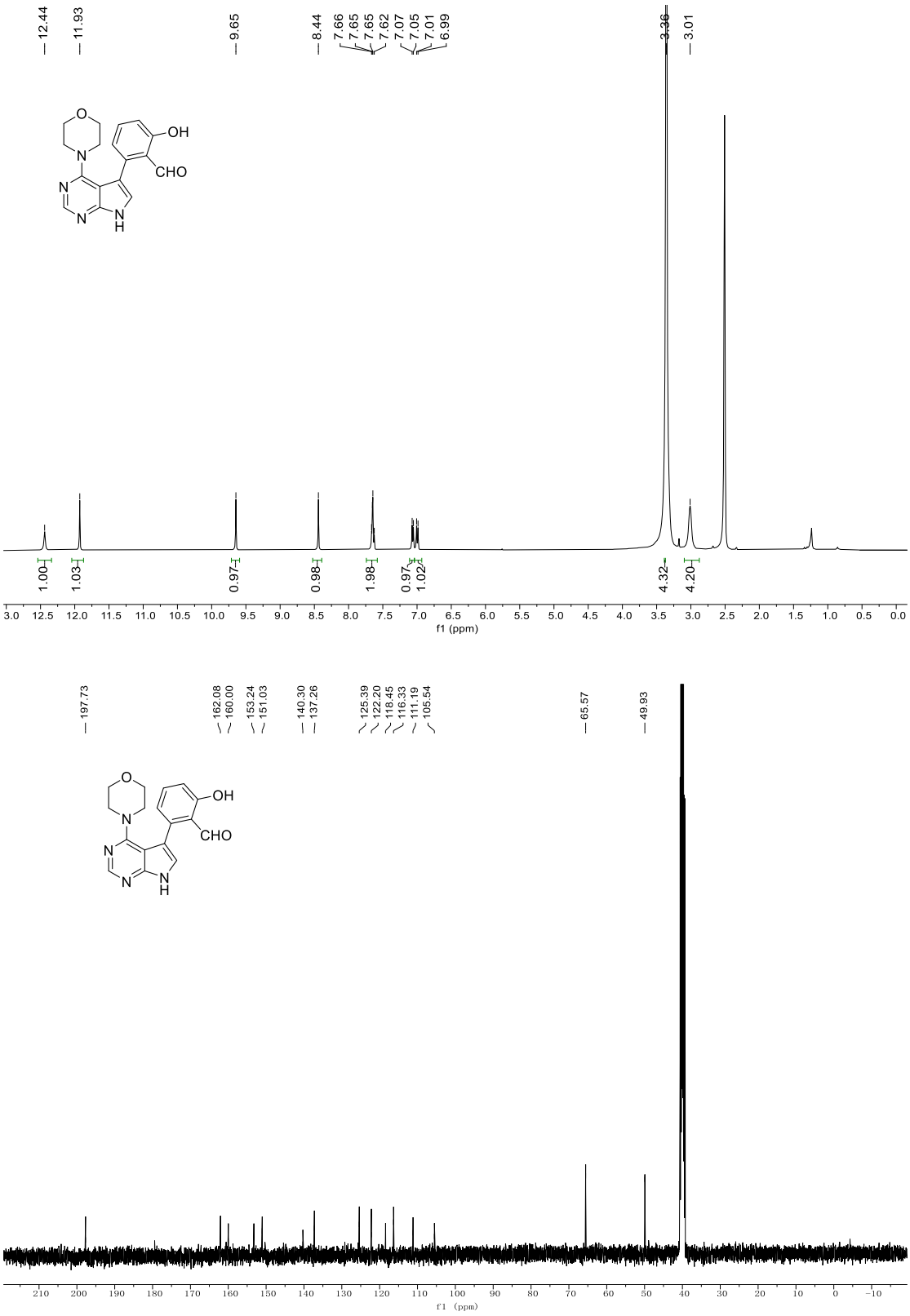


Fig. S11. The ^{1}H-NMR spectrum and ^{13}C-NMR spectrum of compound **A4**

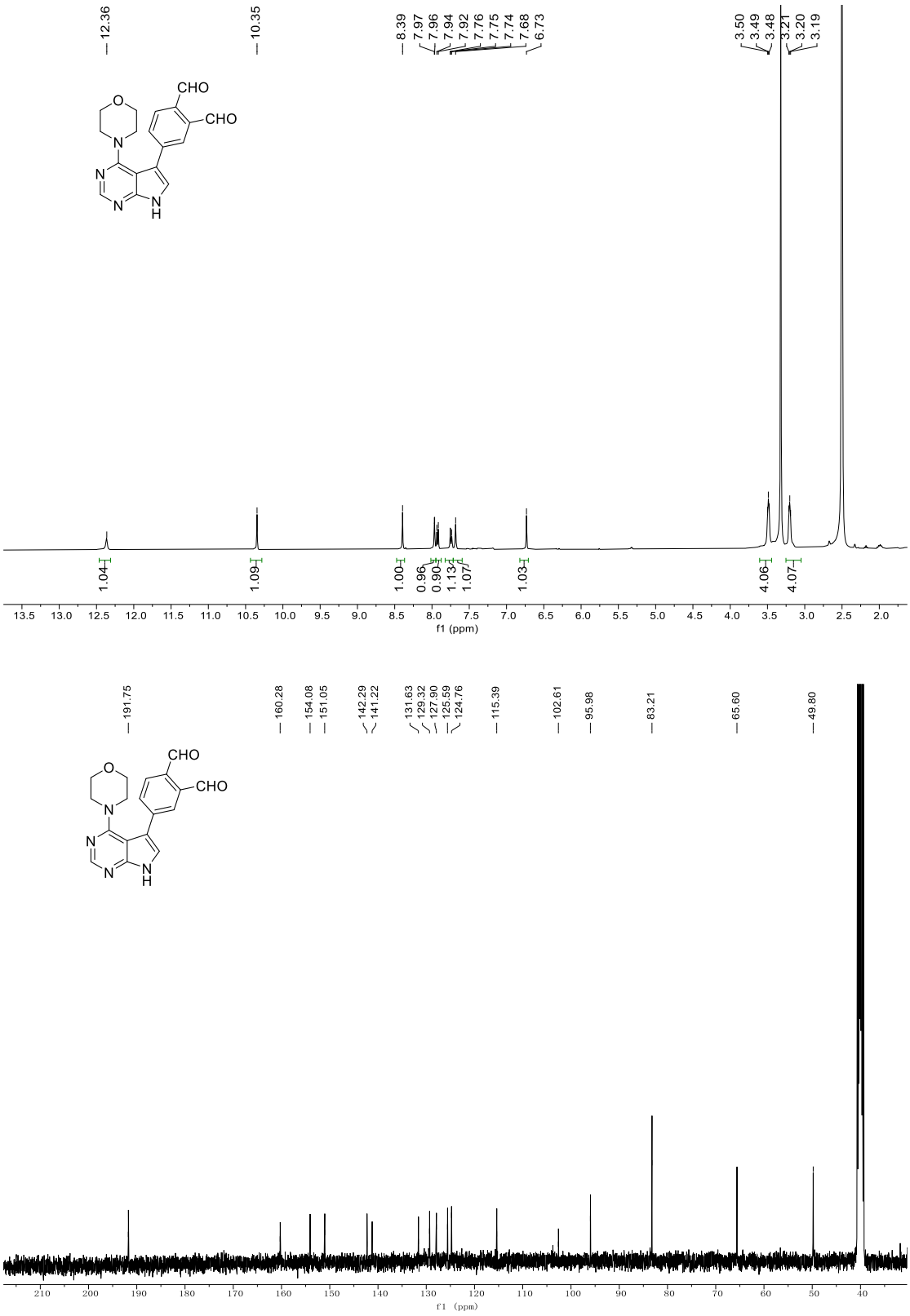


Fig. S12. The ^{1}H-NMR spectrum and ^{13}C-NMR spectrum of compound **A5**

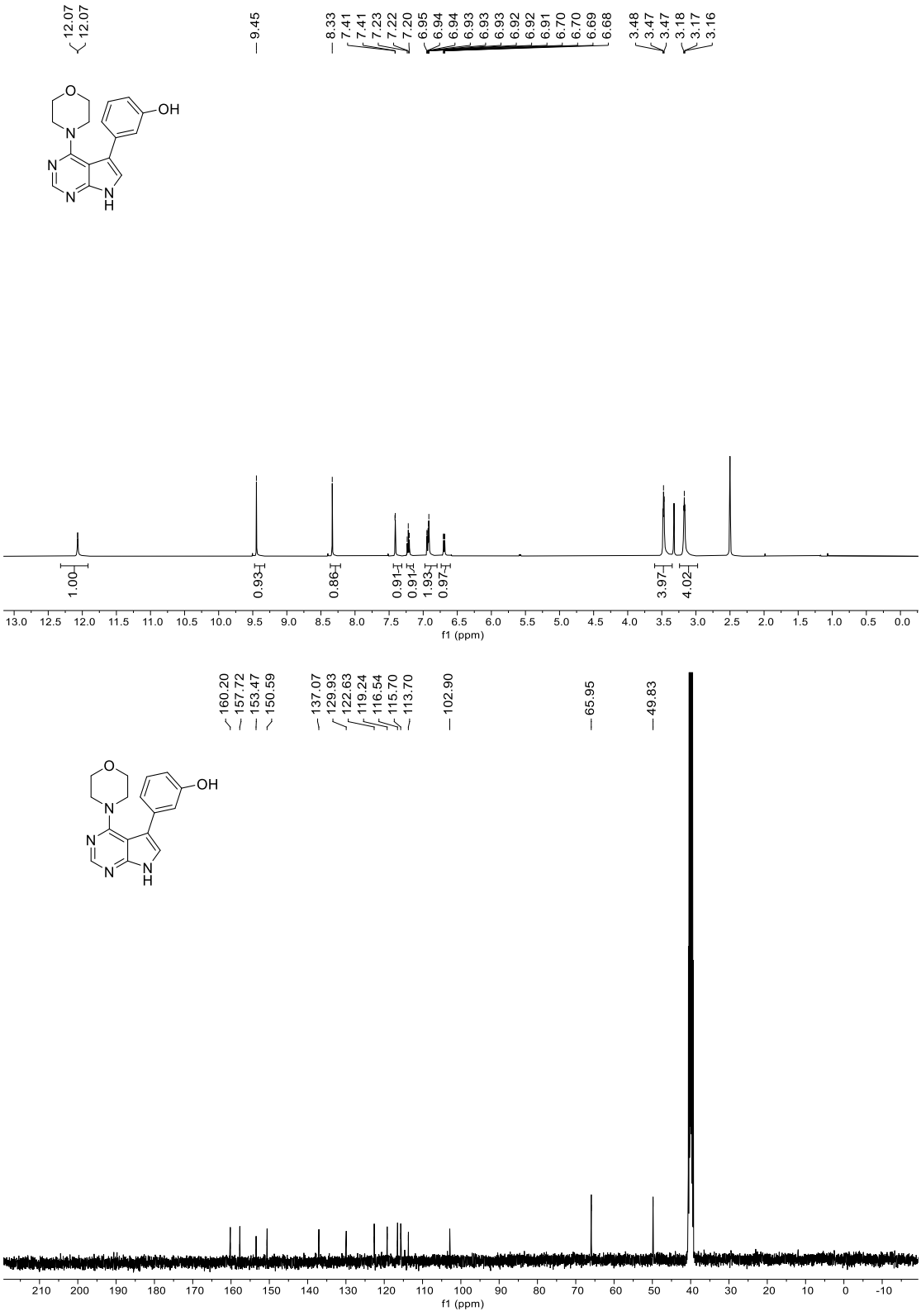
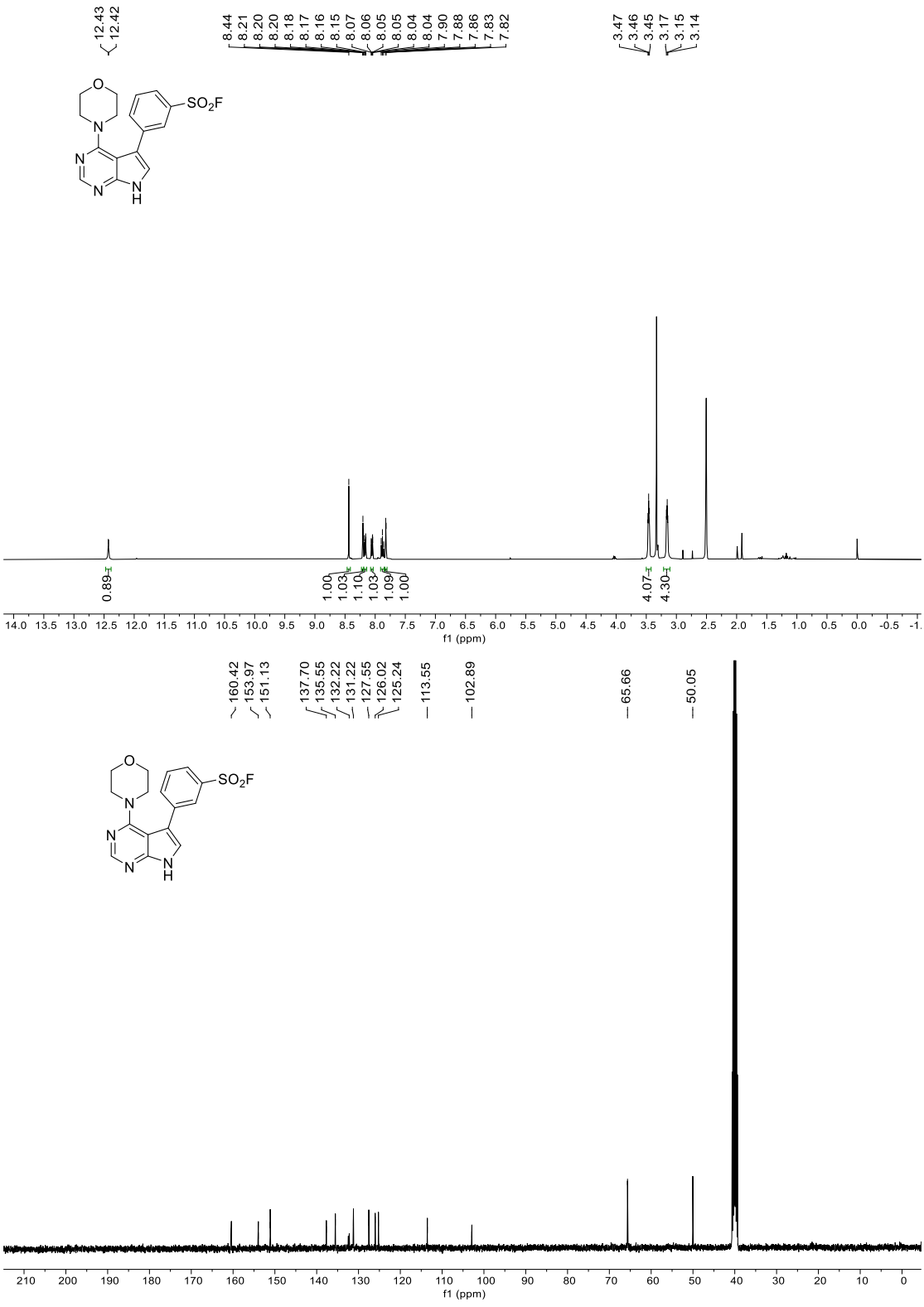


Fig. S13. The ^{1}H-NMR spectrum and ^{13}C-NMR spectrum of compound **A6**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12.42
8.44
8.21
8.20
8.20
8.18
8.17
8.16
8.15
8.07
8.06
8.05
8.05
8.04
8.04
7.90
7.88
7.86
7.83
7.82
3.47
3.46
3.45
3.17
3.15
3.14
SO2F
O
N
N
N
H
0.89
1.00
1.03
1.10
1.03
1.09
1.00
4.07
4.30
14.0 13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.
f1 (ppm)
160.42
153.97
151.13
137.70
135.55
132.22
131.22
127.55
126.02
125.24
113.55
102.89
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N
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f1 (ppm)

