Supporting Information for

Original article

Discovery of novel exceptionally potent and orally active c-MET PROTACs for the treatment of tumors with *MET* alterations

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Running title c-MET PROTACs for the treatment of tumors with MET alterations

1. Supplementary Figures

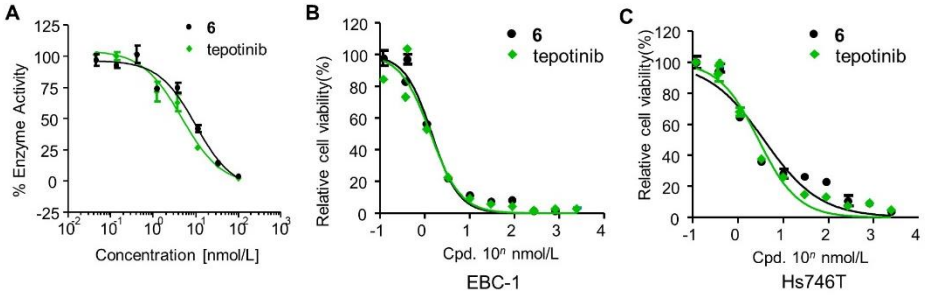
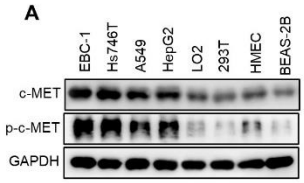


Figure S1. (A) The binding affinities of tepotinib and compound **6** to c-MET. (B-C) Effects of tepotinib and compound **6** in EBC-1 (B) and Hs746T (C) cells. cell viability was measured 72 h after treatment with the indicated doses of the drugs. Data shown are mean ± SD of triplicate measurements that were repeated 3 times with similar results.

A



B

Cpd.	IC_{50} (μmol/L)			
	LO2	293T	HMEC	BEAS-2B
D1	>100	>100	>100	>100
D2	>100	>100	>100	>100
D3	>100	>100	>100	>100
D4	>100	>100	>100	>100
D5	>100	>100	>100	>100
D6	>100	>100	>100	>100
D7	>100	>100	>100	>100
D8	>100	>100	>100	>100
D9	>100	>100	>100	>100
D10	>100	>100	>100	>100
D11	>100	>100	>100	>100
D12	>100	>100	>100	>100
D13	>100	>100	>100	>100
D14	>100	>100	>100	>100
D15	>100	>100	>100	>100
tepotinib	3.37 ± 0.53	5.29 ± 1.11	4.43 ± 0.69	3.67 ± 0.74

Figure S2. (A) The expression of c-MET and p-c-MET in c-MET-sensitive EBC-1 and Hs746T cells, c-MET-insensitive A549 and HepG2 cells and normal LO2, 293T, HMEC and BEAS-2B cells. (B) The antiproliferative effects of our PROTACs and tepotinib in normal LO2, 293T, HMEC and BEAS-2B cells. Cell viability was measured 72 h after treatment with the indicated doses of the drugs. Data shown are mean ± SD of triplicate measurements.

