Supplementary Information

Design, synthesis, and evaluation of BTK-targeting PROTACs with optimized bioavailability *in vitro* and *in vivo*

Yonghui Sun[a], Zimo Yang[a], Zhimin Zhang[b], Zhen Li[a], Liubin Guo[b], Hao Pan[b], Xin Luo[a], Dongzhou Liu*[b] and Yu Rao*[a]

[a]*MOE Key Laboratory of Protein Sciences, School of Pharmaceutical Sciences, MOE Key Laboratory of Bioorganic Phosphorus Chemistry & Chemical Biology, Tsinghua University, Beijing 100084, China*

[b]*Global Drug Development Center, Huadong Medicine Co.,Ltd, No. 866 Mogan Mountain Road, Hangzhou 310011, China*

*E-mail addresses: yrao@tsinghua.edu.cn; jefferydliu@eastchinapharm.com

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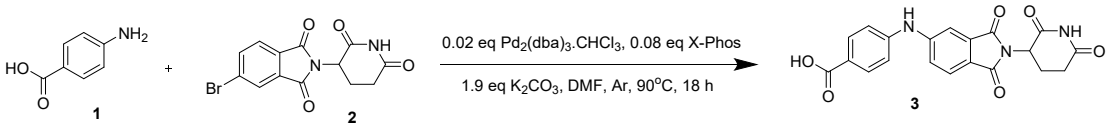
Supplementary information, Data S1

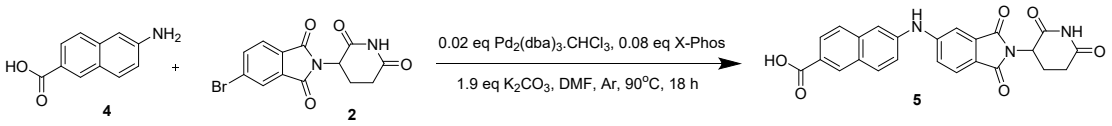
Materials and methods

All commercial materials (Selleck, Alfa Aesar, Aladdin, J&K Chemical LTD, Energy Chemical, Bide Pharmatech LTD and Macklin.) were utilized without further purification. All pomalidomide derivatives, lenalidomide derivatives, ibrutinib derivatives, amino acids were purchased. All solvents were analytical grade. The pomalidomide derivatives were prepared by Crews' procedure. The NMR spectra was generated on a Bruker AVANCE^{III}400 MHz spectrometer in CD_3OD, $CDCl_3$ using solvent peak as standard. ^{13}C-NMR spectra was recorded with complete proton decoupling. Low-resolution mass spectral analyses were performed with an Agilent G6125C or Waters AQUITY UPLC^{TM}/MS. Flash column chromatography was performed on Qingdao Haiyang Chemical Co. Ltd silica gel 60 (200-300 mesh). Analytical TLC was performed on Yantai Chemical Industry Research Institute silica gel 60 F254 plates and the rotavapor was BUCHI's Rotavapor R-3.

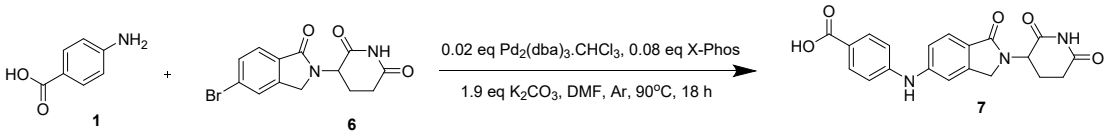
Synthesis of BTK degrader derivatives



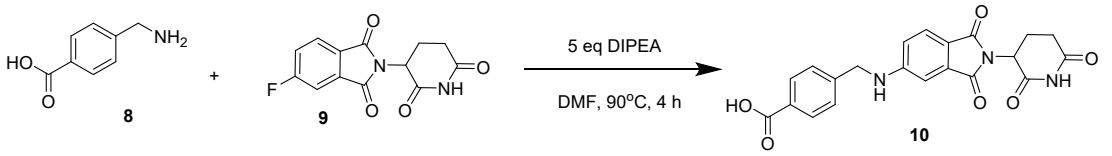
4-aminobenzoic acid (70 mg), [Pd] (0.02 eq), X-Phos (0.08 eq), K_2CO_3 powder (1.9 eq) were added to compound **2** (1.0 eq) solution in DMF (2 ml) and the reaction mixture was stirred at 90^{o}C for 18 h under Ar. The reaction was quenched by saturated aqueous NaCl and the mixture was neutralized by HCl to pH= 1~2, extracted by EtOAc. The organic layer was dried over anhydrous Na_2SO_4, and evaporated in vacuum to afford the crude product, which was further purified by silica gel column chromatography (PE: EA = 2: 1~1: 2 gradient elution) to give the intermediate **3**, the isolated yields roughly equal to 6-20%.



6-amino-2-naphthoic acid (96 mg), [Pd] (0.02 eq), X-Phos (0.08 eq), K_2CO_3 powder (1.9 eq) were added to compound **2** (1.0 eq) solution in DMF (2 ml) and the reaction mixture was stirred at 90°C for 18 h under Ar. The reaction was quenched by saturated aqueous NaCl and the mixture was neutralized by HCl to pH= 1~2, extracted by EtOAc. The organic layer was dried over anhydrous Na_2SO_4, and evaporated in vacuum to afford the crude product, which was further purified by silica gel column chromatography (PE: EA = 2: 1~1: 2 gradient elution) to give the intermediate **5**, the isolated yields roughly equal to 15%.

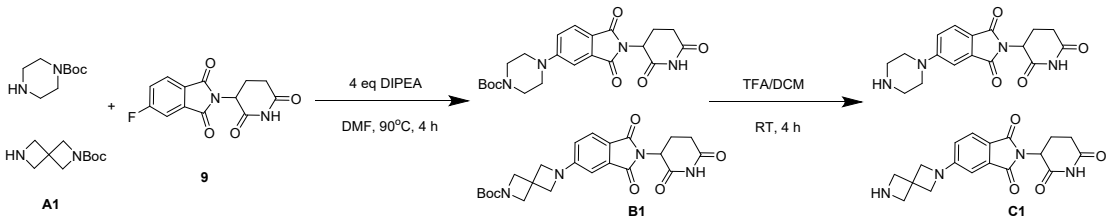


4-aminobenzoic acid (70 mg), [Pd] (0.02 eq), X-Phos (0.08 eq), K_2CO_3 powder (1.9 eq) were added to compound **6** (1.0 eq) solution in DMF (2 ml) and the reaction mixture was stirred at 90°C for 18 h and then 110°C for 5 h under Ar. The reaction was quenched by saturated aqueous NaCl and the mixture was neutralized by HCl to pH= 1~2, extracted by EtOAc. The organic layer was dried over anhydrous Na_2SO_4, and evaporated in vacuum to afford the crude product, which was further purified by silica gel column chromatography (PE: EA = 2: 1~1: 2 gradient elution) to give the intermediate **7**, the isolated yields roughly equal to 33%.

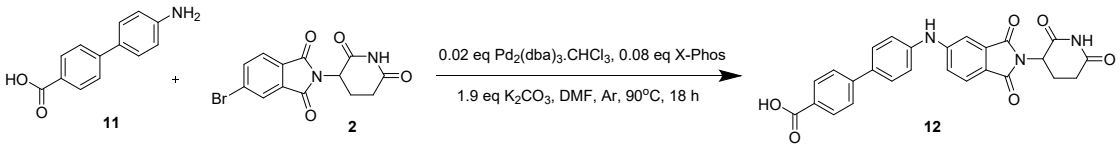


2-(2,6-dioxopiperidin-3-yl)-5-fluoroisoindoline-1,3-dione (276 mg), compound **8** (1.0 eq), DIPEA in dry DMF (5 mL) was stirred in a round bottom flask for 4 h at 90°C. The reaction was quenched by saturated aqueous NaCl and the mixture was neutralized by HCl to pH= 1~2, extracted by EtOAc. The organic layer was dried over anhydrous Na_2SO_4, and evaporated in vacuum to afford the crude product, which was further purified by silica gel column chromatography (DCM: MeOH = 30: 1~20: 1

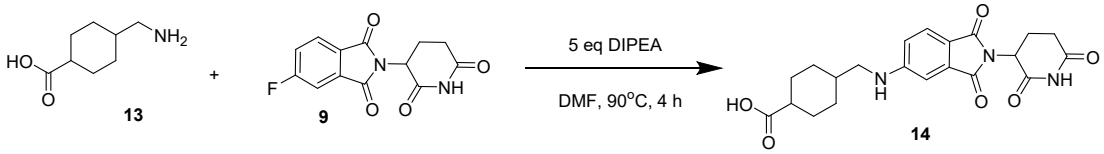
gradient elution) to give the intermediate **10**, the isolated yields roughly equal to 10%.