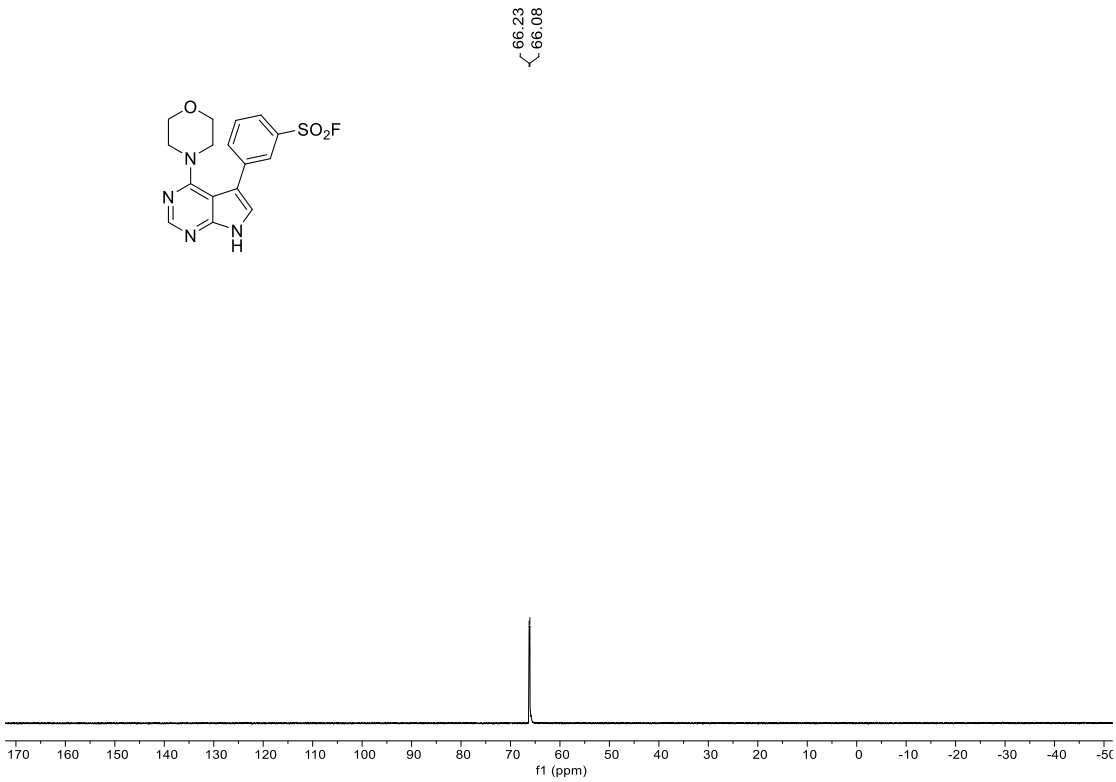


Fig. S14. The ^{1}H-NMR spectrum,^{13}C-NMR and ^{19}F-NMR spectrum of compound **A7**