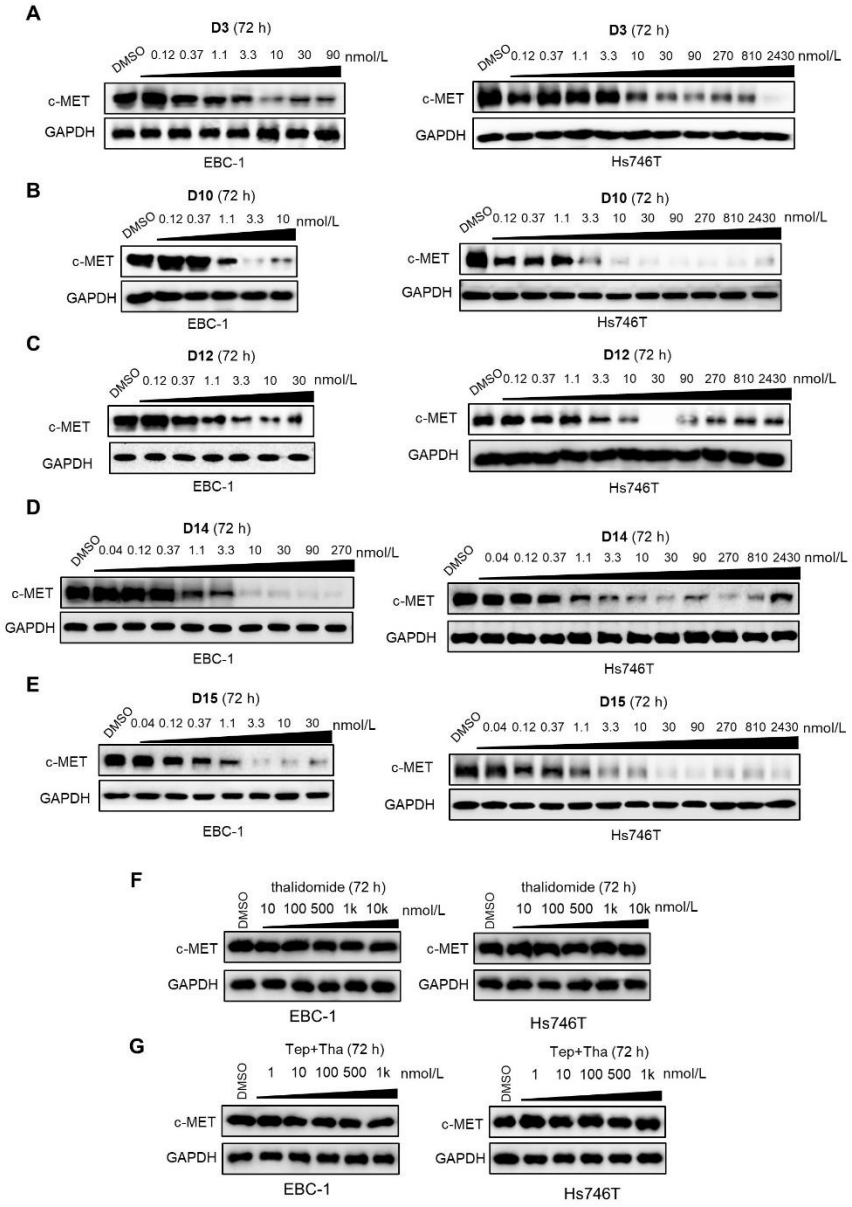


Figure S3. The degradation effects of c-MET-PROTACs on EBC-1 and Hs746T cells. Western blots were used to evaluate the degradation of c-MET proteins by PROTACs. The cell line was treated with compounds **D3** (A), **D10** (B), **D12** (C), **D14** (D), **D15** (E), thalidomide (F), Tep + Tha (tepotinib+thalidomide, G) at indicated concentrations for 72 h.