2-(2,6-dioxopiperidin-3-yl)-5-fluoroisoindoline-1,3-dione (168 mg), intermediate **A1** (1.0 eq), DIPEA in dry DMF (4 mL) was stirred in a round bottom flask for 4 h at 90°C. The reaction was quenched by water and the mixture was washed once with saturated aqueous NaCl, extracted by EtOAc. The organic layer was dried over anhydrous Na_2SO_4, and evaporated in vacuum to afford the crude product, which was further purified by silica gel column chromatography (PE: EA = 2: 1~1: 2 gradient elution) to give one of the intermediate **B1**, the isolated yields roughly equal to 40-60%. Intermediate **B1** was dissolved in DCM and TFA (6: 1) system. The reaction was stirred at room temperature for 4 hours. The liquid was removed by rotary evaporator to give the product intermediate **C1**.

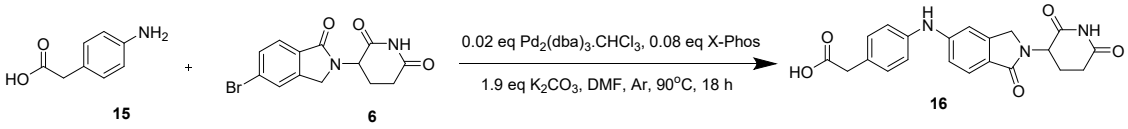


4'-amino-[1,1'-biphenyl]-4-carboxylic acid (20 mg), [Pd] (0.02 eq), X-Phos (0.08 eq), K_2CO_3 powder (1.9 eq) were added to compound **2** (1.0 eq) solution in DMF (2 ml) and the reaction mixture was stirred at 90°C for 18 h under Ar. The reaction was quenched by saturated aqueous NaCl and the mixture was neutralized by HCl to pH= 1~2, extracted by EtOAc. The organic layer was dried over anhydrous Na_2SO_4, and evaporated in vacuum to afford the crude product, which was further purified by silica gel column chromatography (PE: EA = 2: 1~1: 2 gradient elution) to give the intermediate **12**, the isolated yields roughly equal to 20%.



2-(2,6-dioxopiperidin-3-yl)-5-fluoroisoindoline-1,3-dione (1 eq), compound **13** (1 eq),

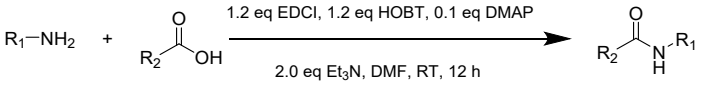
DIPEA (5 eq) in dry DMF was stirred in a round bottom flask for 4 h at 90°C. The reaction was quenched by saturated aqueous NaCl and the mixture was neutralized by HCl to pH= 1~2, extracted by EtOAc. The organic layer was dried over anhydrous Na_2SO_4, and evaporated in vacuum to afford the crude product, which was further purified by silica gel column chromatography (DCM: MeOH = 30: 1~20: 1 gradient elution) to give the intermediate **14**, the isolated yields roughly equal to 10%.



15 6 16

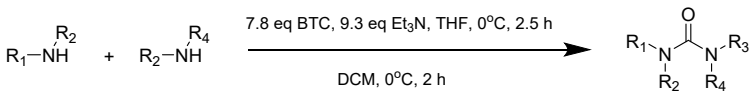
2-(4-aminophenyl)acetic acid (155 mg), [Pd] (0.02 eq), X-Phos (0.08 eq), K_2CO_3 powder (1.9 eq) were added to compound **6** (1.0 eq) solution in DMF (4 ml) and the reaction mixture was stirred at 100°C for 5 h under Ar. The reaction was quenched by saturated aqueous NaCl and the mixture was neutralized by HCl to pH= 1~2, extracted by EtOAc. The organic layer was dried over anhydrous Na_2SO_4, and evaporated in vacuum to afford the crude product, which was further purified by silica gel column chromatography (DCM: MeOH = 30: 1~20: 1 gradient elution) to give the intermediate **16**, the isolated yields roughly equal to 60%.

I General procedure for amide derivatives preparation

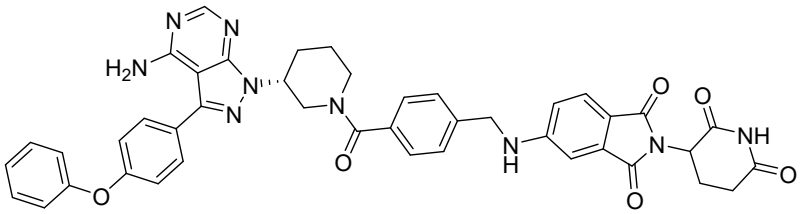


A mixture of carboxylic acid derivative (1 eq), EDCI (1.2 eq), HOBT (1.2 eq), DMAP (0.1 eq), in DMF was stirred in a round bottom flask for 2 min. Then Et_3N (2 eq) was added. The mixture was stirred at room temperature for 10 min. Then amine derivative (1 eq) was added. The mixture was stirred at room temperature for 12 h. The reaction was quenched by saturated aqueous NaCl and the mixture was extracted by EtOAc. The organic layer was dried over anhydrous Na_2SO_4, and evaporated in vacuum to afford the crude product, which was further purified by silica gel column chromatography (DCM: MeOH = 30: 1~20: 1 gradient elution) to give one of the amide derivatives.

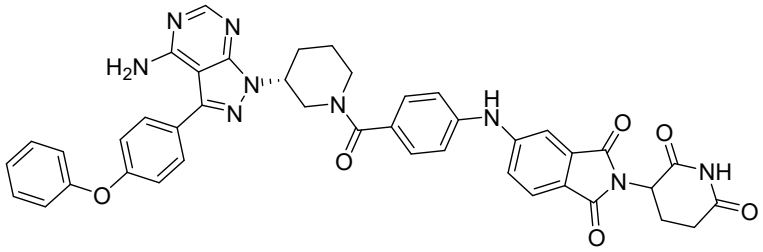
II General procedure for carbamide derivatives preparation



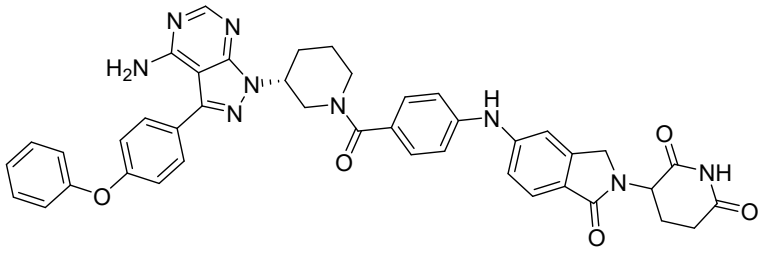
A mixture of amine derivative 1 (1 eq) in THF (dry) was added BTC (7.8 eq), Et_3N (9.3 eq) in a round bottom flask at 0°C. The mixture was stirred at 0°C for 2.5 h. Solvent was removed by rotary evaporator. DCM was added to the mixture. Amine derivative 2 (1 eq) in DCM was added to the mixture dropwise at 0°C. The mixture was stirred at 0°C for 2 h. The reaction was quenched by water and the mixture was washed once with saturated aqueous $NaHCO_3$, extracted by DCM. The organic layer was dried over anhydrous Na_2SO_4, and evaporated in vacuum to afford the crude product, which was further purified by silica gel column chromatography (DCM: MeOH = 30: 1~20: 1 gradient elution) to give one of the carbamide derivatives.