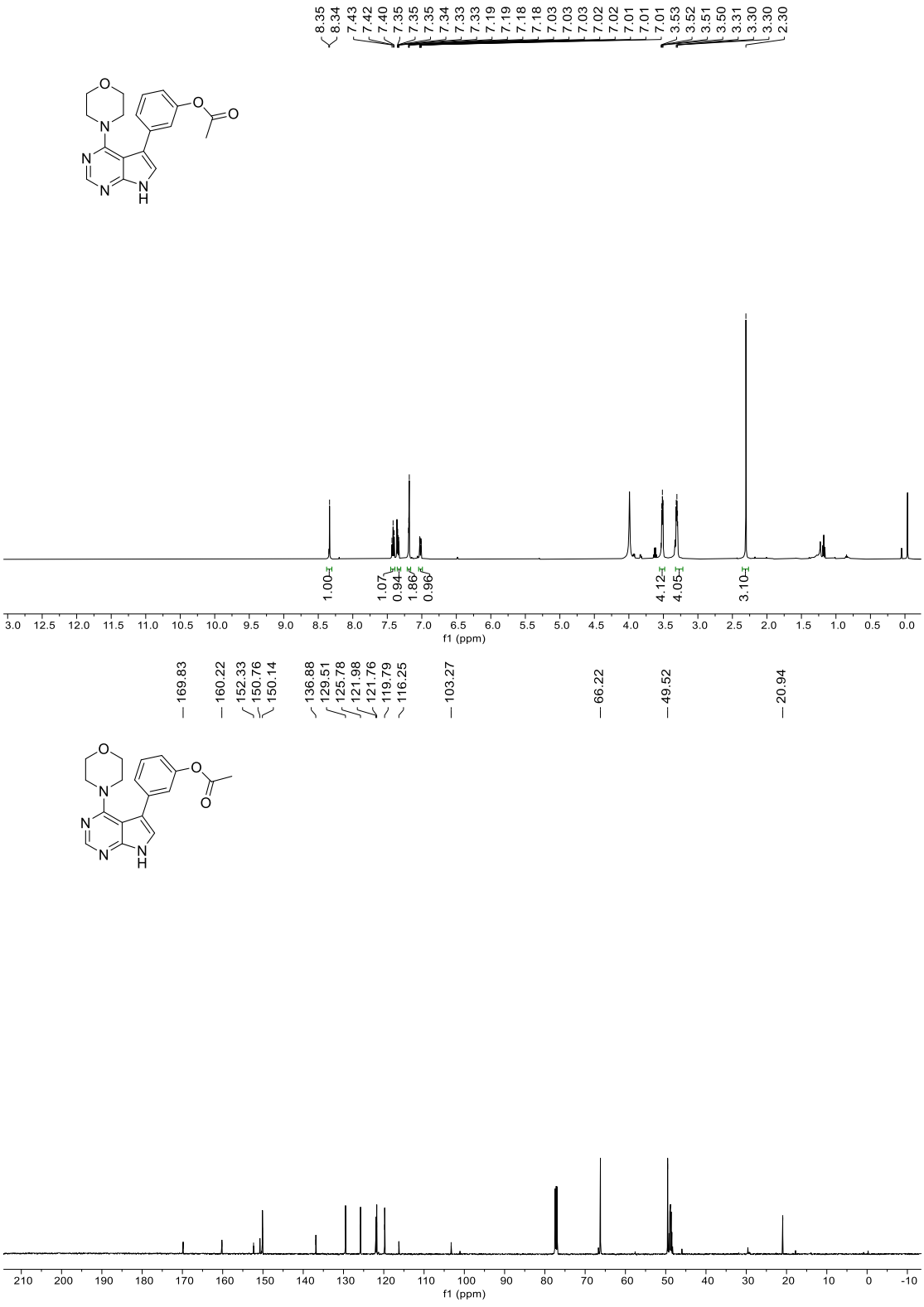


Fig. S15. The ^{1}H-NMR spectrum and ^{13}C-NMR spectrum of compound **A8**

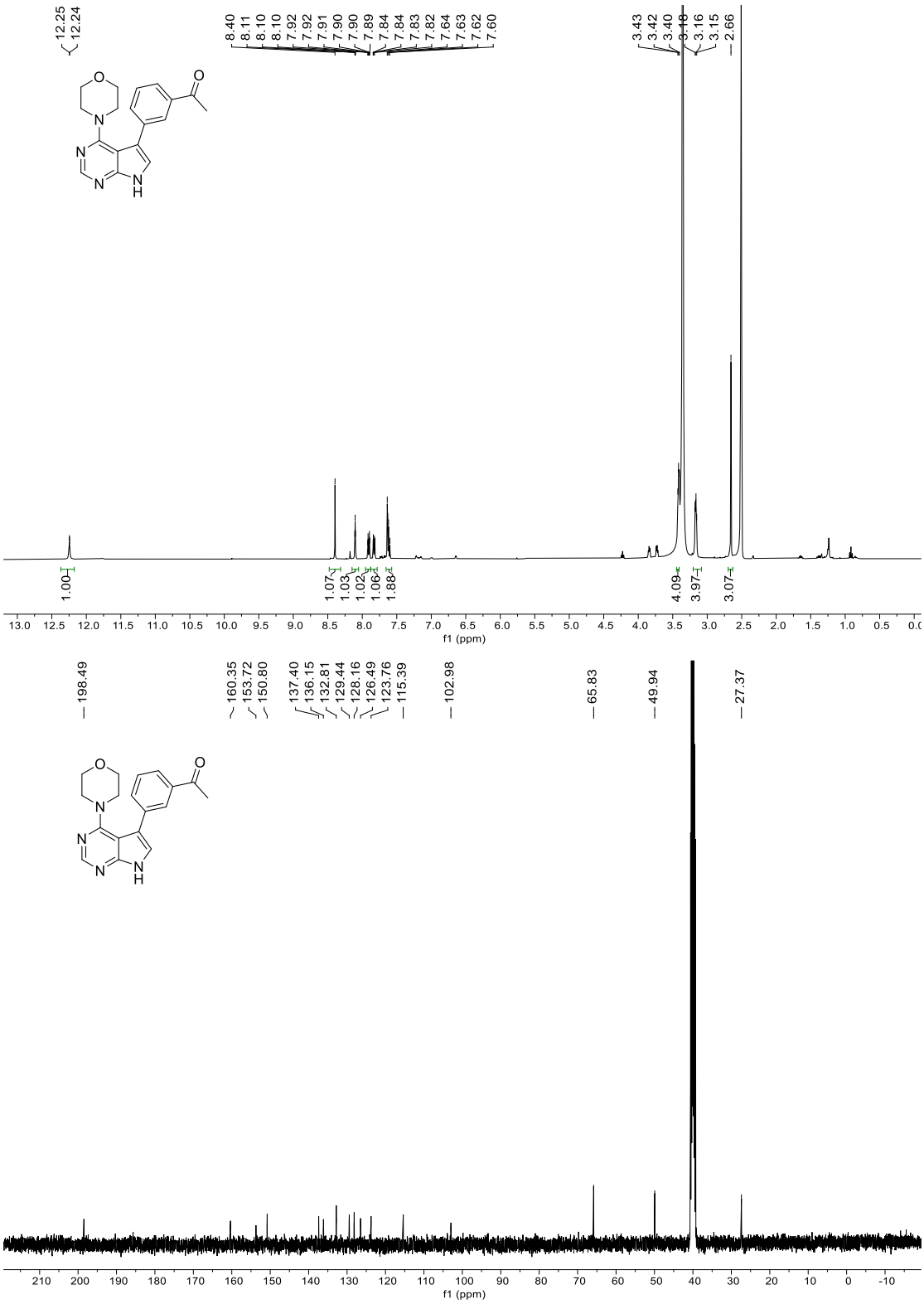


Fig. S16. The ^{1}H-NMR spectrum and ^{13}C-NMR spectrum of compound **A9**

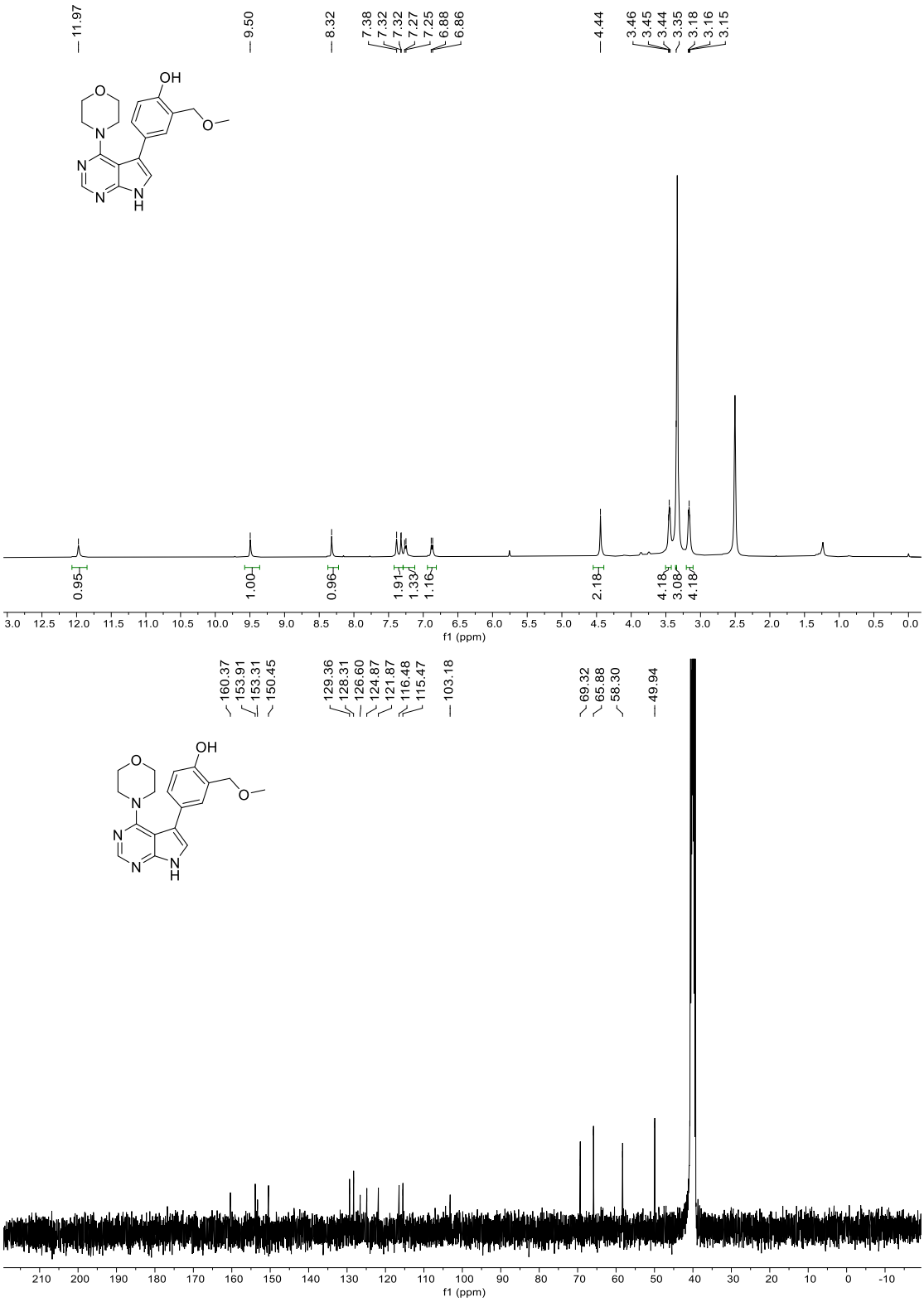


Fig. S17. The ^{1}H-NMR spectrum and ^{13}C-NMR spectrum of compound **A10**

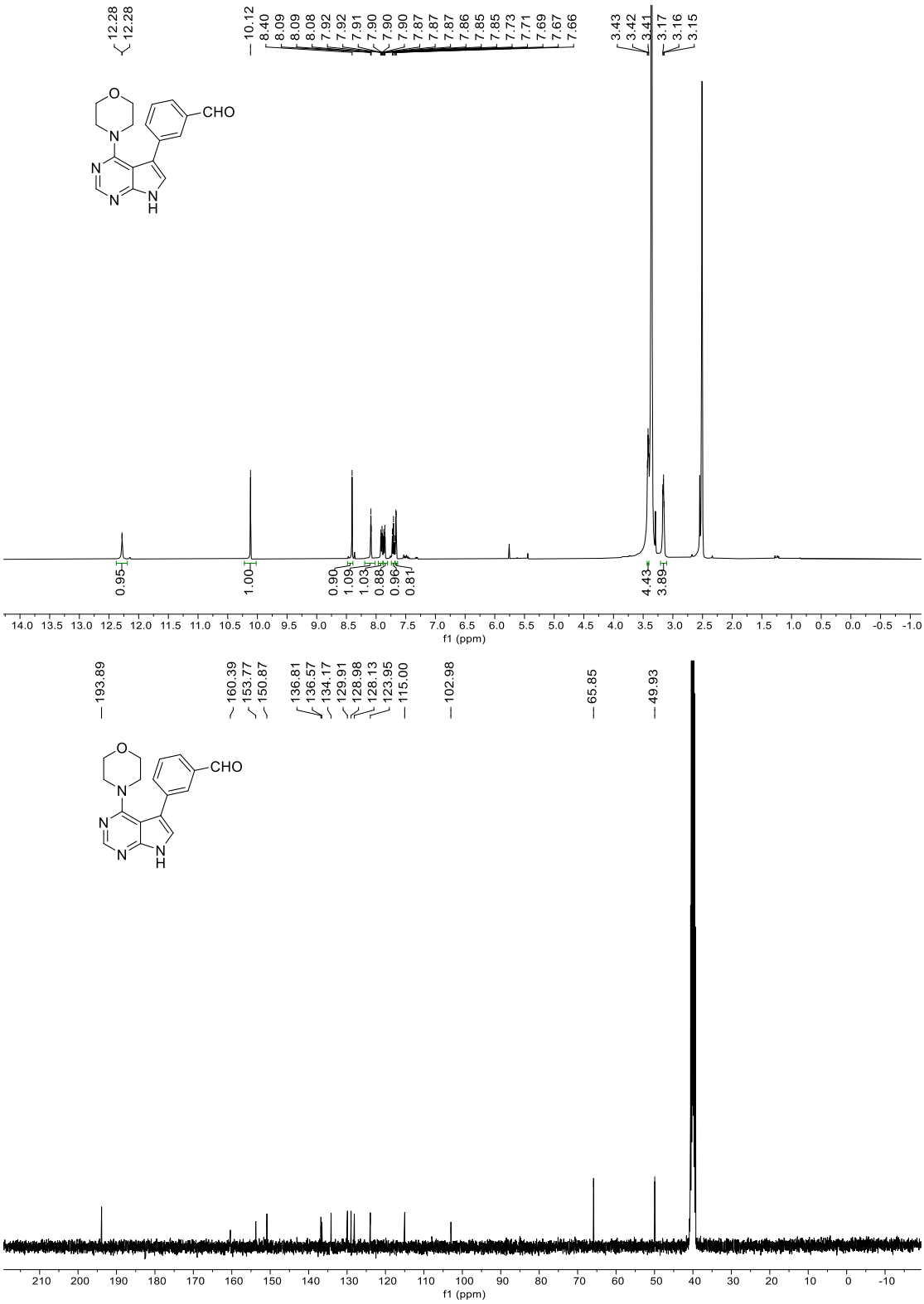


Fig. S18. The ^{1}H-NMR spectrum and ^{13}C-NMR spectrum of compound **A11**

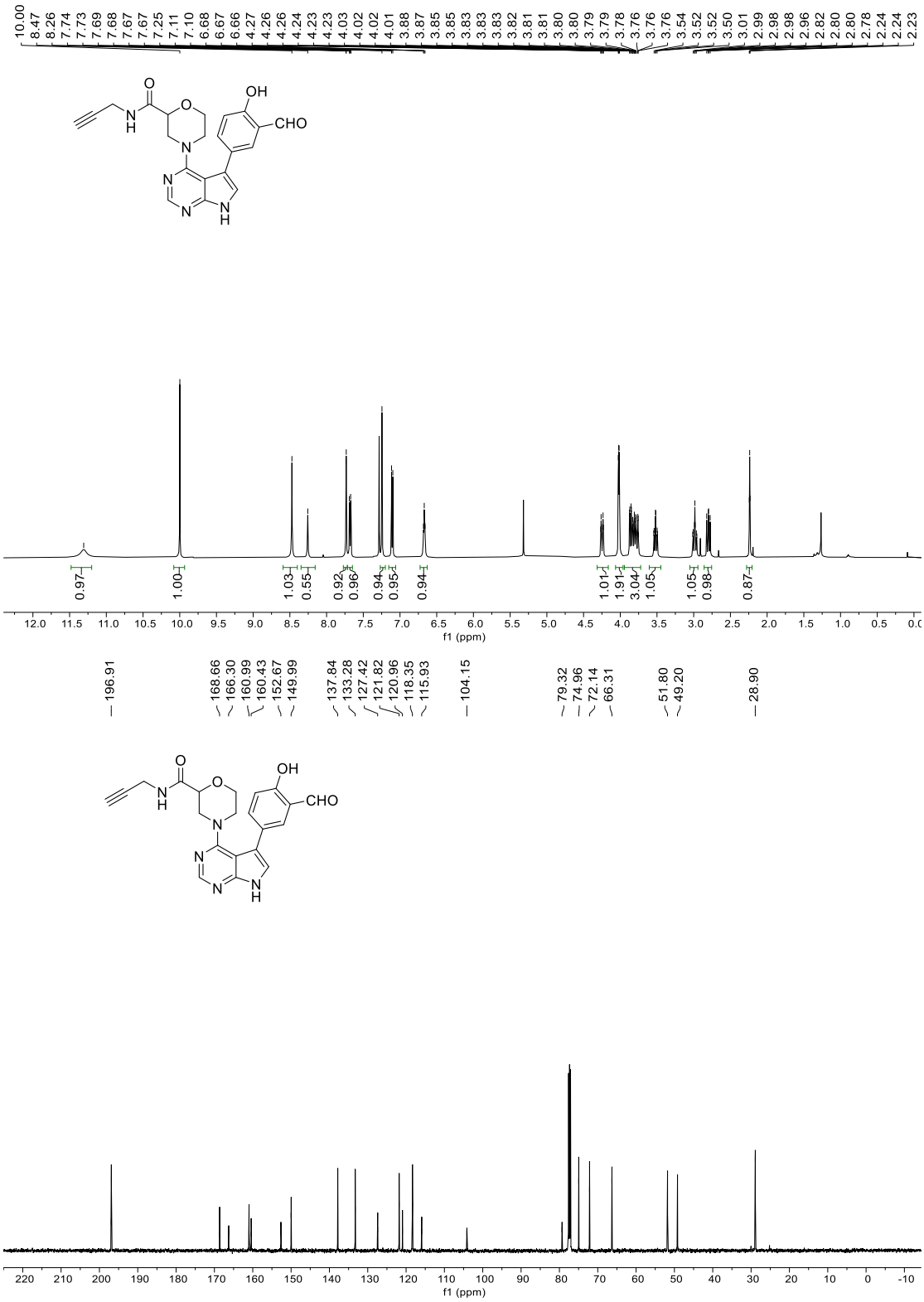
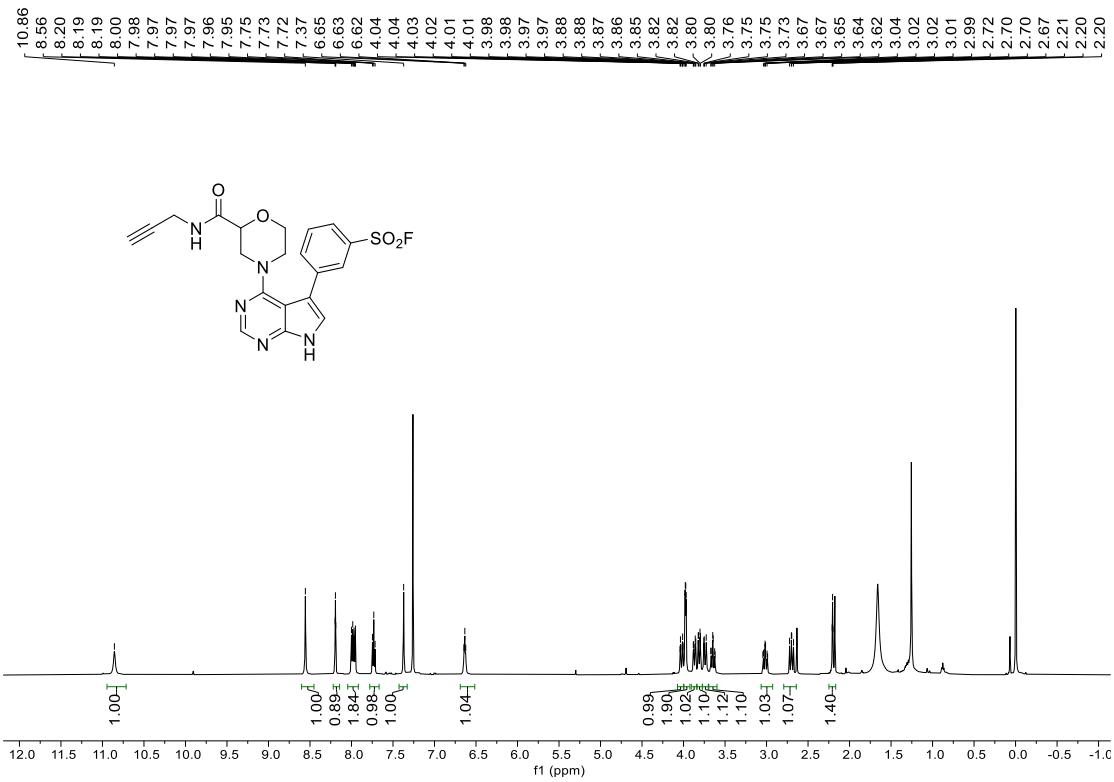


Fig. S19. The ^{1}H-NMR spectrum and ^{13}C-NMR spectrum of compound **ABPA2**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8.20
8.19
8.19
8.00
7.98
7.97
7.97
7.97
7.96
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7.73
7.72
7.37
6.65
6.63
6.62
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4.04
4.03
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3.67
3.65
3.64
3.62
3.04
3.02
3.02
3.01
2.99
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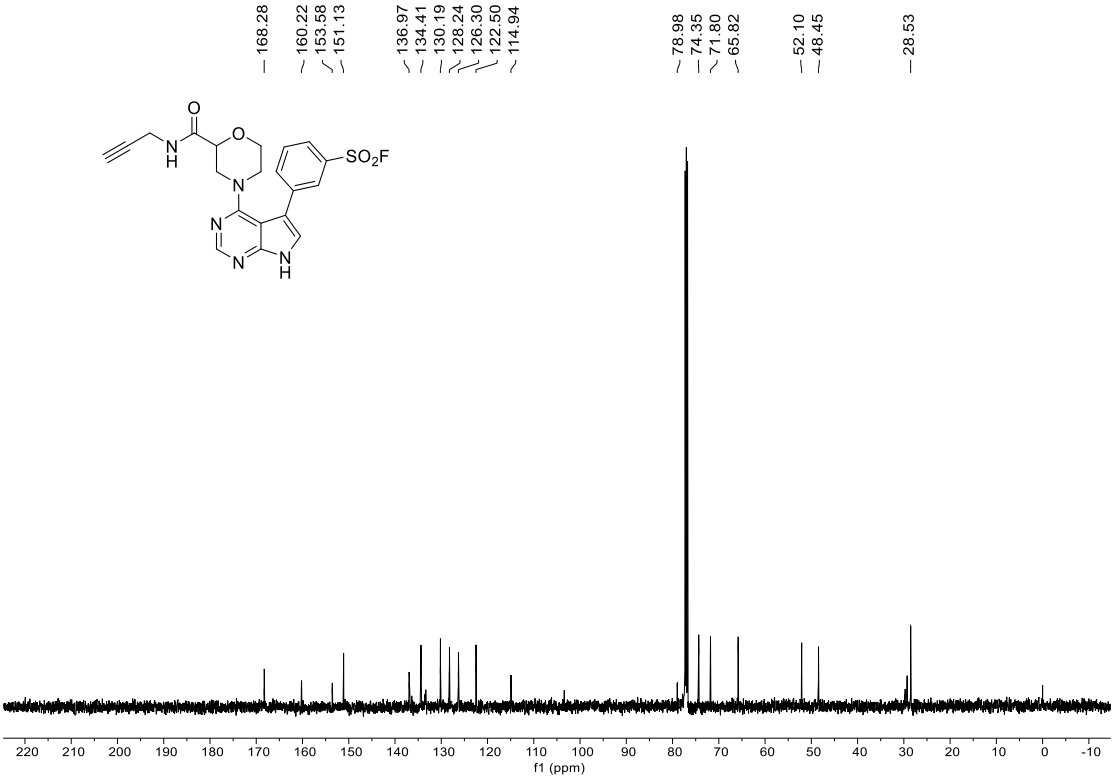
2.70
2.67
2.21
2.20
2.20
O
N
H
O
N
SO2F
N
N
N
H
1.00
1.00
0.89
1.84
0.98
1.00
1.04
0.99
1.90
1.02
1.10
1.12
1.10
1.03
1.07
1.40
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)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153.58
151.13
136.97
134.41
130.19
128.24
126.30
122.50
114.94
78.98
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71.80
65.82
52.10
48.45
28.53
O
N
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H
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f1 (ppm)

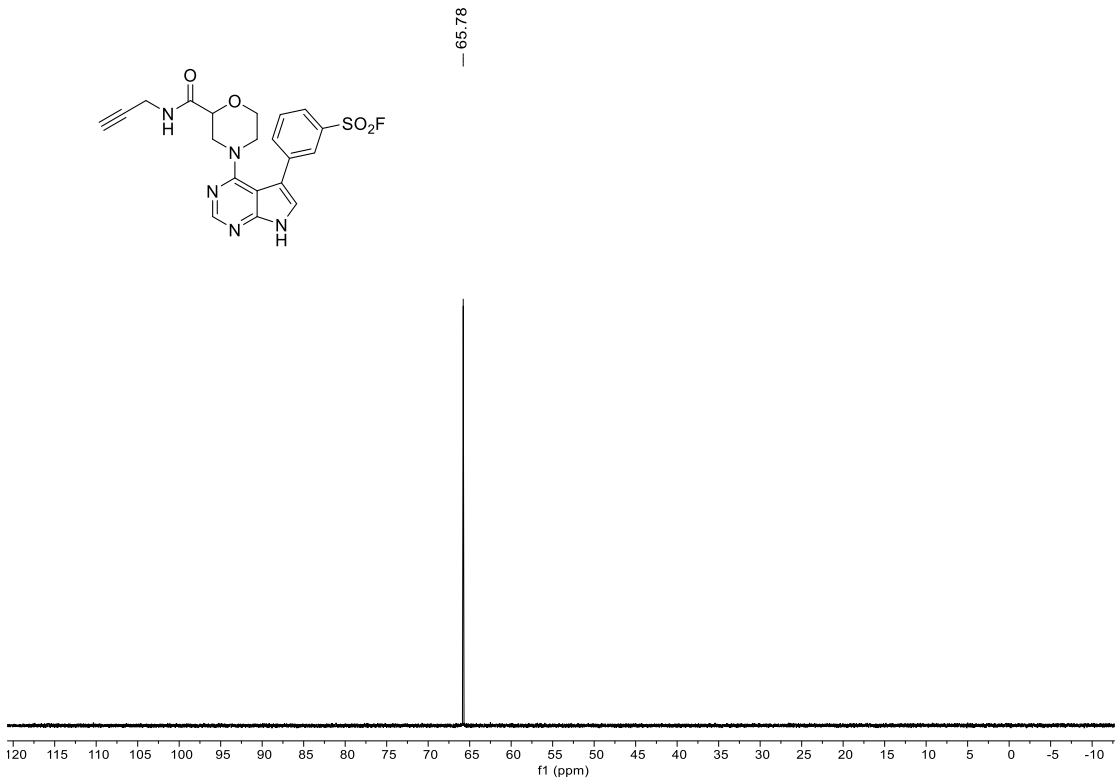


Fig. S20. The ^{1}H-NMR spectrum, ^{13}C-NMR spectrum and ^{19}F spectrum of compound **ABPA7**