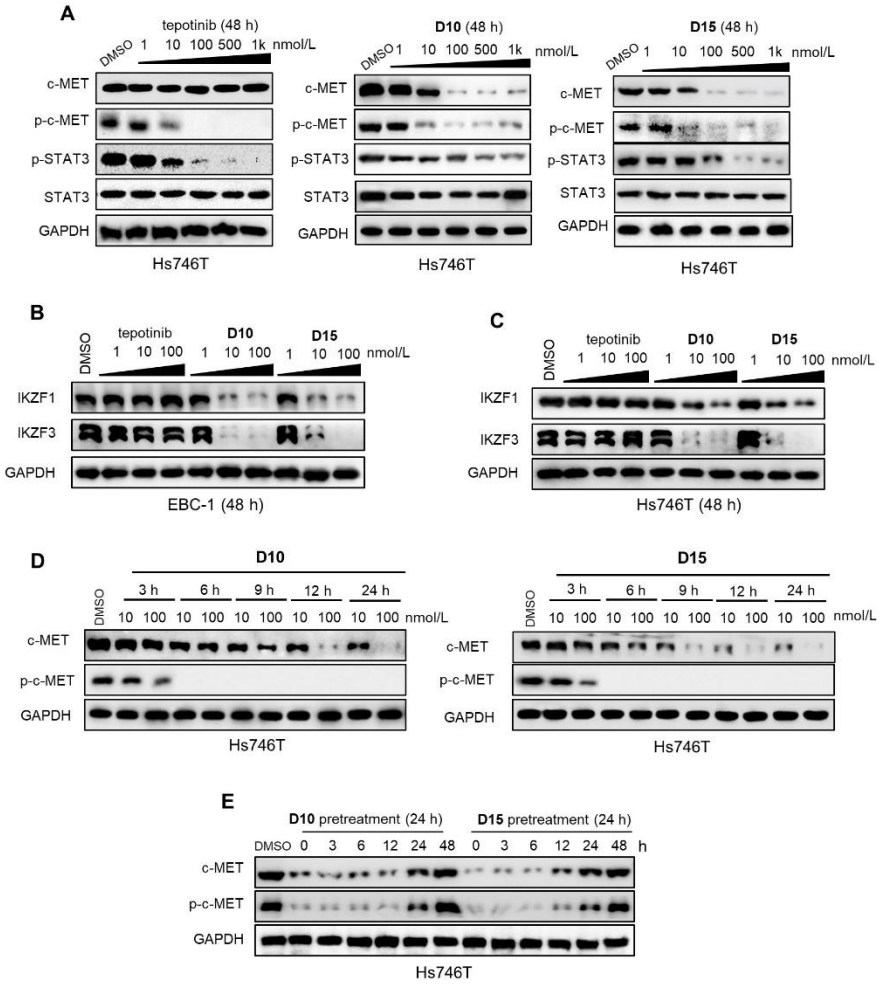
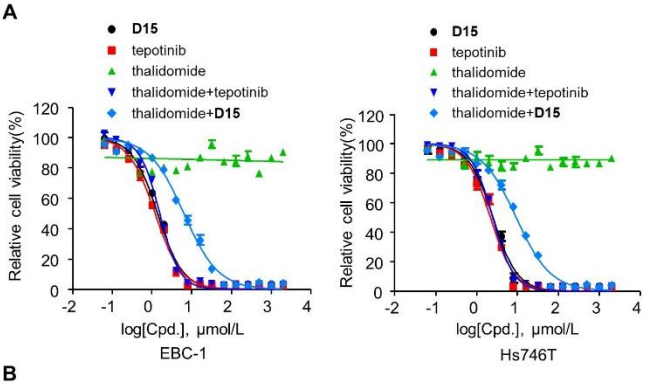


Figure S4. **D10** and **D15** showed dose- and time-dependent degradation effects on c-MET and inhibitory effects on p-c-MET. (A) The effects of **D10** and **D15** on c-MET and its downstream signaling pathways in Hs746T cells. Cells were treated with **D10** and **D15** for 48 h at the indicated concentration. Tepotinib was utilized for comparison. (B, C) The effects of **D10** and **D15** on CRBN substrates (IKZF1 and IKZF3) in EBC-1 (B) and Hs746T (C) cells. Cells were treated with **D10** and **D15** for 48 h at the indicated concentration. Tepotinib was utilized for comparison. (D) The effects of **D10** and **D15** on c-MET and c-MET phosphorylation in Hs746T cells. Cells were treated with **D10** and **D15** (10, 100 nmol/L) at indicated time. (E) Hs746T cells were pretreated with **D10** and **D15** at 100 nmol/L for 24 h, then washed with PBS three times, and harvested at the indicated time points for western blot analysis.

A



B

Compound	IC_{50} (nmol/L)	
	EBC-1	Hs746T
D15	2.11 ± 0.31	3.50 ± 0.55
tepotinib	1.32 ± 0.39	2.63 ± 0.72
thalidomide	>100k	>100k
D15+Tha	8.29 ± 2.53	12.3 ± 1.9
Tep+Tha	1.55 ± 0.33	2.77 ± 0.30

Figure S5. The antiproliferative effects of **D15**, thalidomide, tepotinib, thalidomide +tepotinib (Tep + Tha), and thalidomide + **D15** (**D15** + Tha) on EBC-1 and Hs746T cells. Data shown are mean ± SD of triplicate measurements.