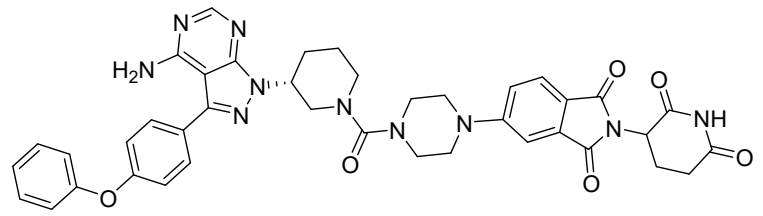
5-((4-((R)-3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidine-1-carbonyl)benzyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (15-271). ^{1}H-NMR (400 MHz, $CDCl_3/CD_3OD$=3:1, ppm): 8.36-8.14 (m, 1H), 7.72-7.49 (m, 3H), 7.49-7.24 (m, 6H), 7.23-7.13 (m, 3H), 7.09 (d, *J* = 8.0Hz, 2H), 6.99 (s, 1H), 6.89-6.67 (m, 1H), 5.03-4.77 (m, 2H), 4.56-4.38 (m, 2H), 3.89-3.71 (m, 3H), 3.22 (t, *J* = 11.9Hz, 1H), 2.92-2.66 (m, 3H), 2.49-1.95 (m, 5H); ^{13}C-NMR (100 MHz, $CDCl_3/CD_3OD$=3:1, ppm): 173.0, 171.3, 169.8, 168.6, 168.2, 159.0, 158.4, 156.5, 155.6, 154.3, 154.1, 144.8, 140.5, 134.8, 130.6, 130.2, 127.8, 127.7, 127.4, 125.8, 124.4, 119.8, 119.3, 118.3, 116.5, 106.8, 98.7, 53.7, 47.0, 32.1, 31.6, 31.5, 30.4, 29.9, 29.6, 23.0, 22.9, 14.2; LC-MS: calculated for $C_{43}H_{37}N_9O_6$ $[M+H]^+$, 776.3; found, 776.4.



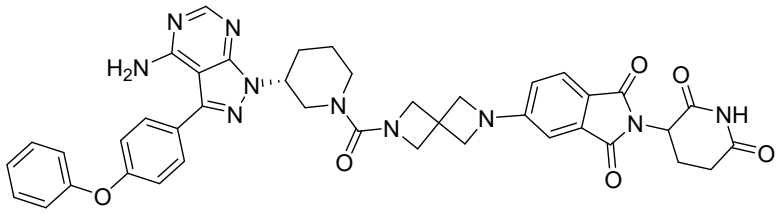
5-((4-((R)-3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidine-1-carbonyl)phenyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (15-59). ^{1}H-NMR (400 MHz, $CDCl_3/CD_3OD$=3:1, ppm): 8.40-8.18 (m, 1H), 7.76-7.60 (m, 3H), 7.53 (s, 1H), 7.50-7.34 (m, 5H), 7.34-7.13 (m, 5H), 7.10 (d, *J* = 8.1Hz, 2H), 5.08-4.73 (m, 2H), 4.02-3.82 (m, 3H), 3.34-3.25 (m, 1H), 2.91-2.70 (m, 2H), 2.55-1.92 (m, 6H); LC-MS: calculated for $C_{42}H_{35}N_9O_6$ $[M+H]^+$, 762.27; found, 762.66.



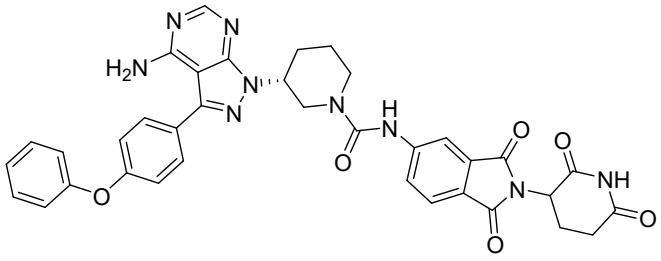
3-(5-((4-((R)-3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidine-1-carbonyl)phenyl)amino)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (15-81). ^{1}H-NMR (400 MHz, $CDCl_3/CD_3OD$=3:1, ppm): 8.29 (s, 1H), 7.75-7.58 (m, 3H), 7.50 (s, 1H), 7.48-7.29 (m, 4H), 7.29-7.13 (m, 6H), 7.10 (d, *J* = 7.7Hz, 2H), 5.20-4.83 (m, 3H), 4.23-3.91 (m, 4H), 3.68-3.58 (m, 1H), 2.94-2.79 (m, 1H), 2.50-1.98 (m, 7H); LC-MS: calculated for $C_{42}H_{37}N_9O_5$ $[M+H]^+$, 748.29; found, 748.69.



5-(4-((R)-3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidine-1-carbonyl)piperazin-1-yl)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (15-83-1). ^{1}H-NMR (400 MHz, $CDCl_3/CD_3OD$=3:1, ppm): 8.32 (s, 1H), 7.71 (d, *J* = 8.5Hz, 1H), 7.65 (d, *J* = 8.6Hz, 2H), 7.44-7.38 (m, 2H), 7.30 (d, *J* = 2.2Hz, 1H), 7.23-7.14 (m, 3H), 7.09 (d, *J* = 7.6Hz, 3H), 5.03-4.86 (m, 2H), 3.99-3.75 (m, 2H), 3.53-3.41 (m, 8H), 3.00 (t, *J* = 11.9Hz, 1H), 2.90-2.73 (m, 3H), 2.42-1.92 (m, 5H), 1.90-1.72 (m, 1H); LC-MS: calculated for $C_{40}H_{38}N_{10}O_6$ $[M+H]^+$, 755.30; found, 755.72.

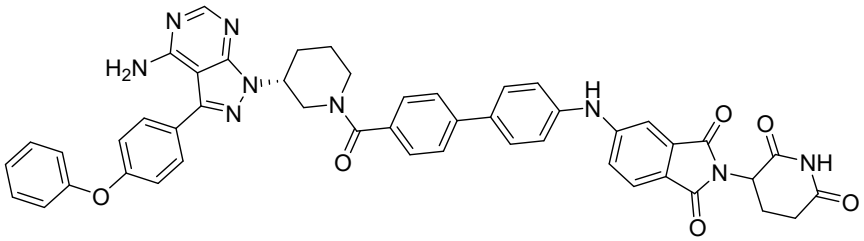


5-(6-((R)-3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidine-1-carbonyl)-2,6-diazaspiro[3.3]heptan-2-yl)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (15-83-2). ^{1}H-NMR (400 MHz, $CDCl_3/CD_3OD$=3:1, ppm): 8.34-8.25 (m, 1H), 7.73-7.56 (m, 3H), 7.47 (s, 2H), 7.44-7.34 (m, 2H), 7.24-7.05 (m, 5H), 5.01-4.78 (m, 2H), 4.24 (s, 2H), 4.17 (s, 2H), 4.09-3.85 (m, 2H), 3.36 (s, 4H), 3.10-2.66 (m, 4H), 2.45-1.89 (m, 6H); LC-MS: calculated for $C_{41}H_{38}N_{10}O_6$ $[M+H]^+$, 767.30; found, 767.69.