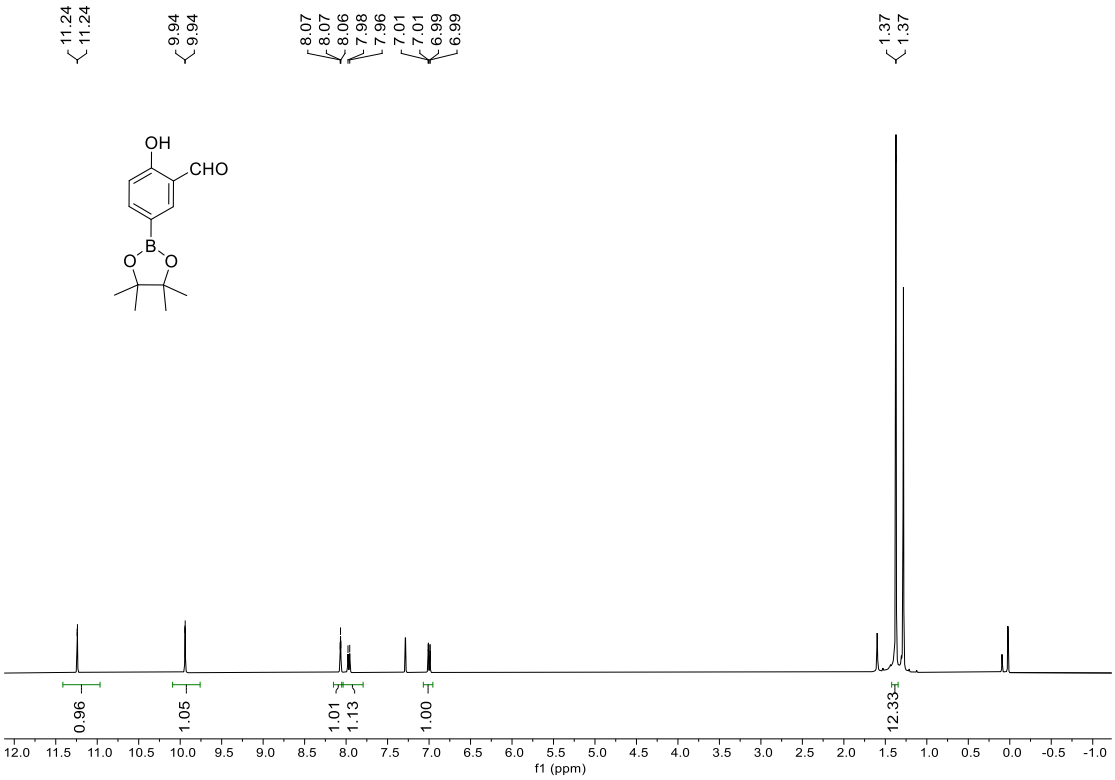


Fig. S21. The ^{1}H-NMR spectrum and ^{13}C-NMR spectrum of compound **B1**

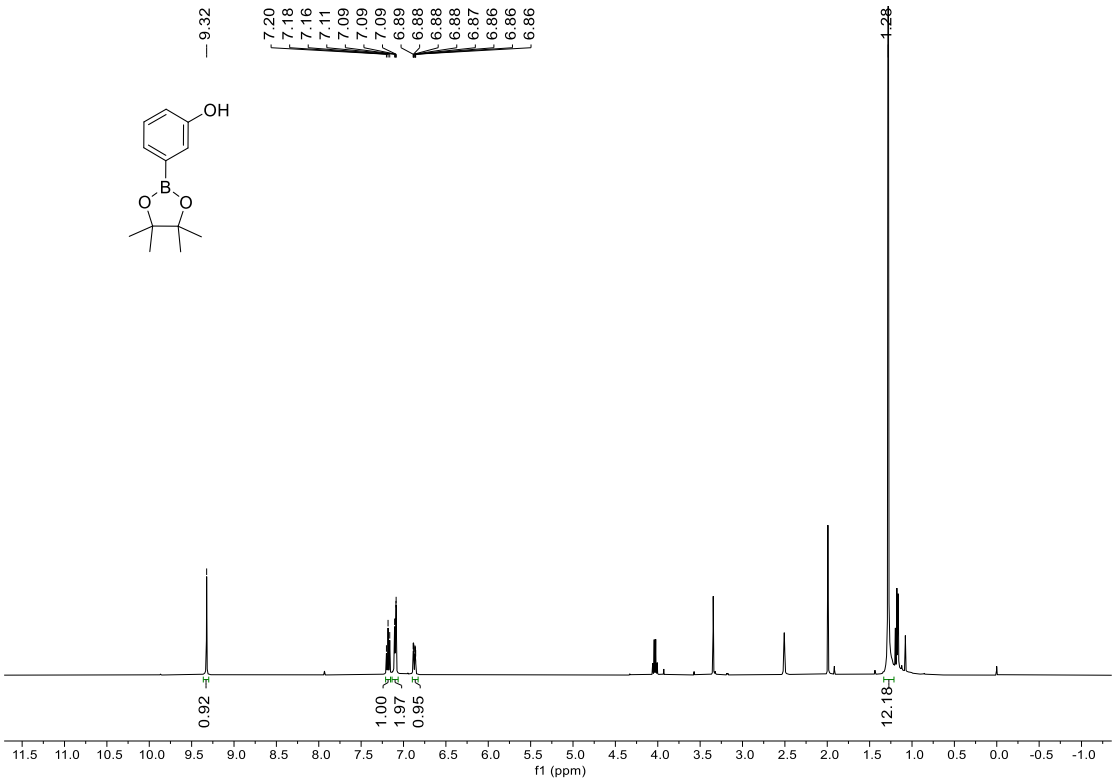


Fig. S22. The ^{1}H-NMR spectrum of compound **B2**

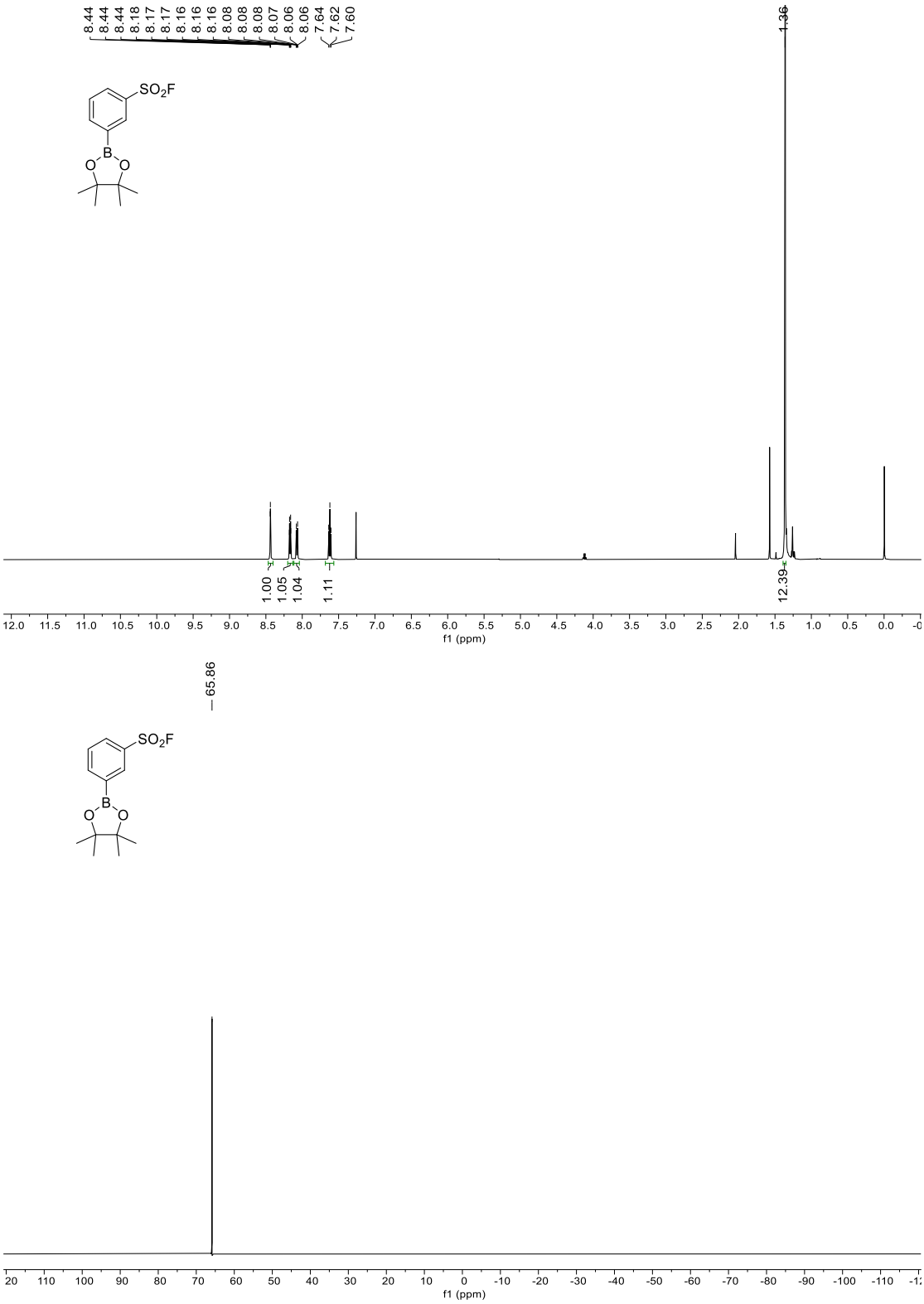


Fig. S23. The ^{1}H-NMR spectrum and ^{19}F-NMR spectrum of compound **B3**

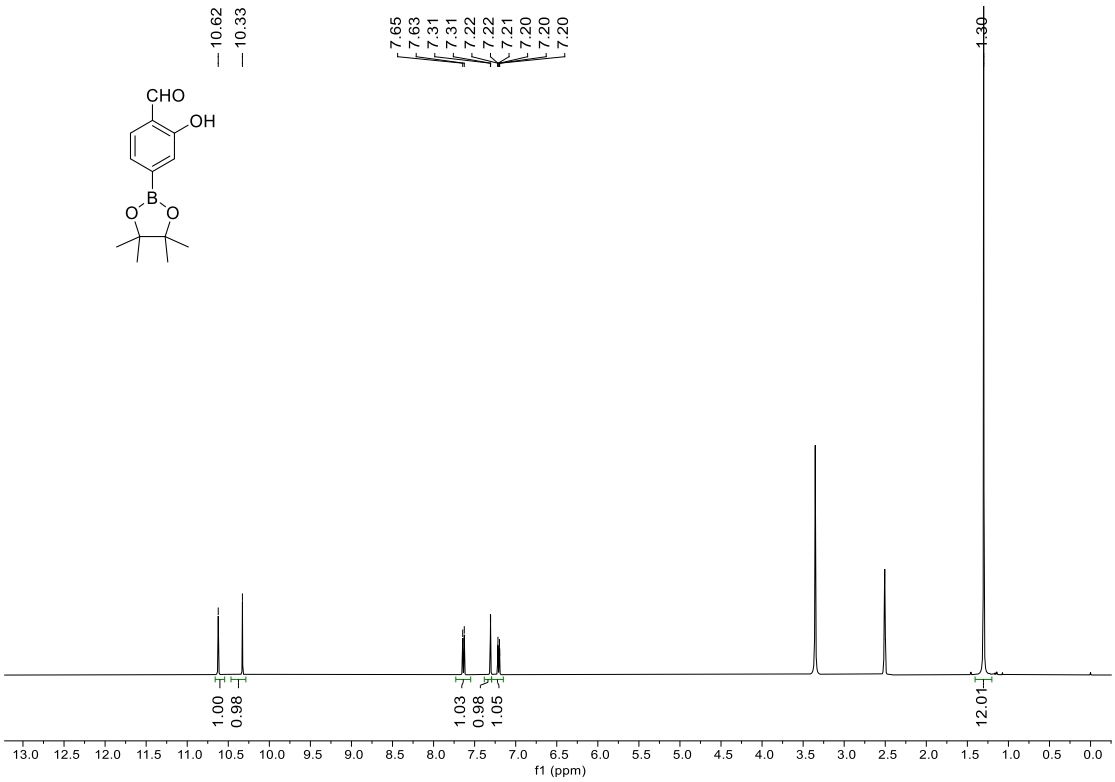


Fig. S24. The [1]H-NMR spectrum of compound **B5**

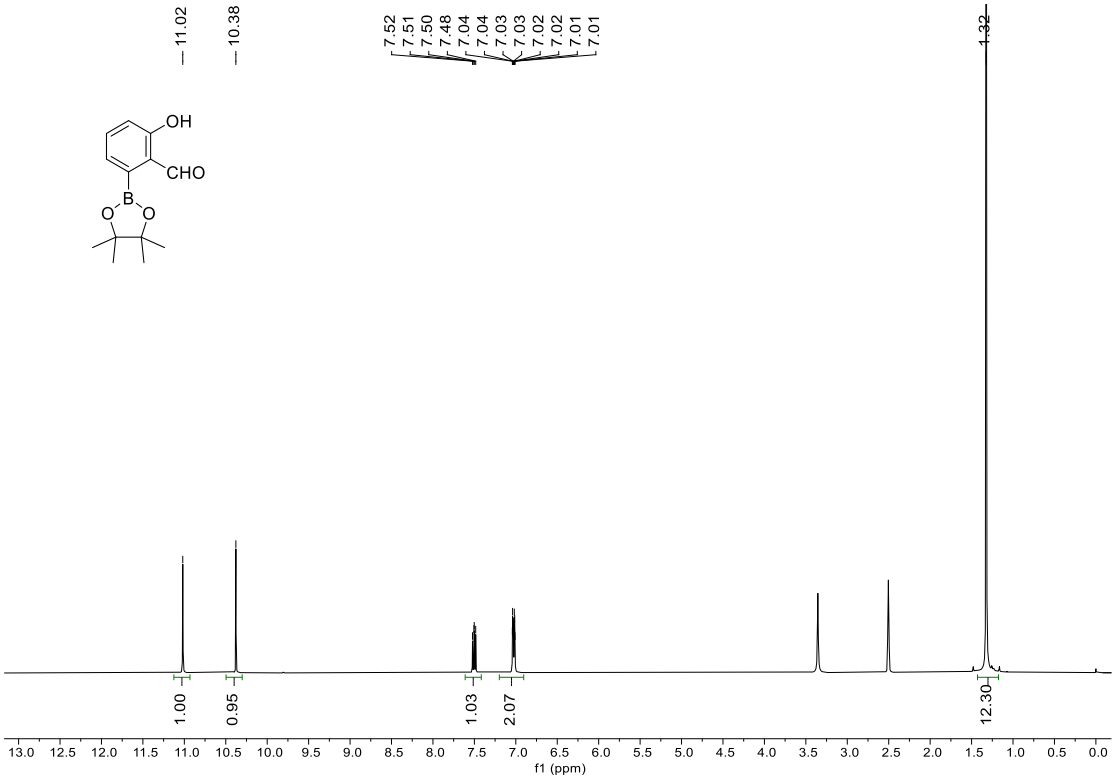


Fig. S25. The [1]H-NMR spectrum of compound **B6**

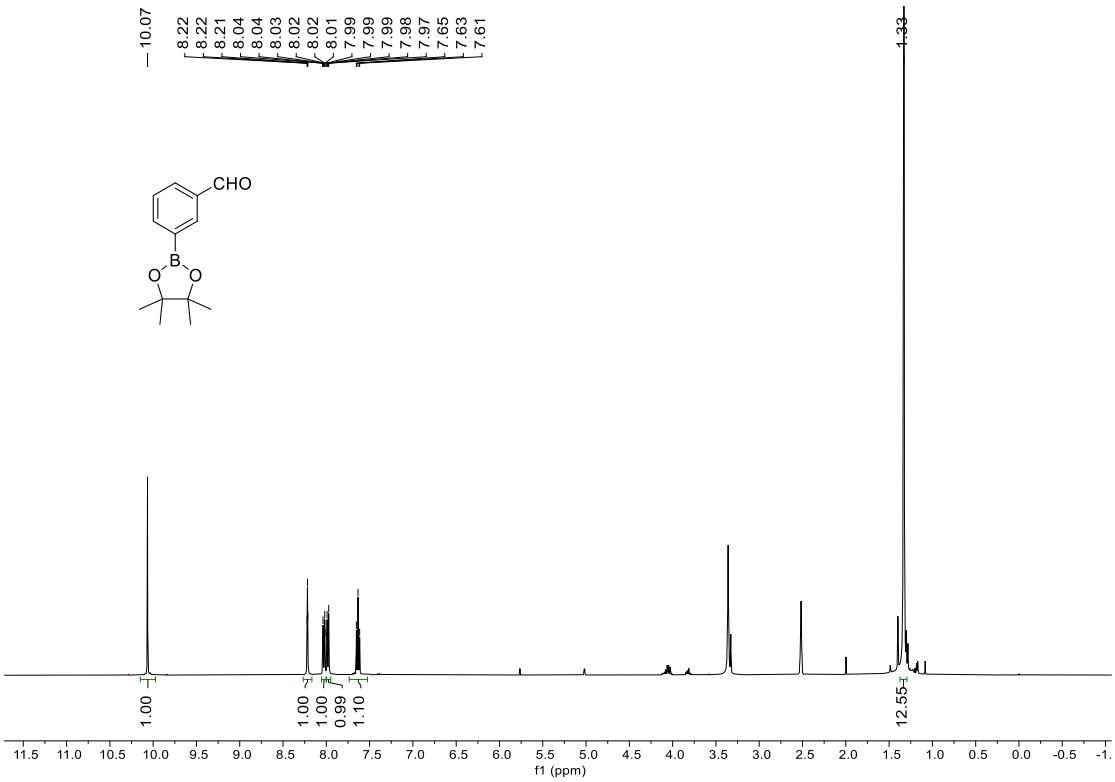


Fig. S26. The [1]H-NMR spectrum of compound **B7**

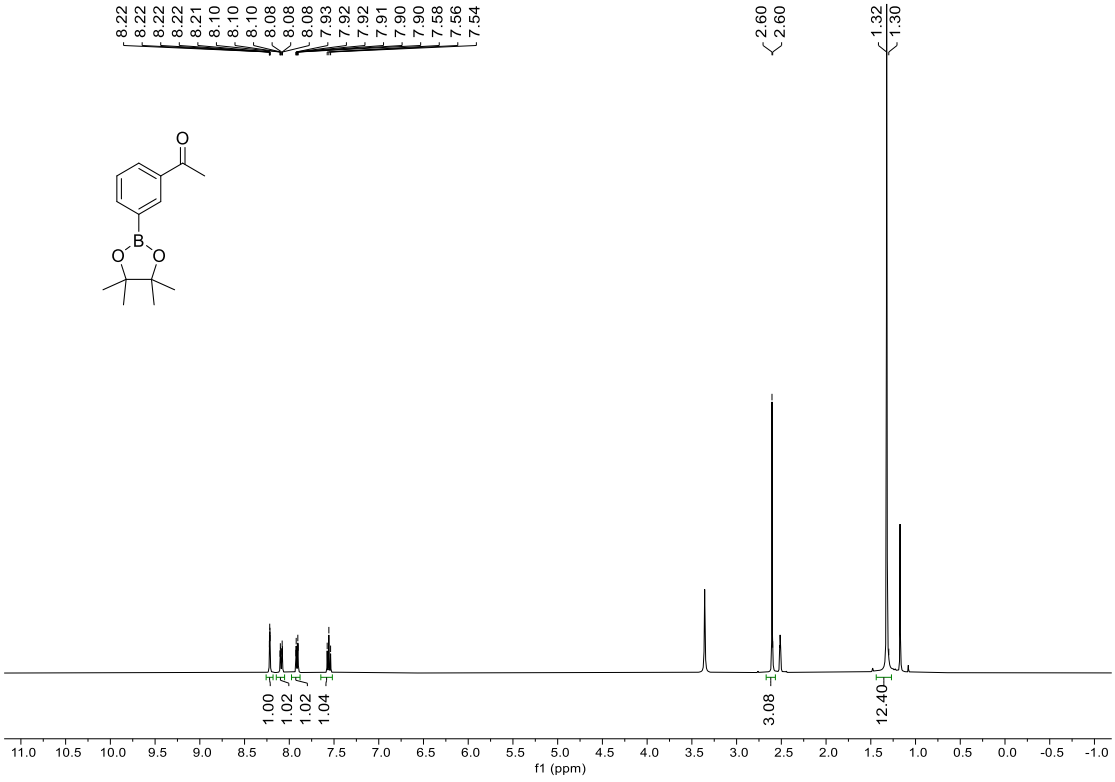


Fig. S27. The [1]H-NMR spectrum of compound **B8**

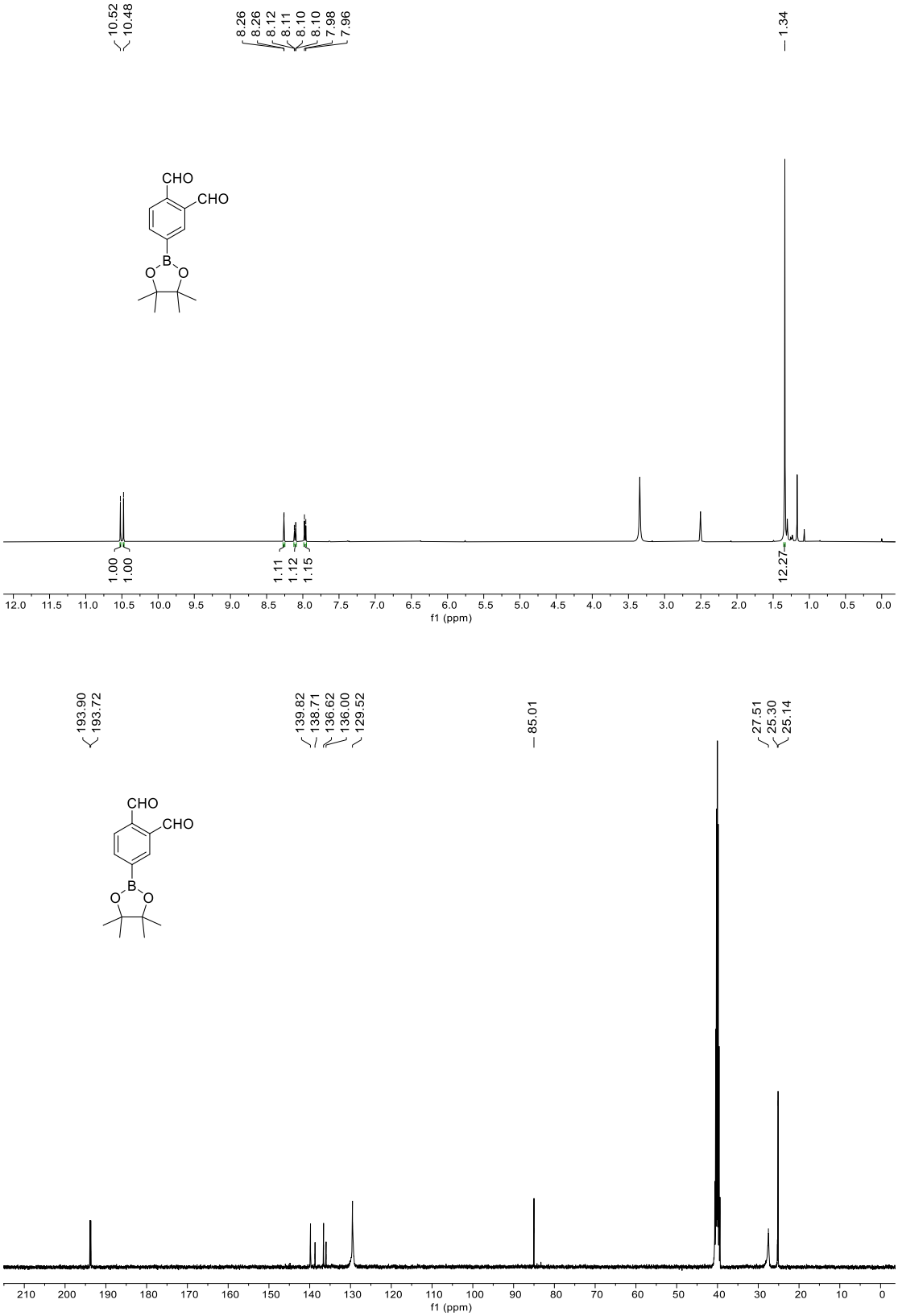


Fig. S28. The ^{1}H-NMR spectrum and ^{13}C-NMR spectrum of compound **B9**