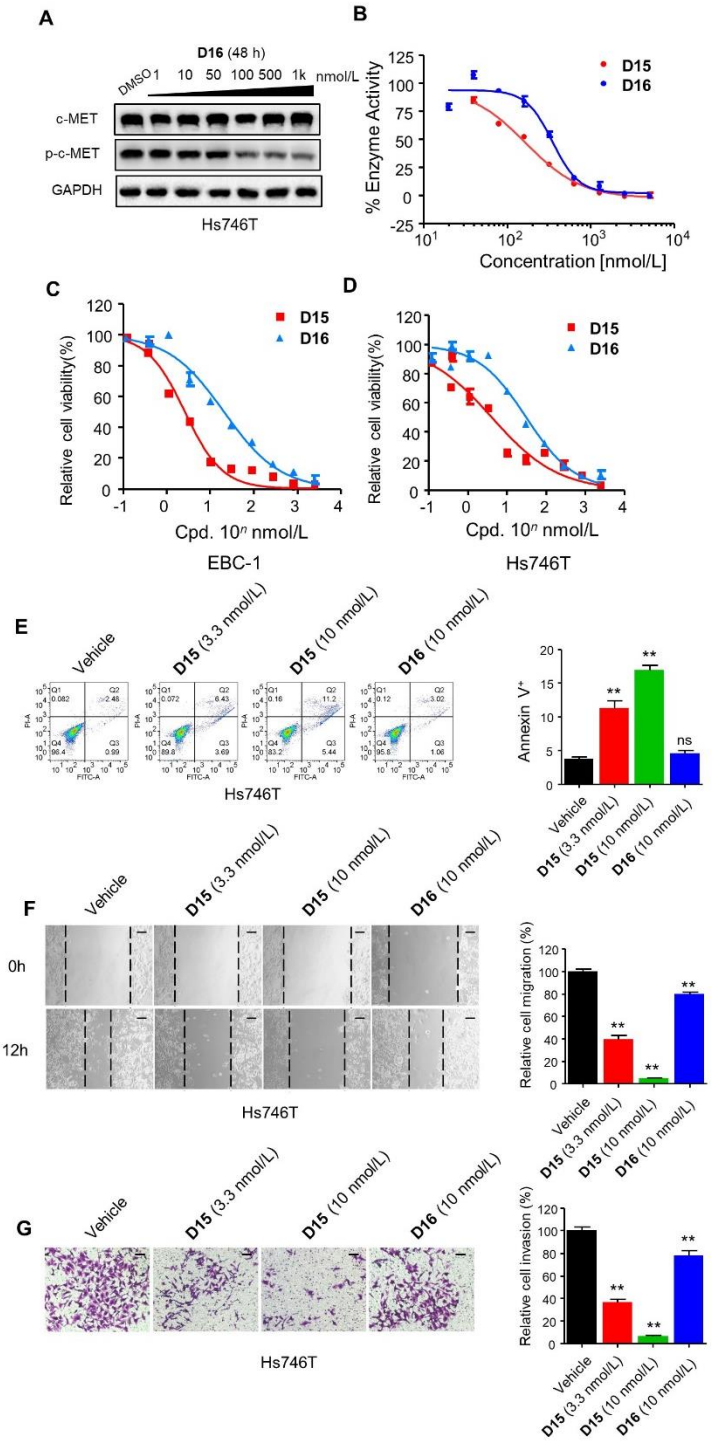


Figure S6. Degradation of c-MET mainly contributes to the anticancer effect of **D15**. (A) The effects of c-MET and p-c-MET by **D16** in Hs746T cells. (B) The binding affinities of **D15** and **D16** to c-MET. (C-D) Effects of **D15** and **D16** in EBC-1 (C) and Hs746T (D) cells. cell viability was measured 72 h after treatment with the indicated doses of the drugs. (E) Representative images of flow cytometry analysis of apoptosis Hs746T cells treated with **D15**, **D16** and vehicle control (DMSO) at the indicated dose for 48 h. Histograms show the relative cell percentage of apoptosis in Hs746T cells (right). (F,

G) wound healing assay (F) and transwell assay (G) in Hs746T cells treated with **D15**, **D16** and vehicle control (DMSO) at the indicated dose for 12 h. Histograms show the relative cell migration and cell invasion (right). Scale bars:100 mm. Data are mean ± SD, $n = 3$, $^{**}P < 0.01$ (*t* test).