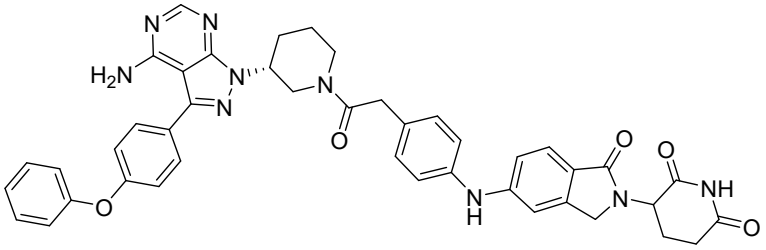


(3R)-3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)piperidine-1-carboxamide (15-155). ^{1}H-NMR (400 MHz, $CDCl_3/CD_3OD$=3:1, ppm): 8.33 (s, 1H), 7.89 (d, *J* = 1.2Hz, 1H), 7.71 (s, 1H), 7.64 (d, *J* = 8.6Hz, 2H), 7.45-7.36 (m, 2H), 7.23-7.06 (m, 5H),

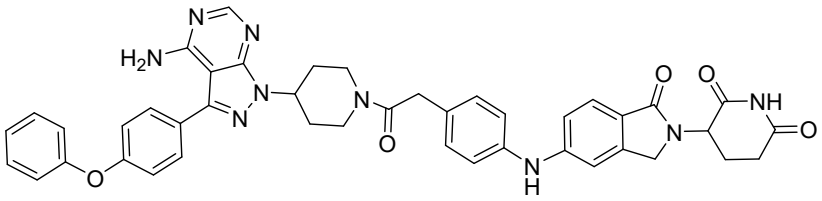
5.03-4.83 (m, 2H), 4.29-4.09 (m, 2H), 3.82 (s, 1H), 3.67-3.55 (m, 1H), 3.19-3.07 (m, 1H), 2.91-2.71 (m, 3H), 2.57-2.43 (m, 1H), 2.32-1.97 (m, 4H); LC-MS: calculated for $C_{36}H_{31}N_9O_6$ $[M+H]^+$, 686.24; found, 686.62.



5-((4'-((R)-3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidine-1-carbonyl)-[1,1'-biphenyl]-4-yl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (15-177). ^{1}H-NMR (400 MHz, $CDCl_3/CD_3OD$=3:1, ppm): 8.39-8.18 (m, 1H), 7.78-7.46 (m, 11H), 7.41 (t, *J* = 7.8Hz, 2H), 7.37-7.24 (m, 2H), 7.24-7.14 (m, 3H), 7.10 (d, *J* = 7.7Hz, 2H), 5.04-4.73 (m, 2H), 3.97-3.62 (m, 2H), 3.28-3.14 (m, 1H), 2.94-2.71 (m, 2H), 2.49-1.95 (m, 7H); LC-MS: calculated for $C_{48}H_{39}N_9O_6$ $[M+H]^+$, 838.30; found, 838.79.

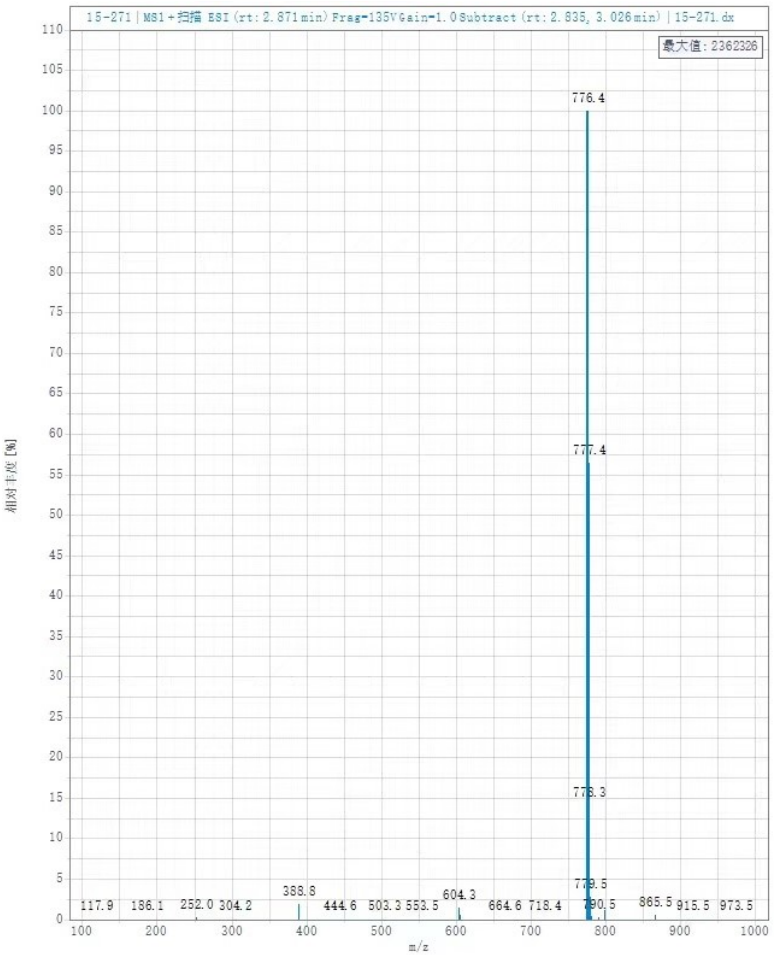


3-(5-((4-(2-((R)-3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)-2-oxoethyl)phenyl)amino)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (15-293). ^{1}H-NMR (400 MHz, $CDCl_3/CD_3OD$=3:1, ppm): 8.35-8.23 (m, 1H), 7.73-7.58 (m, 3H), 7.46-7.35 (m, 2H), 7.27-6.98 (m, 11H), 5.11 (dt, *J* = 13.1Hz, *J* = 4.1Hz, 1H), 4.86-4.46 (m, 2H), 4.44-4.27 (m, 2H), 4.19-3.95 (m, 1H), 3.91-3.32 (m, 4H), 3.26-2.81 (m, 3H), 2.45-2.13 (m, 4H), 2.09-1.88 (m, 1H); LC-MS: calculated for $C_{43}H_{39}N_9O_5$ $[M+H]^+$, 762.31; found, 762.09.