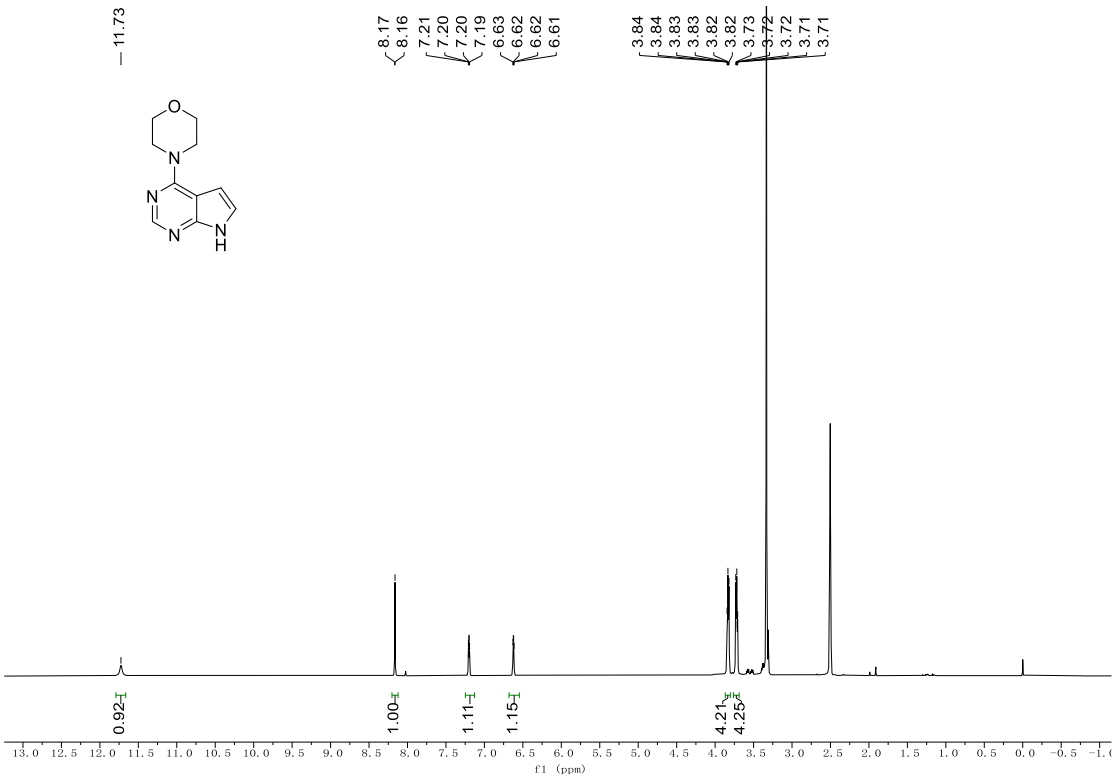


Fig. S29. The ^{1}H-NMR spectrum of compound **B10**

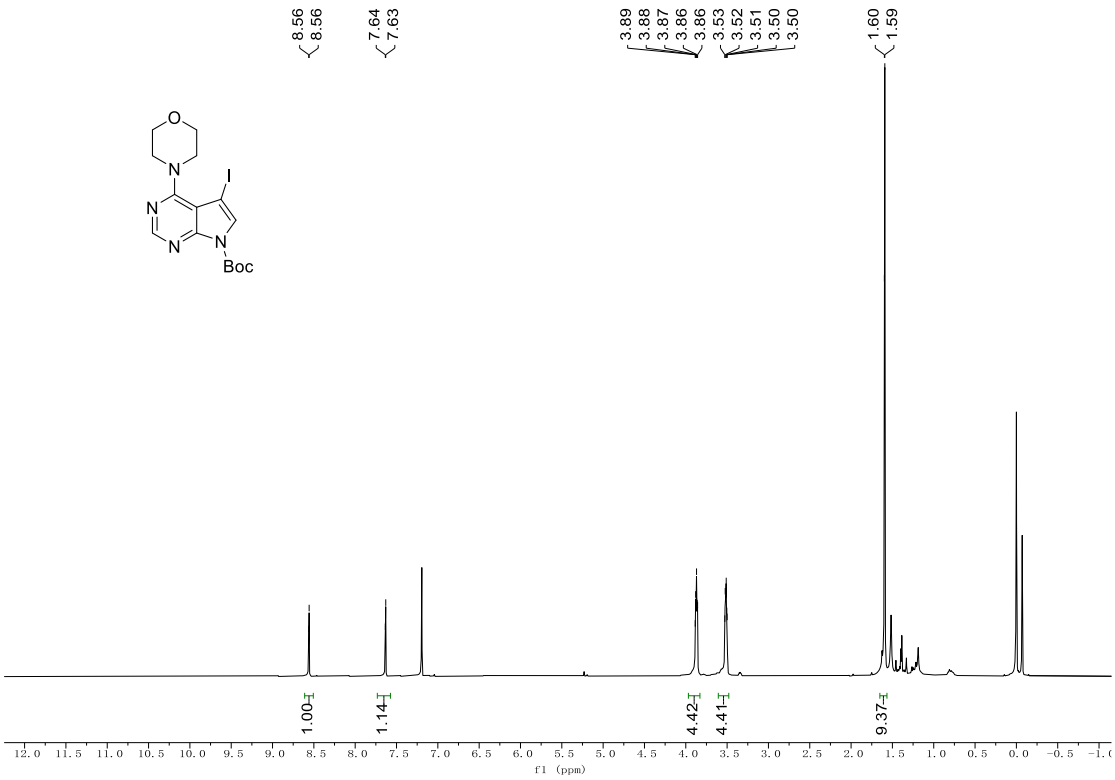


Fig. S30. The ^{1}H-NMR spectrum of compound **B12**

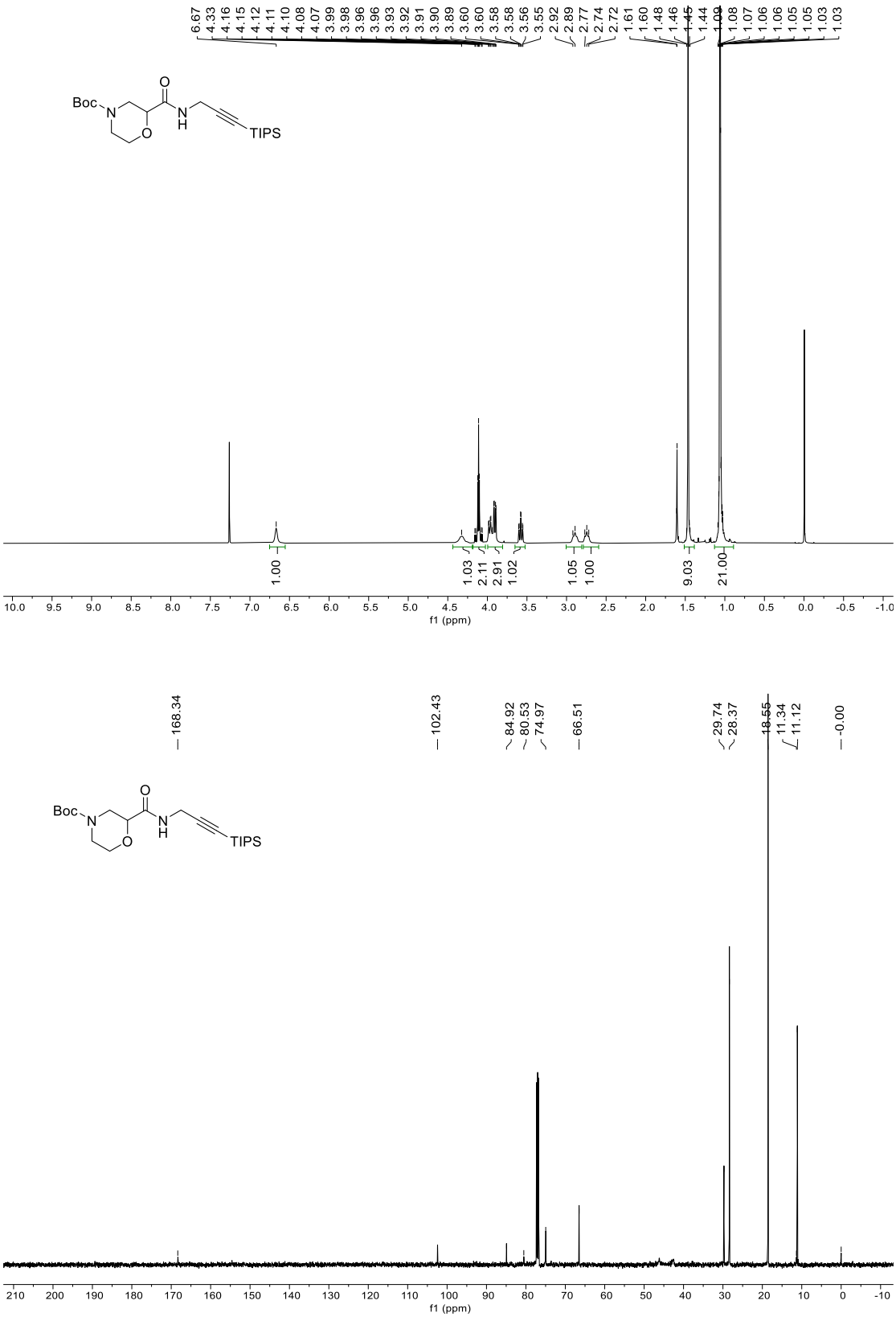


Fig. S31. The ^{1}H-NMR spectrum and ^{13}C-NMR spectrum of compound **B15**

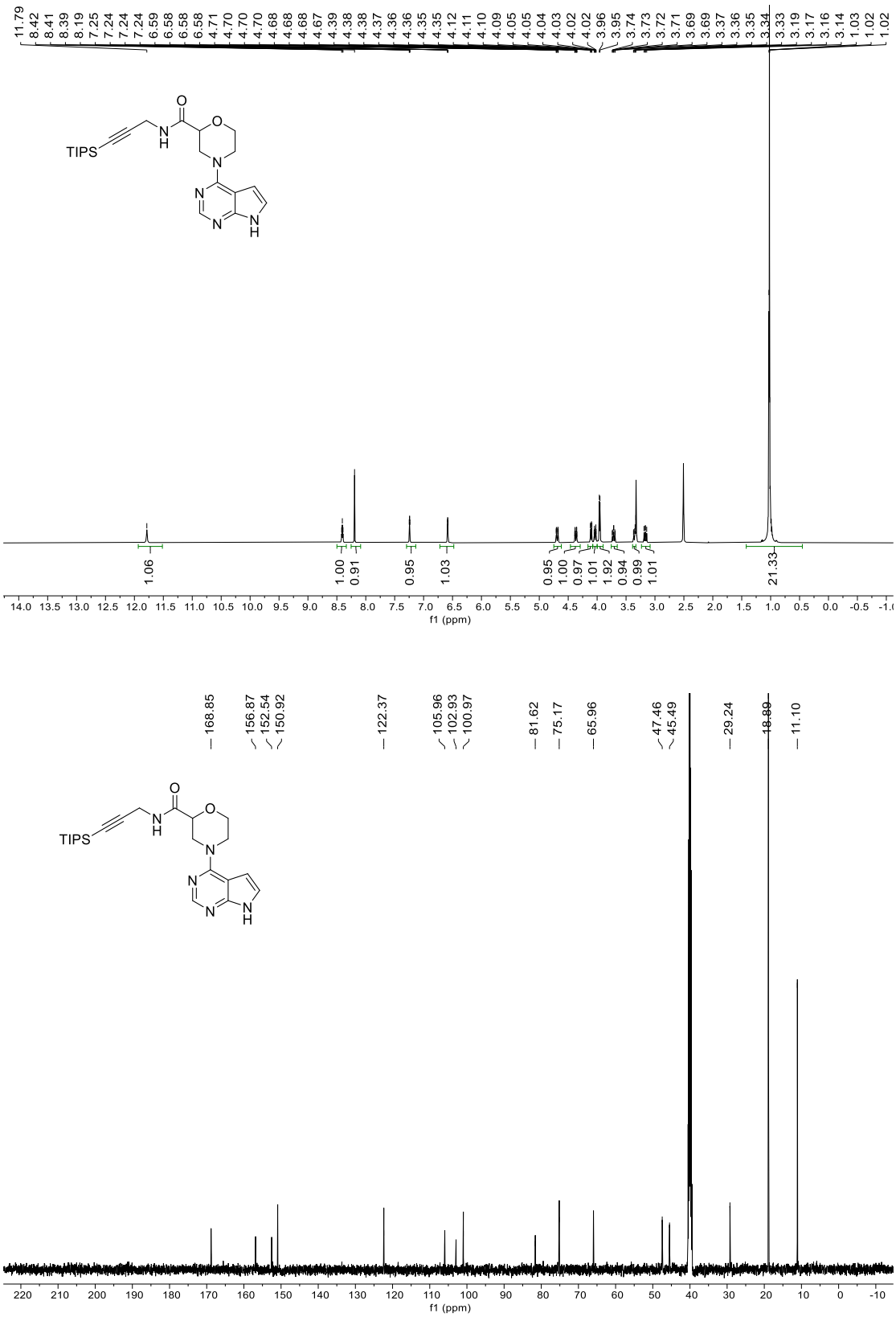


Fig. S32. The ^{1}H-NMR spectrum and ^{13}C-NMR spectrum of compound **B17**

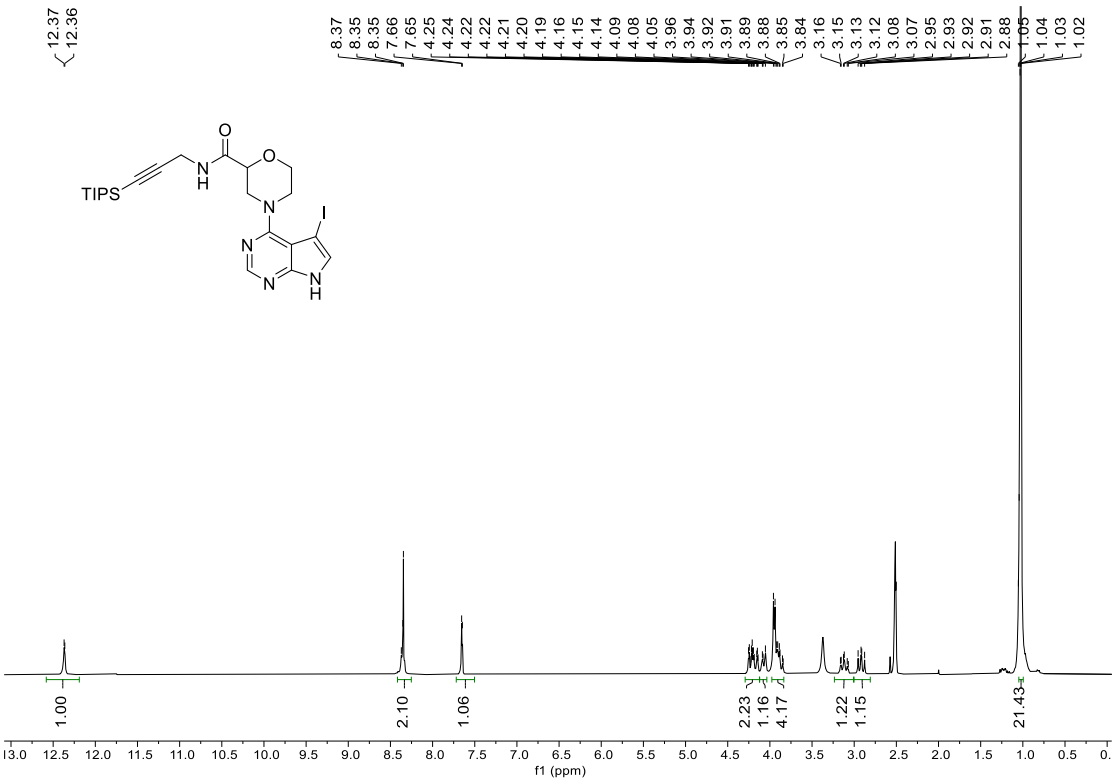


Fig. S33. The ^{1}H-NMR spectrum of compound **B18**

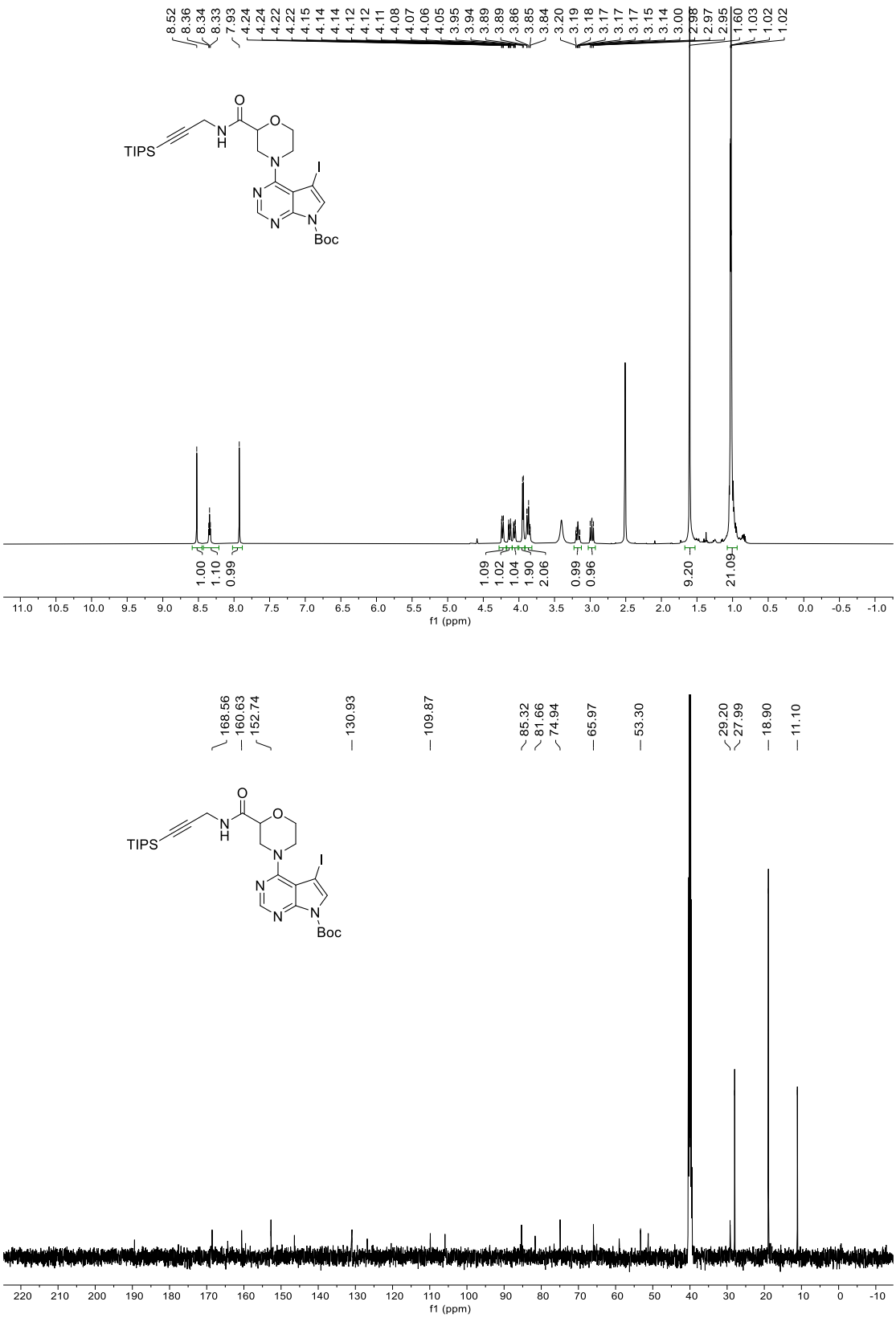


Fig. S34. The ^{1}H-NMR spectrum and ^{13}C-NMR spectrum of compound **B19**.

7. Raw data of electrophoretic gels and blots

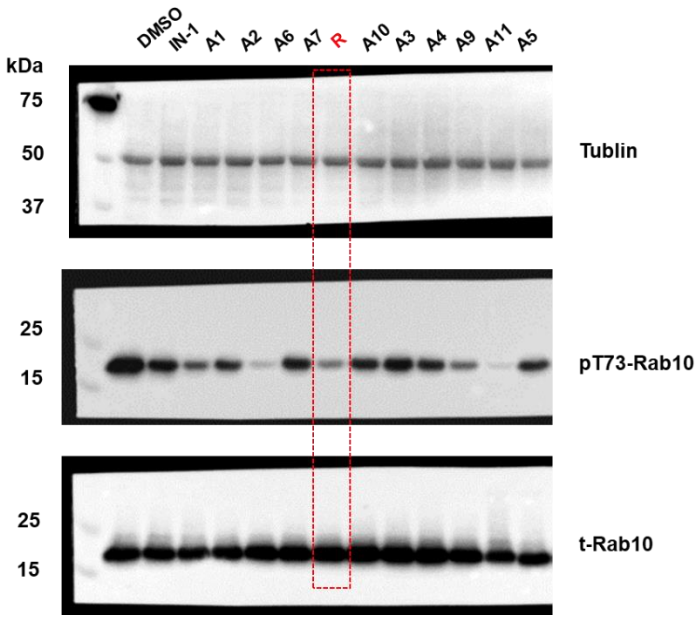