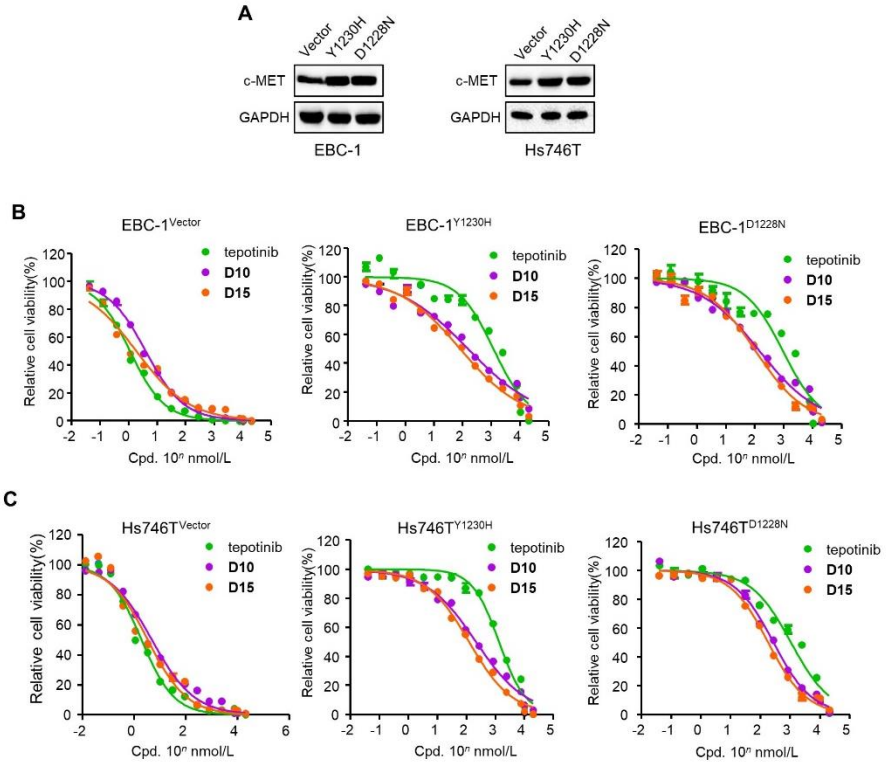


Figure S7. c-MET degradation overcame acquired type Ib c-MET TKI resistance. (A) Immunoblots for c-MET and GAPDH in EBC-1^{Vector}, EBC-1^{Y1230H} and EBC-1^{D1228N} cells. (A) Immunoblots for c-MET and GAPDH in Hs746T^{Vector}, Hs746T^{Y1230H} and Hs746T^{D1228N} cells. (B-C) Effects of tepotinib, **D10** and **D15** in EBC-1 (B) and Hs746T(C) cells with Y1230H and D1228N mutation, respectively. Cell viability was measured 72 h after treatment with the indicated doses of the drugs. Data shown are mean ± SD of triplicate measurements.