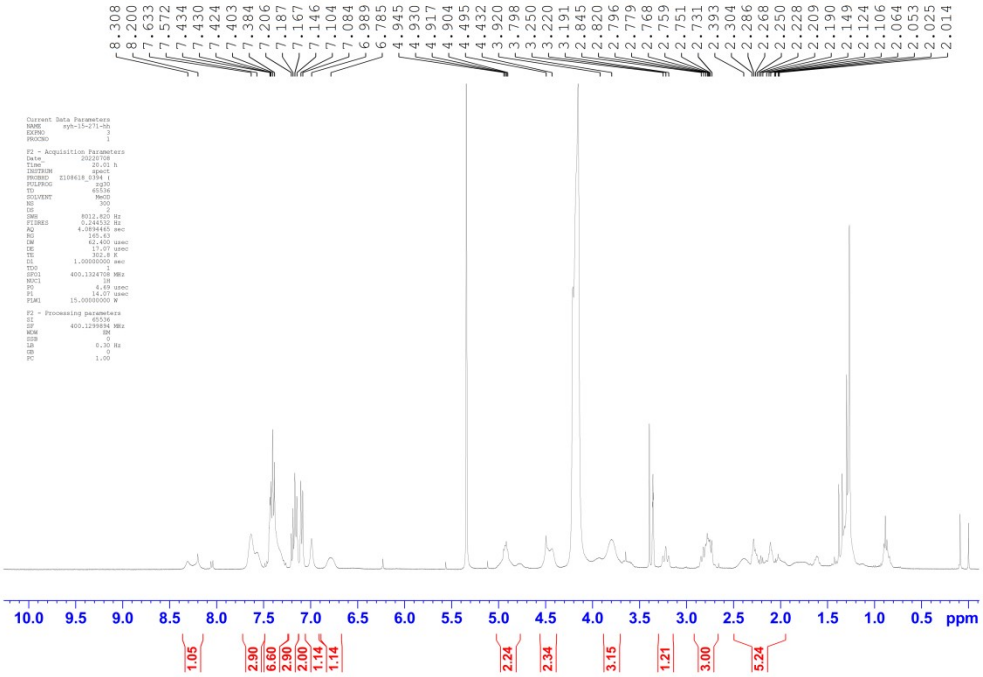


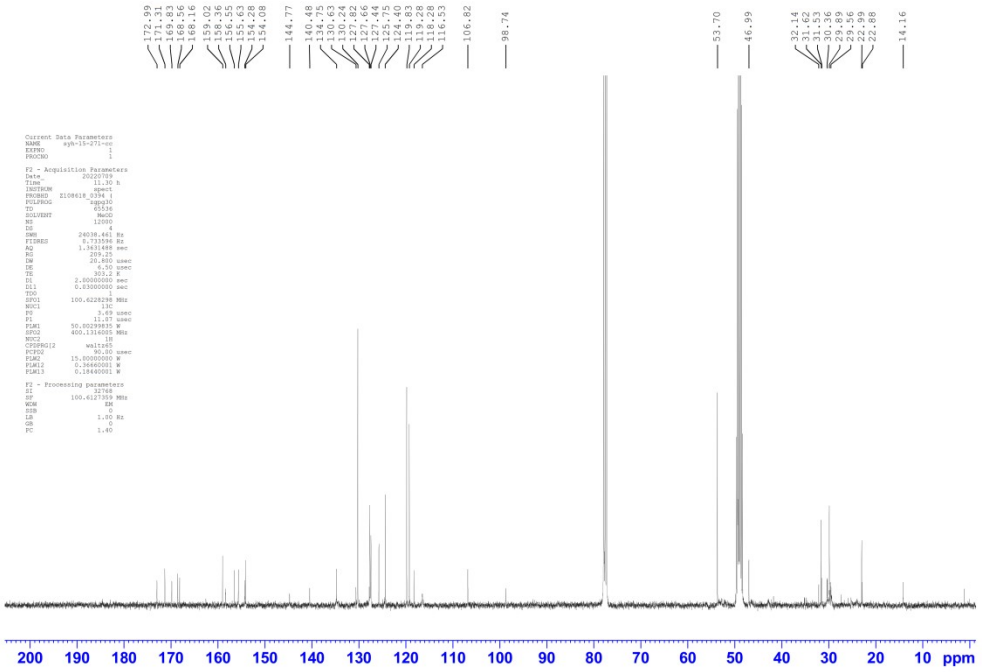
3-(5-((4-(2-(4-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)-2-oxoethyl)phenyl)amino)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (15-295). ^{1}H-NMR (400 MHz, $CDCl_3/CD_3OD$=3:1, ppm): 8.29 (s, 1H), 7.73-7.57 (m, 3H), 7.44-7.37 (m, 2H), 7.31-6.99 (m, 11H), 5.19-4.71 (m, 3H), 4.42-4.27 (m, 1H), 4.16 (d, *J* = 13.1Hz, 1H), 3.78 (s, 2H), 3.41-3.27 (m, 1H), 3.04-2.79 (m, 3H), 2.46-1.93 (m, 7H); LC-MS: calculated for $C_{43}H_{39}N_9O_5$ $[M+H]^+$, 762.31; found, 762.37.