Fig. S35. Raw WB data for **fig. 2A,** cellular inhibition of A1-A11, the maker was Precision Plus Protein™ Dual Color Standards, 1610374, BIO-RAD, **compound R** was not used in this manuscript, all the data of compound R was cut off from the **Fig.2A**

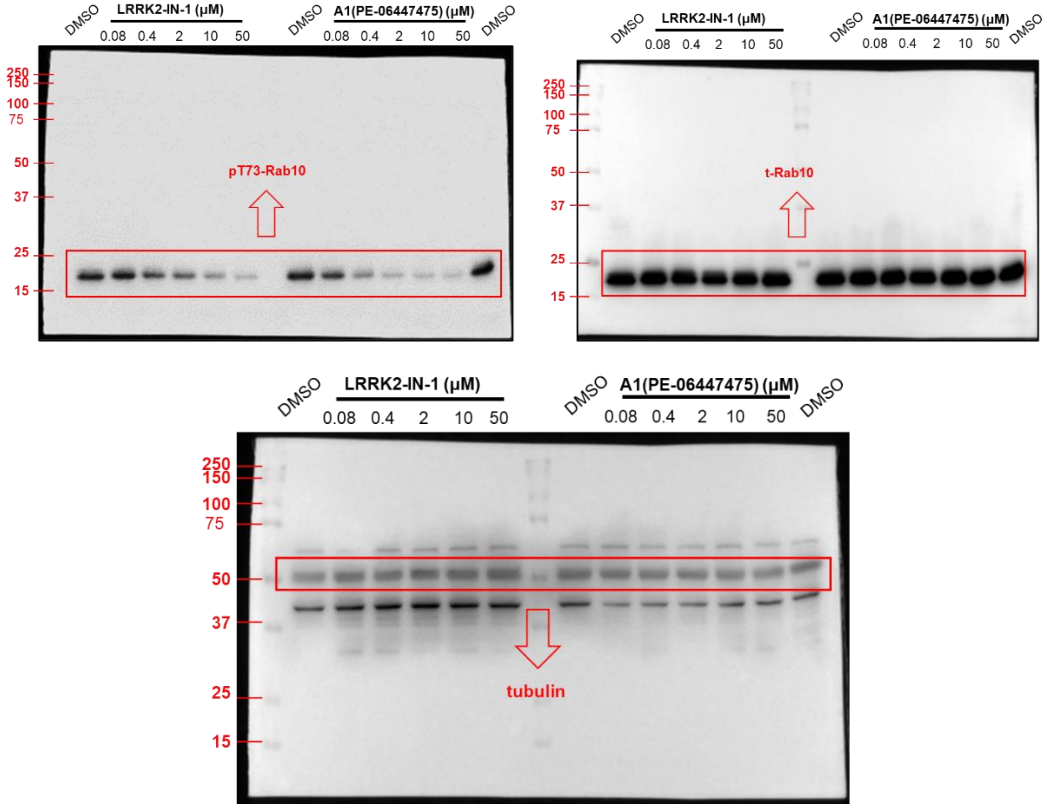


Fig. S36. Raw WB data for cellular inhibition in A549 of compound lrrk2-IN-1 and compound **A1** (PF-06447475) in **fig. S3,** the maker was Precision Plus Protein™ Dual Color Standards, 1610374, BIO-RAD.

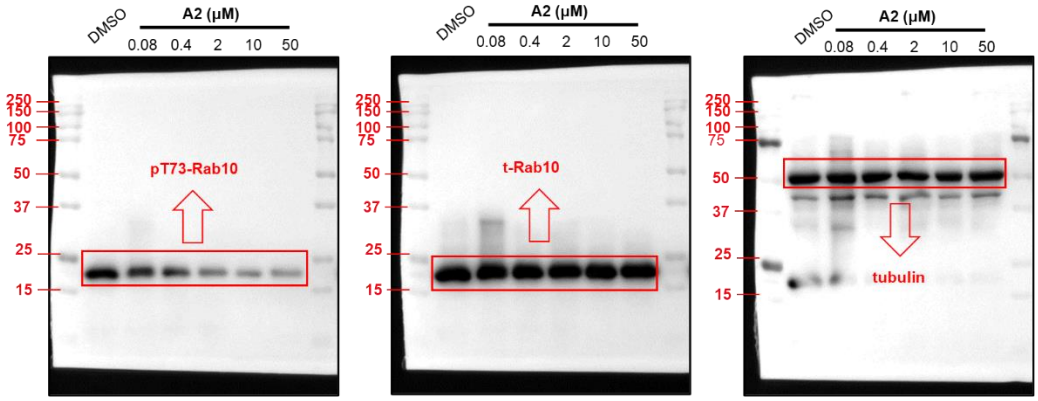


Fig. S37. Raw WB data for cellular inhibition in A549 of compound **A2** in **fig. S3,** the maker was Precision Plus Protein™ Dual Color Standards, 1610374, BIO-RAD.

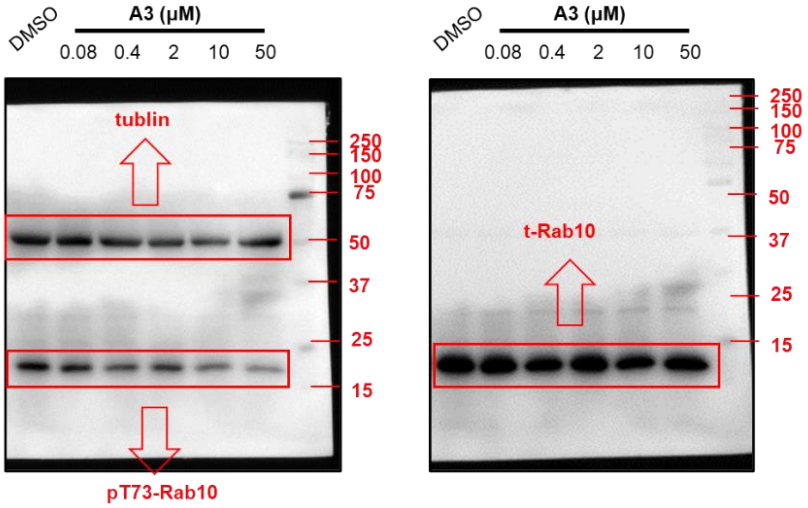


Fig. S38. Raw WB data for cellular inhibition in A549 of compound **A3** in **fig. S3,** the maker was Precision Plus Protein™ Dual Color Standards, 1610374, BIO-RAD.