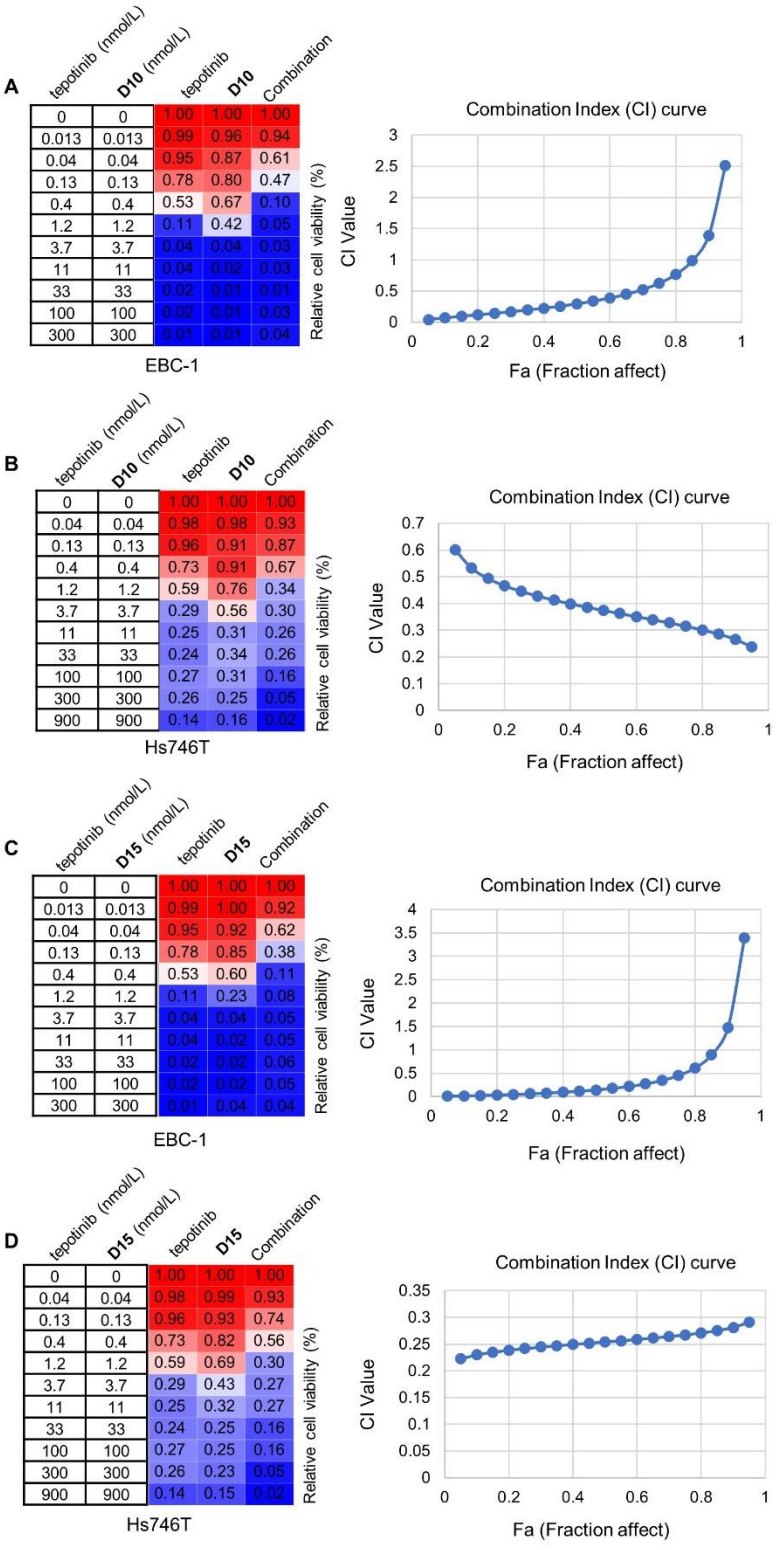


Figure S8. (A-B) Effects of tepotinib and **D10** as single agents or drug combinations in EBC-1(A) and Hs746T(B) cells. (C-D) Effects of tepotinib and **D15** as single agents or drug combinations in EBC-1(C) and Hs746T(D) cells. Cell viability was measured 72 h after treatment with the indicated doses of the drugs (left). Combinational Index (CI)

was calculated by the Chou-Talalay equation using multiple doses and response points. Data shown are mean ± SD of triplicate measurements that were repeated 3 times with similar results.