Mass spectrum data of compound **15-271**

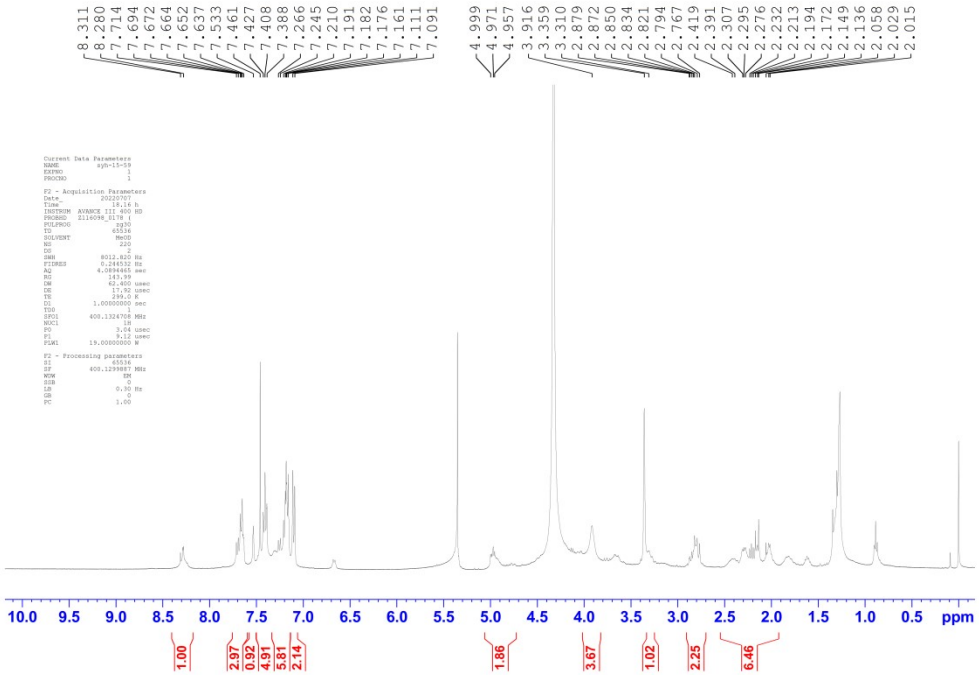


NMR data of compound **15-271**

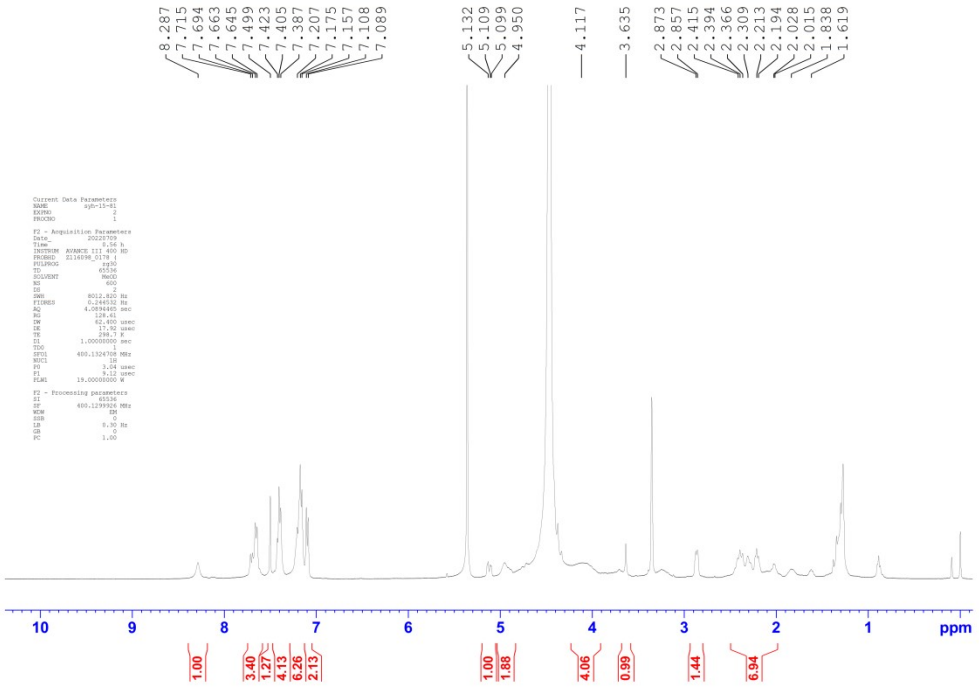




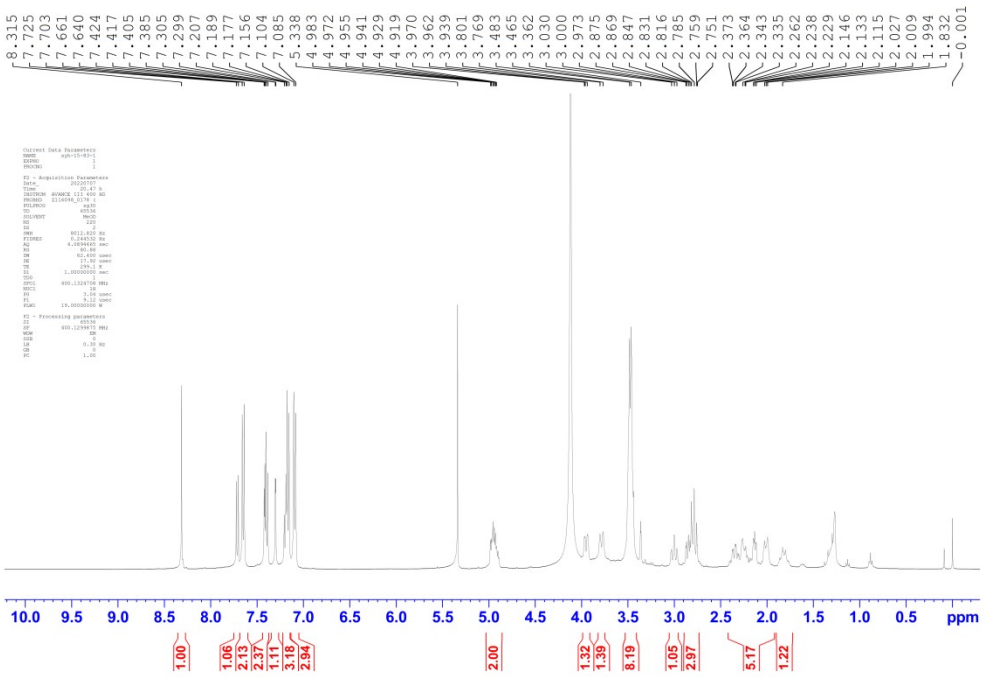
NMR data of compound **15-59**



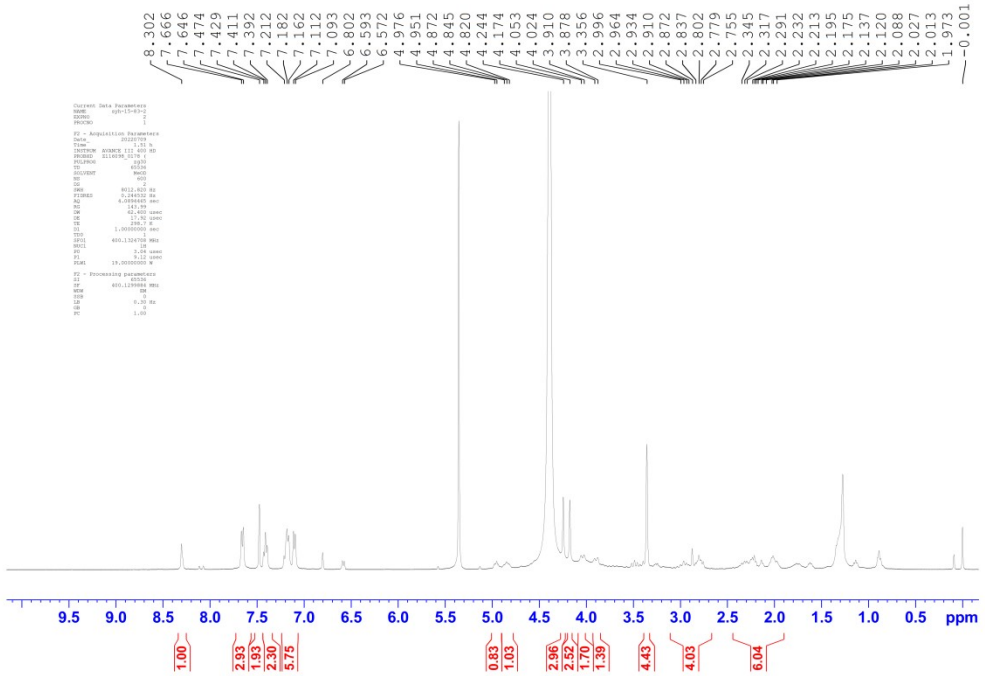
NMR data of compound **15-81**



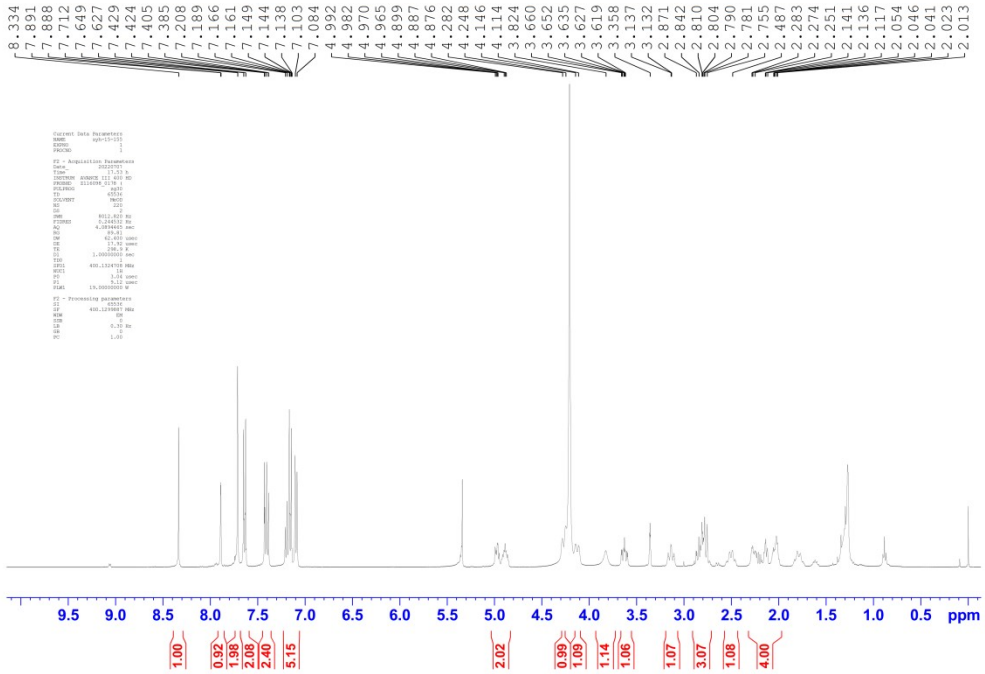
NMR data of compound **15-83-1**



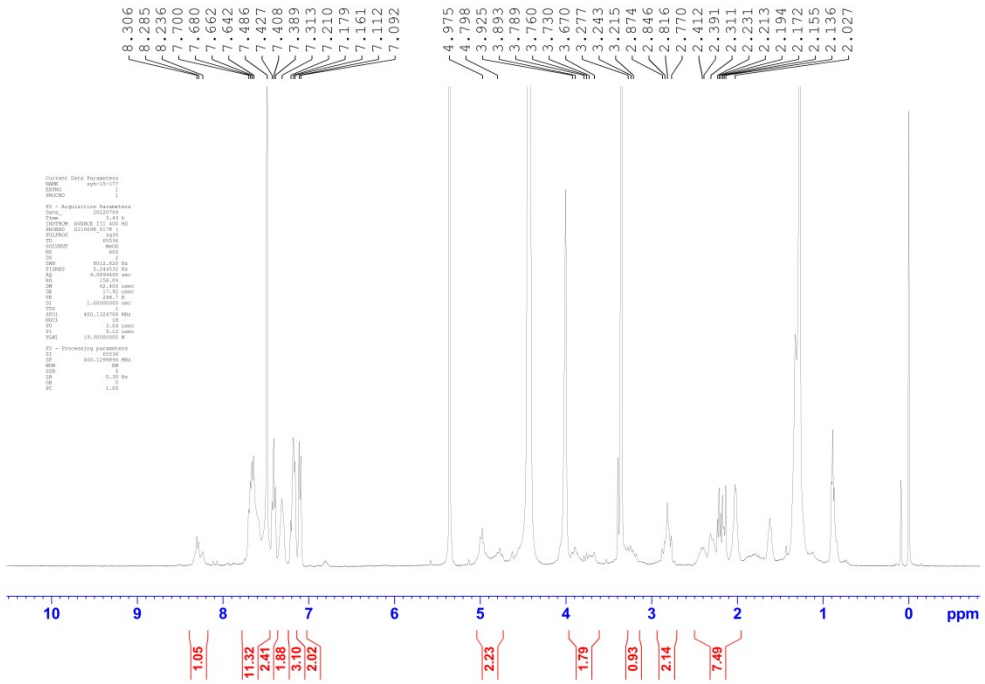
NMR data of compound **15-83-2**



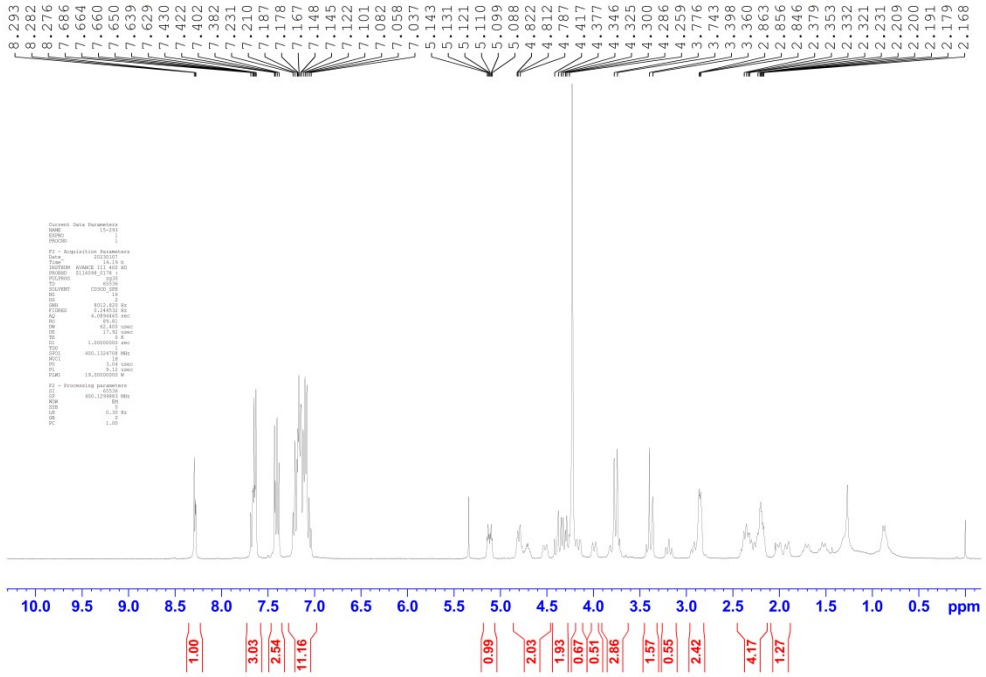
NMR data of compound **15-155**



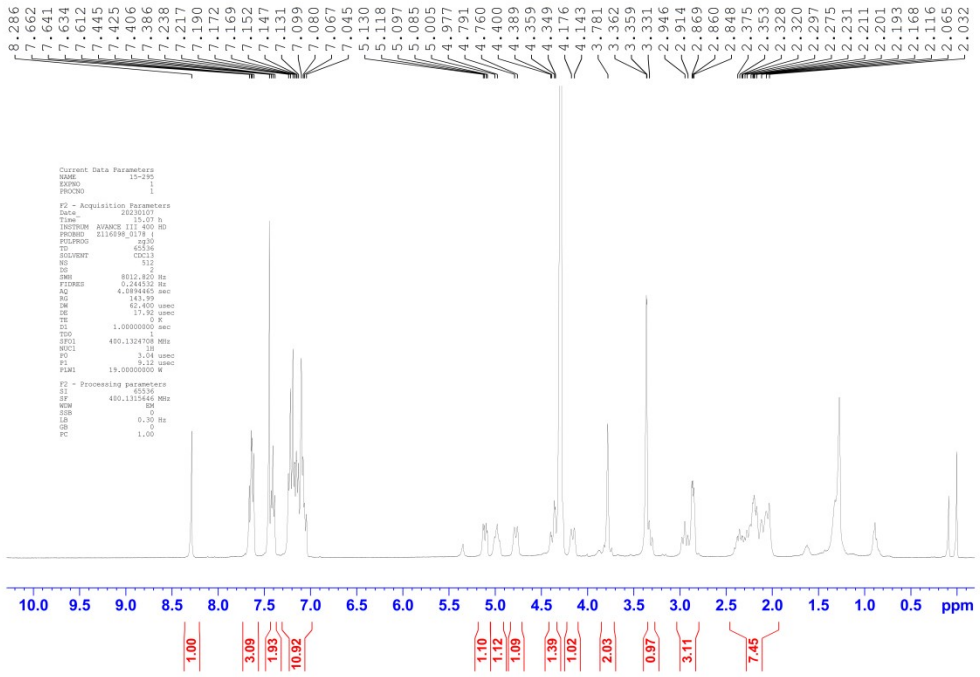
NMR data of compound **15-177**



NMR data of compound **15-293**



NMR data of compound **15-295**



Supplementary information, Data S2

Cell Culture Conditions

The human Ramos cell line was provided by Prof. Susan K. Pierce (NIH). DOHH2 cell line was provided by Prof. Ning Ding (Peking University Cancer Hospital & Institute). RPMI 1640, DMEM, Pen Strep and 2-Mercaptoethanol were purchased from Gibco®, MEM nonessential amino acid solution (10 mM, 100×) was purchased from Solarbio®, DMSO (Cat. No. D2650-100ML) was purchased from SIGMA®, FBS was purchased from Gemini. Ramos and DOHH2 cell lines were cultured in RPMI 1640 media with 10% FBS, 1% penicillin-streptomycin, 1% nonessential amino acid and 50 μM 2-Mercaptoethanol at 37°C incubator containing 5% CO_2.

Immunoblotting

Cells were suspended in 1 mL of culture medium in 12-well cell culture plate (3×10^5 cells/well), which were treated with compound at various concentrations. After the indicated time, cells were lysed with 2X SDS sample buffer (50 mM Tris-HCl (pH6.8), 2% (W/V) SDS, 0.1% (W/V) Bromophenol Blue, 10% (V/V) Glycerin and 1% (W/V) 2-Mercaptoethanol in ddH_2O) and heated at 100°C for 15 min. 10 μL of the cell lysate were loaded onto 