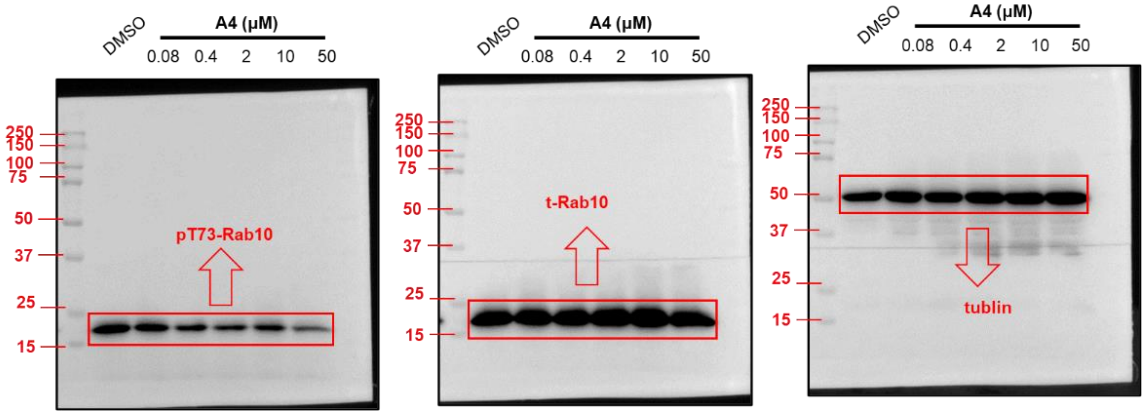


Fig. S39. Raw WB data for cellular inhibition in A549 of compound **A4** in **fig. S3,** the maker was Precision Plus Protein™ Dual Color Standards, 1610374, BIO-RAD.

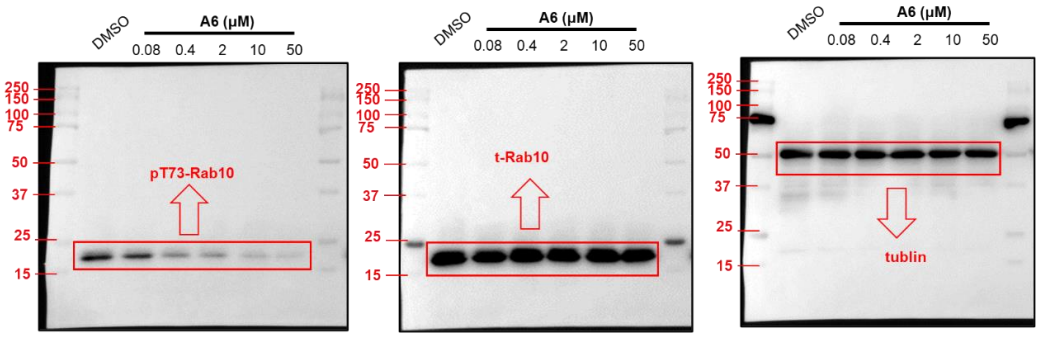


Fig. S40. Raw WB data for cellular inhibition in A549 of compound **A6** in **fig. S3,** the maker was Precision Plus Protein™ Dual Color Standards, 1610374, BIO-RAD.

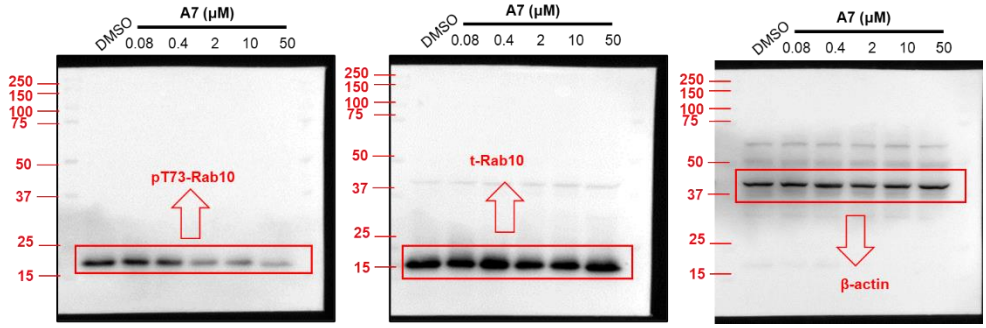


Fig. S41. Raw WB data for cellular inhibition in A549 of compound **A7** in **fig. S3,** the maker was Precision Plus Protein™ Dual Color Standards, 1610374, BIO-RAD.

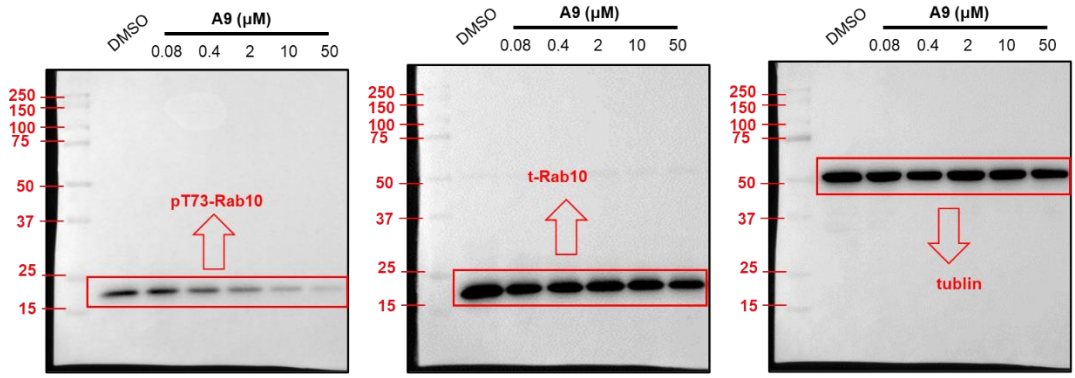


Fig. S42. Raw WB data for cellular inhibition in A549 of compound **A9** in **fig. S3,** the maker was Precision Plus Protein™ Dual Color Standards, 1610374, BIO-RAD.

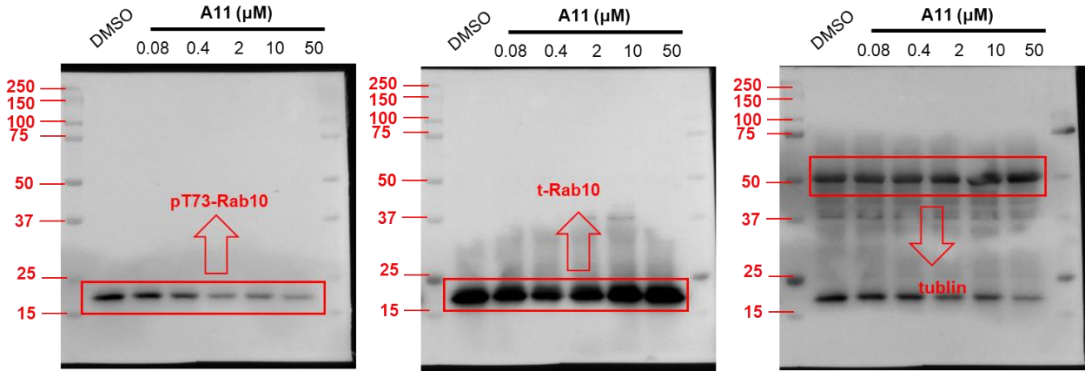


Fig. S43. Raw WB data for cellular inhibition in A549 of compound **A11** in **fig. S3,** the maker was Precision Plus Protein™ Dual Color Standards, 1610374, BIO-RAD.

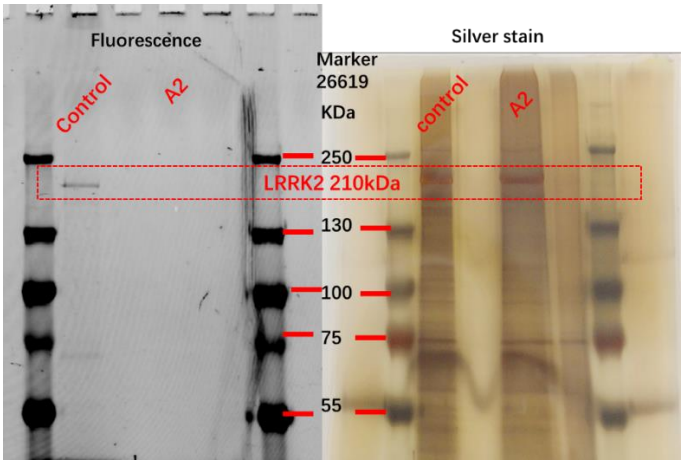


Fig. S44. Raw data for **fig. 2C,** gel-based recombinant LRRK2 labelling with control (Fluorescein-5-EX N-hydroxysuccinimide ester) and **ABPA2**, the maker was PageRuler Plus Prestained Protein Ladder (Thermo Scientific, 26619).

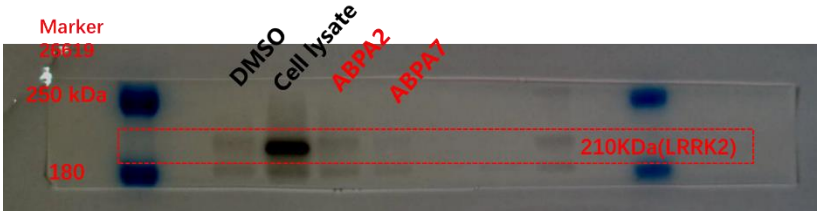


Fig. S45. Raw date for **fig. 2D,** Pull-down/Western blotting experiments against endogenous LRRK2, the maker was PageRuler Plus Prestained Protein Ladder (Thermo Scientific, 26619)

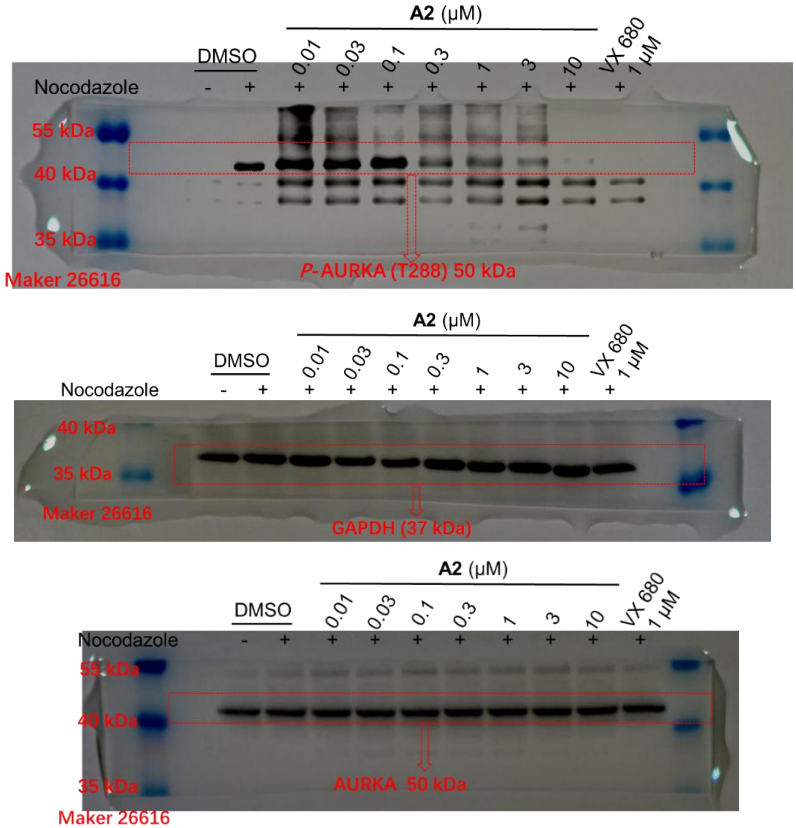


Fig. S46. Raw data for **fig. 4B,** WB analysis of **A2** on inhibition of AURKA autophosphorylation (T288), the maker was PageRuler Plus Prestained Protein Ladder (Thermo Scientific, 26616).

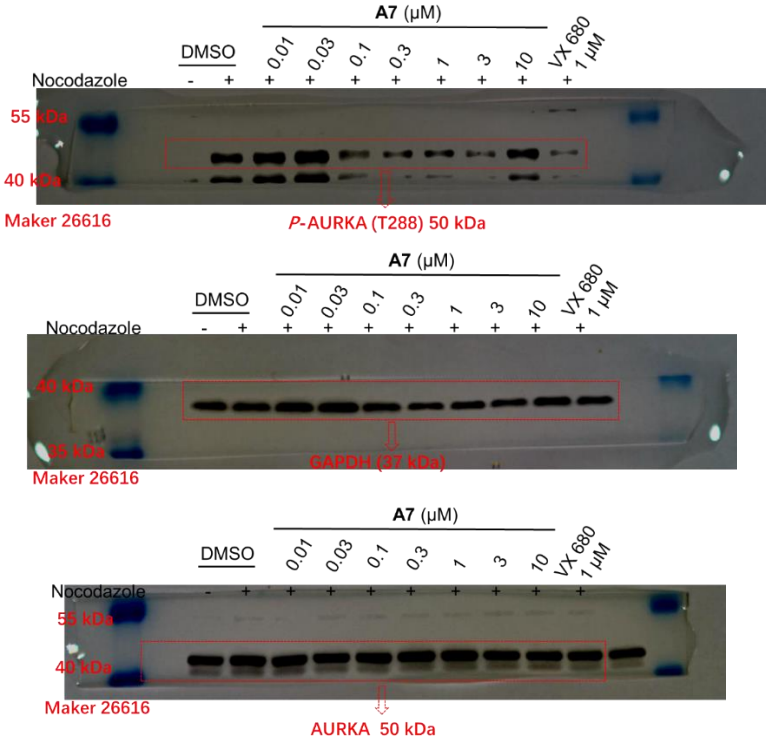


Fig. S47. Raw date for **fig. S8**, WB analysis of **A7** on inhibition of AURKA autophosphorylation (T288), the exposure time for the first gel was 1 min, the exposure time for the second gel was 5 min; the maker was PageRuler Plus Prestained Protein Ladder (Thermo Scientific, 26616).

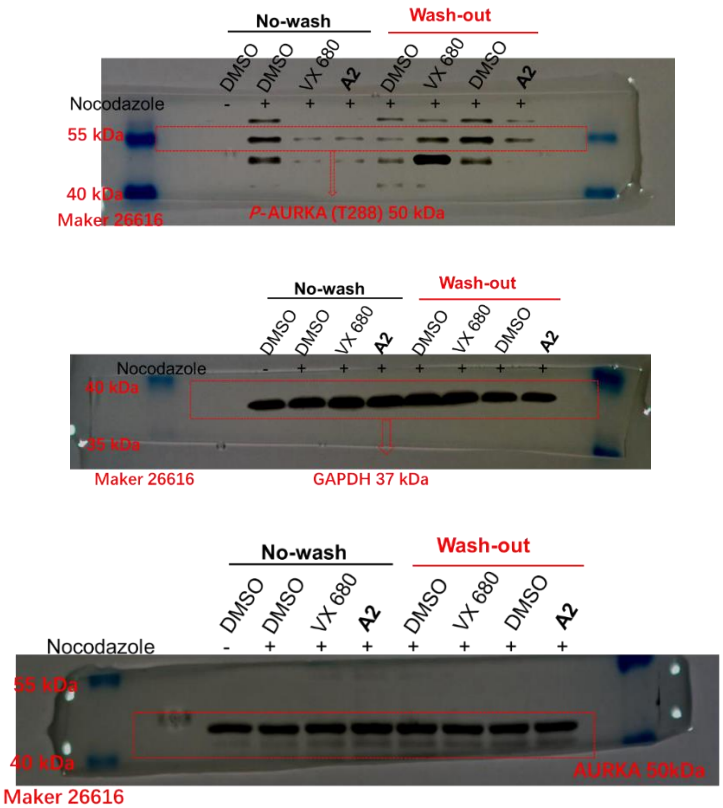


Fig. S48. Raw date for **fig. 4D**, Washout experiments in live K562 cells (**A2**: 20 μM, VX 680: 1 μM), determined by WB of AURKA (T288) autophosphorylation, the maker was PageRuler Plus Prestained Protein Ladder (Thermo Scientific, 26616).

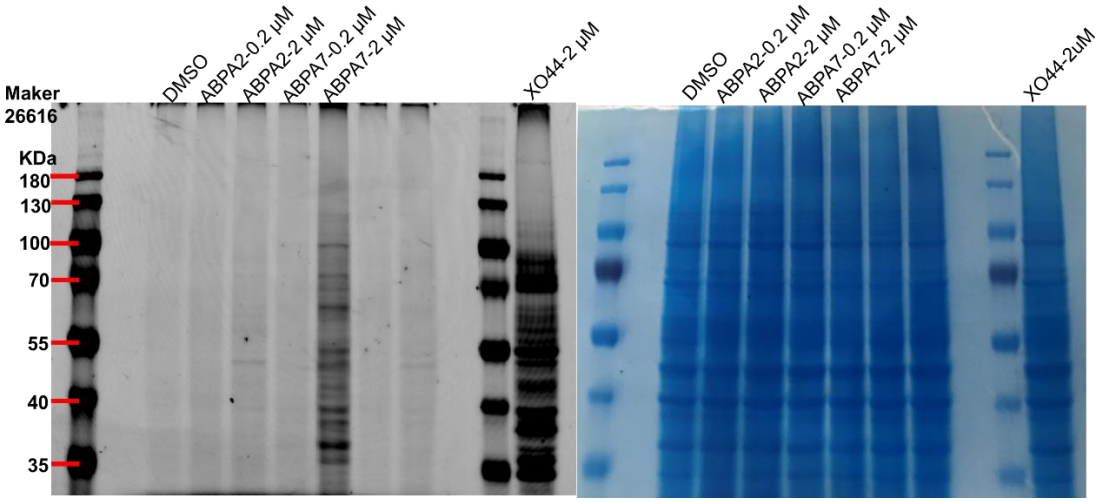


Fig. S49. Raw date for **fig. 4E**, gel-based proteome labelling by **ABPA2** and **ABPA7** (left: fluorescence, right: CBB) the maker was PageRuler Plus Prestained Protein Ladder (Thermo Scientific, 26616).