10% SDS-PAGE gel for the protein band separation, and the gel then electrotransfered to PVDF membranes at 4°C, 100V for 1.5 h. Incubated with primary antibody at 4°C, overnight. Primary antibodies: BTK (Rabbit mAb, CST, D3H5, #8547S 1: 1000) was purchased from CST. GSPT1 (Rabbit mAb, #A1621, 1: 1000) was purchased from Abclonal. *β*-actin from Easybio (Rabbit, BE0022-100, 1: 3000). Rabbit (Invitrogen, Prod#31460, 1:4000)/mouse (Invitrogen, Prod#31430, 1:4000) secondary antibodies were incubated at room temperature for 1 h. Blots were imaged with M5 Hiper ECL Western HRP Substrate (MF074-05) on AllCap ECL instrument. For grayscale analysis, we use the imageJ 1.50i. GraphPad Prism 5 software was used for calculating DC_{50} by nonlinear regression analysis.

Permeability on MDCK monolayer

Madin-Darby canine kidney cells type II (MDCKII) were purchased from BNCC. MDCK cells were seeded onto 24-well Corning Transwell plates at a density of 5 x 10^4 cells/ cm^2. The assay was performed at day 5 after seeding when confluent cell monolayer was formed.

Test compounds were diluted with the transport buffer (HBSS with 10mM Hepes, pH7.40) to a concentration of 10 μM. After washing the cells twice with HBSS, test compound and reference compound will be dosed to the apical or basolateral side of the cell monolayer. The plate was incubated at 37±1℃, 100 μL samples were aliquoted from the receiver side at 120 minutes as well as 2 μL of samples from the donor sides. Concentration of test compounds were determined by LC-MS/MS based on the peak area ratio of analyte/Interal standard. Permeation of lucifer yellow through the monolayer was measured to evaluate the cellular integrity.

The percentage of lucifer yellow in the basolateral side is less than 1% indicating the system is verified for testing.

Liver microsome stability

The liver microsomal incubations consisted of 100 mM PBS (pH 7.4) containing test compound (final concentration was 1 μM), 1 mM NADPH and 0.5 mg/mL liver microsomes (mouse, rat, dog, monkey and human, respectively). The reaction mixtures were pre-incubated for 10 min at 37 °C before the addition of corresponding working solution. Reaction was terminated at 0, 5, 15, 30, 60 min by adding 3 times volume of ice-cold acetonitrile (containing 100 ng/mL tolbutamide as internal standards). The final concentration of organic solvents was less than 0.5% in all incubations. The quenched samples were centrifuged at 4000 rpm for 15 min at 4 °C, 40 μL of supernatant was mixed well with 100 μL of 0.1% formic acid which is ready for bioanalysis.

In vivo PK test

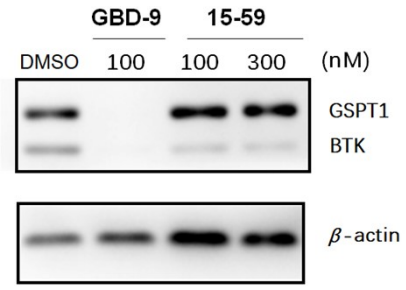
Balb/c mice (9 males each group) were purchased from Vital River Laboratory Animal Technology Co., Ltd (Zhejiang). All animals were treated in accordance with institutional guide for the care and use of laboratory animals. All experiments were performed in compliance with the author's institute's policy on animal use and ethics. Tested compounds (15-271, 15-293, 15-295) were administrated to mice in both intravenous (2 mg/kg) and intragastric (5 mg/kg) respectively. Blood samples of mice

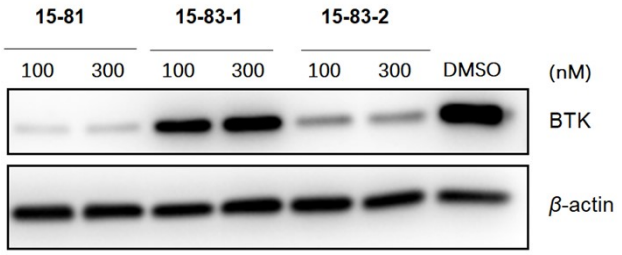
were collected into tubes with EDTAK2 at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 24 h after IV administration and 0.25, 0.5, 1, 2, 4, 6, 8, 24 h after oral administration. Plasma was separated after centrifugation at 2000 g for 10 min. 200 μL aliquot of acetonitrile (containing 200 ng/mL tolbutamide) was added to 50 μL of plasma, followed by vortex and centrifugation for 15 min (4815 g, in 96-well plate). The supernatant was subsequently transferred to a clean 96-well plate for LCMS analysis.

LC-MS/MS consisted of an HPLC system (Sciex exion LCAD) equipped with a binary solvent manager, an auto sampler and 5500+ Qtrap mass spectrometer (Sciex) with electrospray ionization source. MS detection was performed in positive ESI mode (scan mode MRM) with the source temperature maintaining at 550 °C. Other settings included the IonSpray voltage at 5500 V, collision cell exit potential at 10 V (entrance potential 10 V), curtain gas (nitrogen) 35 psi and collision gas 8 psi. Generally, GS 1 and GS 2 were set at 50 psi. The MS transition were 762.4/304.3 for 15-293, 776.4/420.1 for 15-271 and 762.4/304.2 for 15-295. Data acquisition and analysis were performed using Analyst software (AB SCIEX). A Kinetex polar C18 column (50 mm× 2.1mmi.d., 2.6 μm; phenomenex) was used at a column temperature of 35℃. The flow rate was set to 0.4 mL/min. The mobile phase consisted of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). Gradient elution started from 5% (B) held for 0.5 min, followed by a linear gradient to 95% (B) in 1 min and held for another 1 min then zoomed to 5% (B) in the next 0.2 min, and finally re-equilibrated for 0.8 min.

Ethical statement

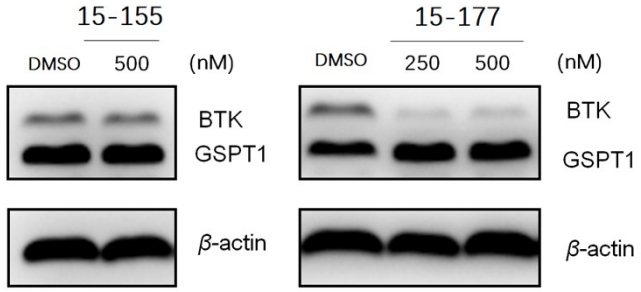
All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Zhejiang University of Technology and approved by the Animal Ethics Committee of Zhejiang University of Technology.





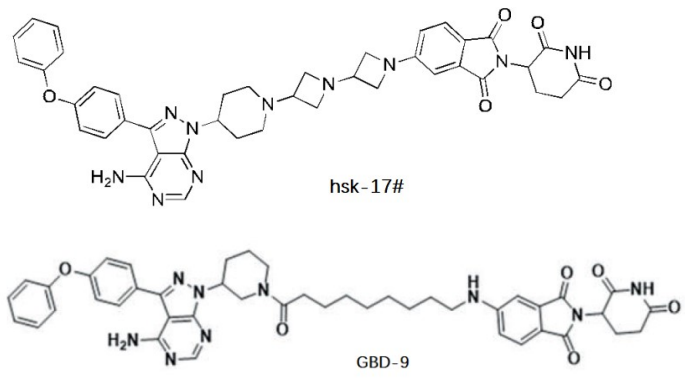
Supplementary information, Fig S1. Degradation efficiency of novel BTK degraders.

Immunoblot for BTK, GSPT1 and β-actin after 24 hours treatment on Ramos cells with the indicated concentrations of compounds. [a*] 3×10^5 cells in each hole (12-well plate).



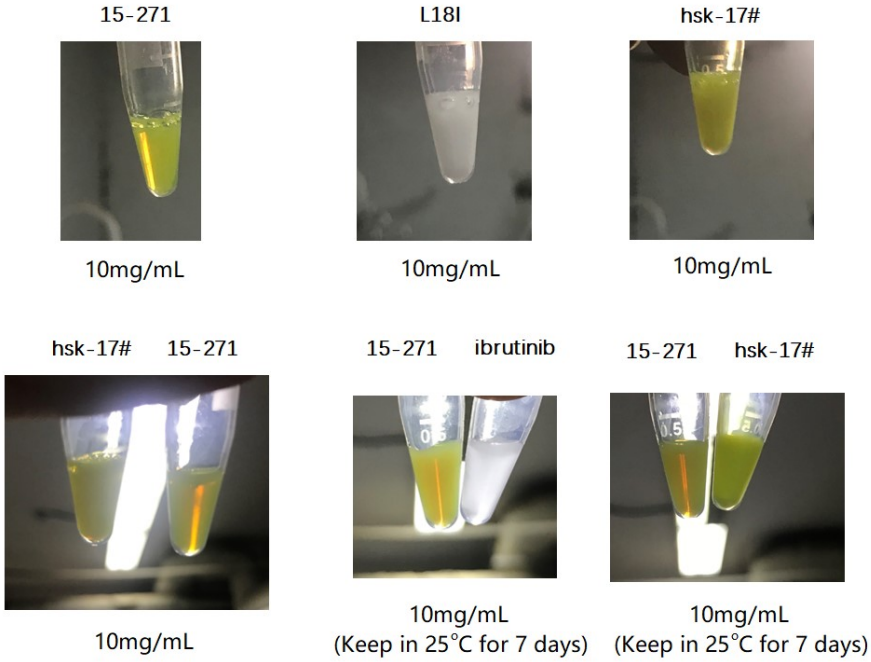
Supplementary information, Fig. S2. Degradation efficiency of BTK degraders with rigid linker.

Immunoblot for BTK, GSPT1 and β-actin after 24 hours treatment on Ramos cells with the indicated concentrations of compounds. [a*] 3×10^5 cells in each hole (12-well plate).



Supplementary information, Fig. S3. Chemical structures of compound hsk-17#

and compound GBD-9.



Supplementary information, Fig. S4. Solubility test for BTK degraders and ibrutinib.

Compound was dissolved in DMSO: Cremophor EL: 1× PBS =1/1/8 (v/v/v) with 10 mg/mL.

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