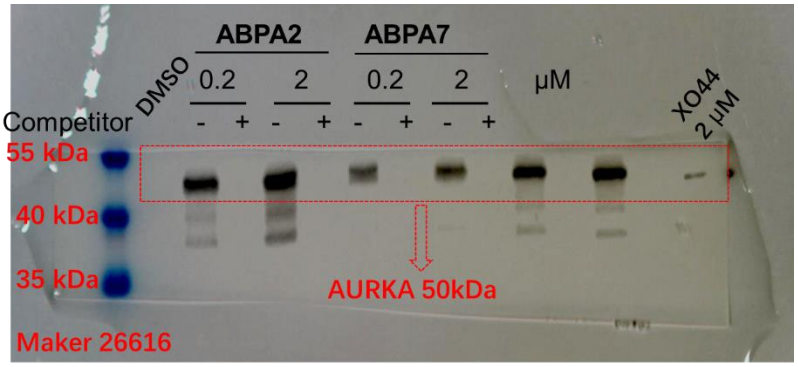
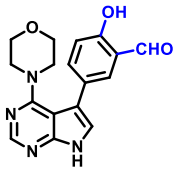
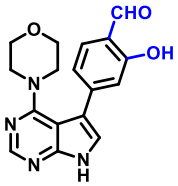
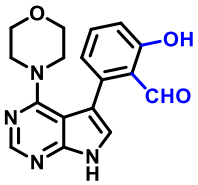
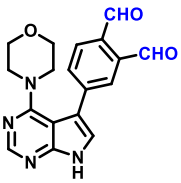
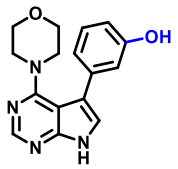
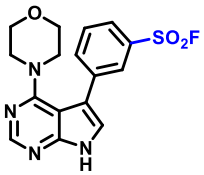
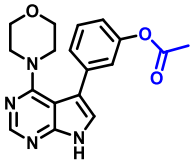
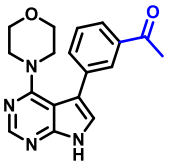
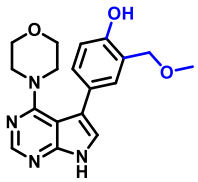
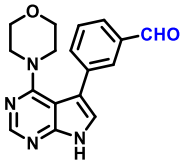
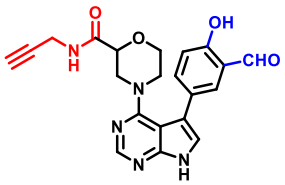
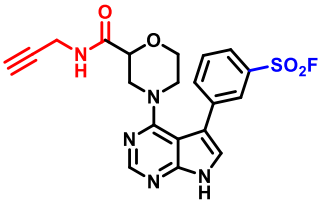


Fig. S50. Raw date for **fig. 4F**, Pull-down/WB experiments against endogenous AURKA, Competitor: **A2** or **A7**, the second gel and the thirthe maker was PageRuler Plus Prestained Protein Ladder (Thermo Scientific, 26616).

8. Original HRMS and summar table.

Table S6. Summary of HRMS (ESI^+) for compound **A2** to compound **A11**

Compound	Chemical Structure	HRMS (ESI^+)
A2		calcd for $C_{17}H_{16}N_4O_3$ ([M+H$^+$]): 325.1301 found:325.1295
A3		calcd for $C_{17}H_{16}N_4O_3$ ([M+H$^+$]): 325.1301 found:325.1300
A4		calcd for $C_{17}H_{16}N_4O_3$ ([M+H$^+$]): 325.1301 found:325.1294
A5		calcd for $C_{18}H_{16}N_4O_3$ ([M+H$^+$]): 337.1301 found:337.1302
A6)		calcd for $C_{16}H_{16}N_4O_2$ ([M+H$^+$]): 297.1352 found:297.1340
A7		calcd for $C_{16}H_{15}FN_4O_3S$ ([M+H$^+$]): 363.0927 found:363.0920
A8		calcd for $C_{18}H_{18}N_4O_3$ ([M+H$^+$]): 339.1457 found: not detected (compoundnot stable)
A9		calcd for $C_{18}H_{18}N_4O_2$ ([M+H$^+$]): 323.1508 found:323.1502

A10		calcd for $C_{18}H_{20}N_4O_3$ ([M+H$^+$]): 341.1614 found: 341.1603
A11		calcd for $C_{17}H_{16}N_4O_2$ ([M+H$^+$]): 309.1352 found:309.1344
ABPA2		calcd for $C_{21}H_{19}N_5O_4$ ([M+H$^+$]): 406.1515 found:406.1511
ABPA7		HRMS (ESI$^+$) calcd for $C_{20}H_{18}FN_5O_4S$ ([M+H$^+$]): 444.1142 found:444.1133

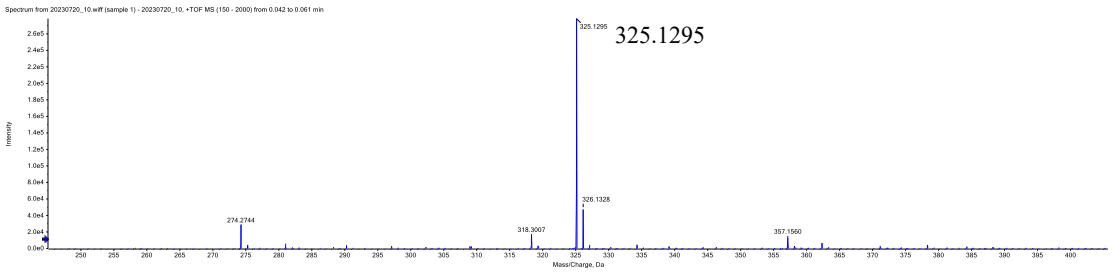


Fig. S51. The HRMS spectrum of compound **A2**

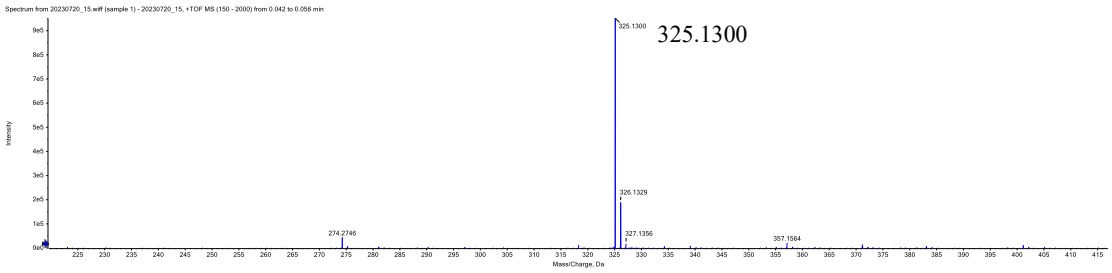


Fig. S52. The HRMS spectrum of compound **A3**

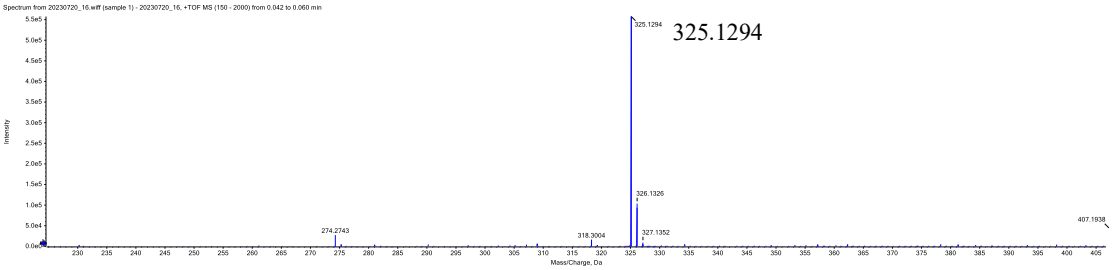


Fig. S53. The HRMS spectrum of compound **A4**

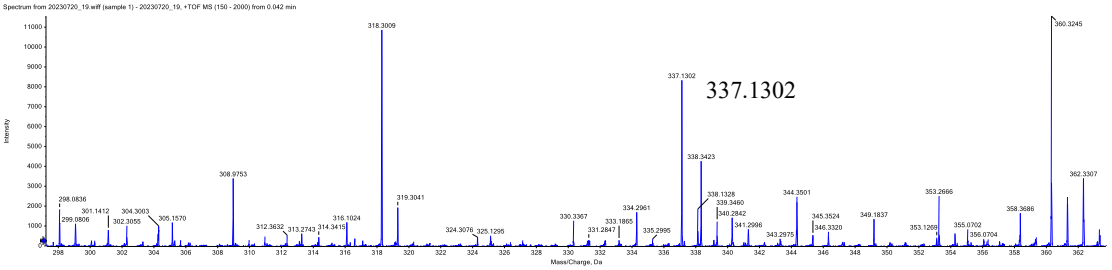


Fig. S54. The HRMS spectrum of compound **A5**

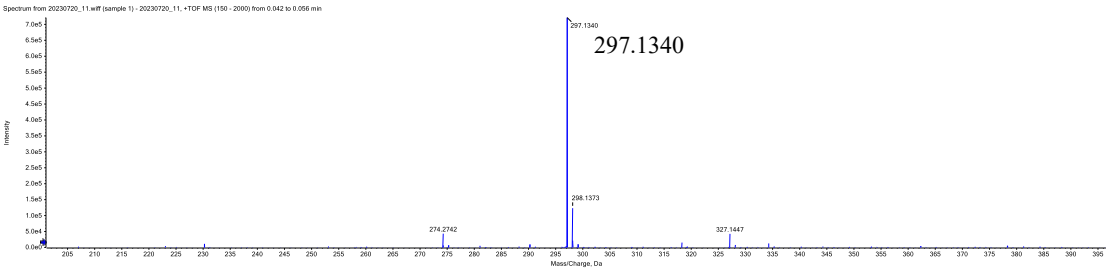


Fig. S55. The HRMS spectrum of compound **A6**

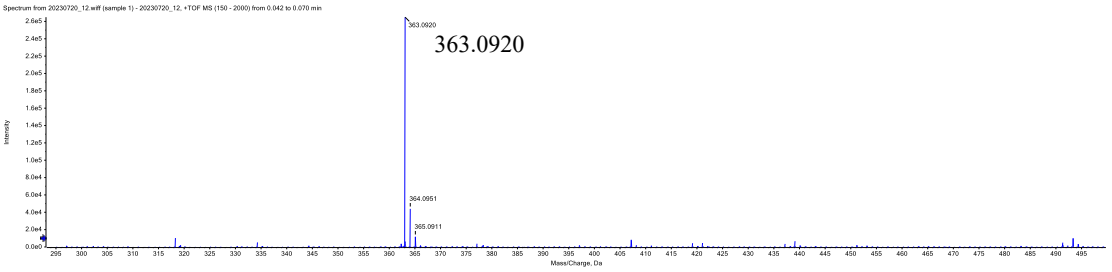


Fig. S56. The HRMS spectrum of compound **A7**

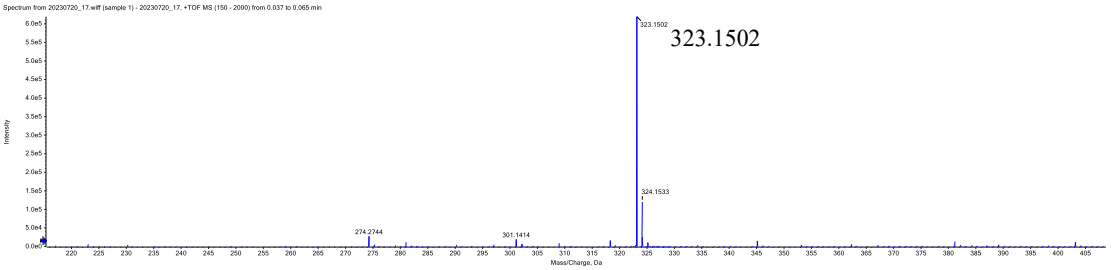


Fig. S57. The HRMS spectrum of compound **A9**

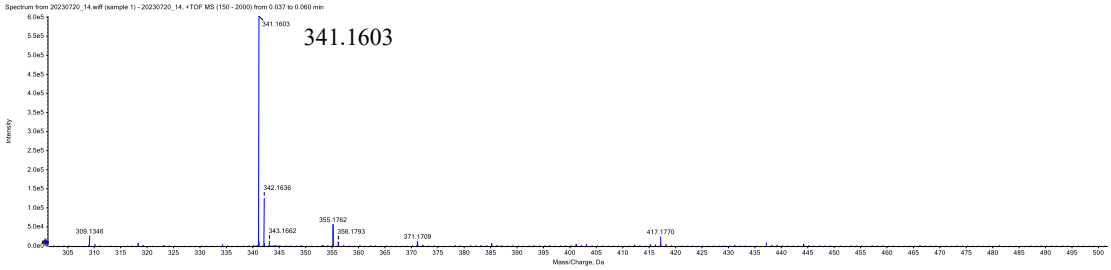


Fig. S58. The HRMS spectrum of compound **A10**

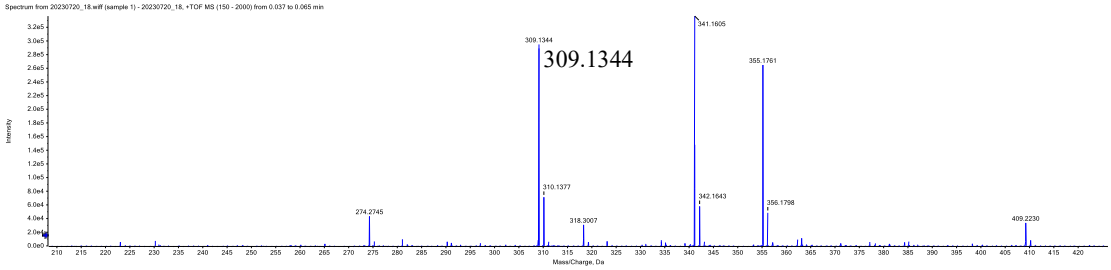


Fig. S59. The HRMS spectrum of compound **A11**

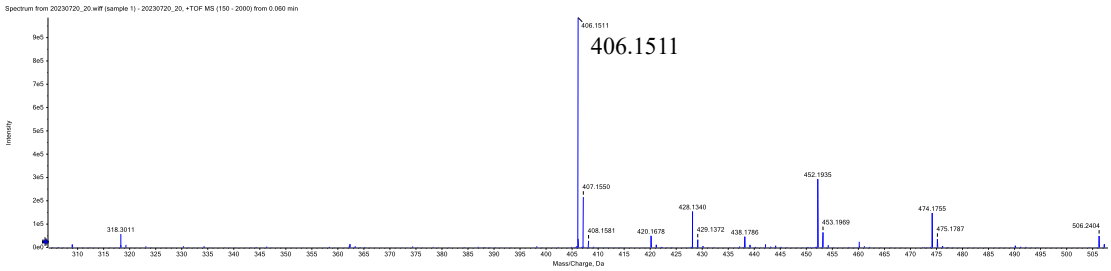


Fig. S60. The HRMS spectrum of compound **ABPA2**

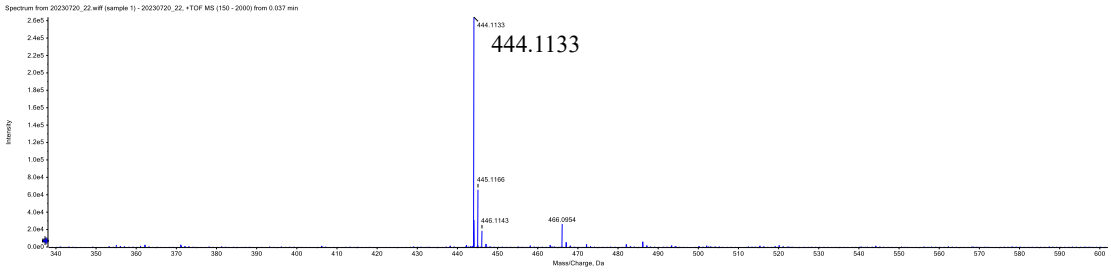


Fig. S61. The HRMS spectrum of compound **ABPA7**