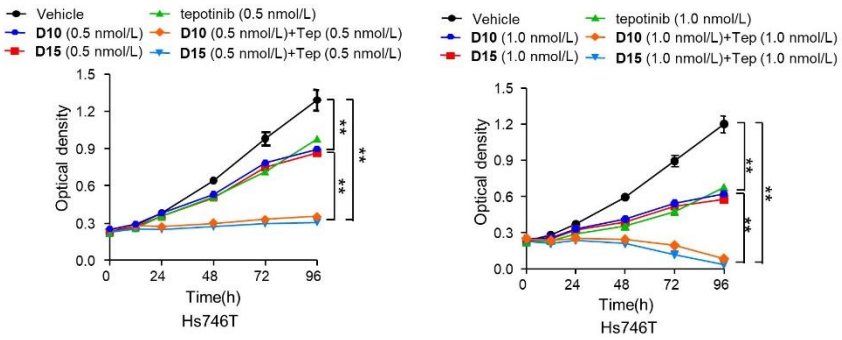


Figure S9. The effects of c-MET and p-c-MET by tepotinib with **D10** and **D15** as single agents or drug combinations in Hs746T cells at the indicated concentration. Data are mean ± SD, $n = 3$. $^{**}P < 0.01$ (one-way ANOVA).

2. Synthesis of compounds and compounds characterization

*2.1. tert-Butyl 4-(((2-chloropyrimidin-5-yl)oxy)methyl)piperidine-1-carboxylate (**2**)*

A mixture of 2-Chloro-5-hydroxypyrimidine (5.00g, 38.31mmol), N-Boc-4-piperidinemethanol (10.05g, 46.61mmol), triphenylphosphine (12.24g, 46.61mmol) were dissolved in anhydrous tetrahydrofuran (100mL), stirred at 0 °C under nitrogen (N_2) protection, and slowly added diisopropyl azodicarboxylate (9.42g, 46.61mmol) dropwise, reacted for 12 h. After the reaction was completed, the mixture was concentrated and purified by column chromatography to obtain a white solid **2** (12.23g, yield 95.12%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.54 (s, 2H), 4.02 (d, J = 6.5 Hz, 2H), 3.96 (s, 2H), 2.73 (s, 2H), 2.00 – 1.89 (m, 1H), 1.77 – 1.67 (m, 2H), 1.40 (s, 9H), 1.15 (dd, J = 12.3, 4.3 Hz, 2H). UPLC–MS (ESI$^+$): calculated for $C_{15}H_{22}ClN_3O_3$ $[M + Na]^+$ 350.12; found 350.11.

*2.2. tert-Butyl 4-(((2-(3-(hydroxymethyl)phenyl)pyrimidin-5-yl)oxy)methyl)piperidine-1-carboxylate (**3**)*

4-((2-Chloropyrimidin-5-yl)oxy)methyl)piperidine-1-carboxylate tert-butyl ester (14.00g, 42.81mmol), 3-hydroxymethylphenylboronic acid pinacol ester (10.00g, 42.81mmol), [1,1'-bis(diphenylphosphino)ferrocene]palladium dichloride (0.91g, 1.31mmol), potassium phosphate (K_3PO_4) (18.12g, 85.60mmol) and water (20 mL) were added in 1,4-dichloride In oxane (100 mL), the mixture was stirred at 80 °C under

N_2 protection for 6 h. After the reaction was completed, Ethyl acetate (EA) was added to dilute, saturated aqueous sodium bicarbonate ($NaHCO_3$) solution (50 mL×3) was added to wash. The EA phase was dried over anhydrous Sodium sulfate (Na_2SO_4), filtered and concentrated. The residue was purified by column chromatography to obtain a white solid **3** (16.02g, yield 95.04%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.64 (s, 2H), 8.30 (d, J = 1.7 Hz, 1H), 8.16 (dd, J = 7.6, 1.6 Hz, 1H), 7.44 (t, J = 7.6 Hz, 1H), 7.40 (t, J = 8.1 Hz, 1H), 5.28 (t, J = 5.7 Hz, 1H), 4.58 (d, J = 5.7 Hz, 2H), 4.07 (d, J = 6.4 Hz, 2H), 4.03 – 3.92 (m, 2H), 2.75 (s, 2H), 2.04 – 1.87 (m, 1H), 1.81 – 1.71 (m, 2H), 1.40 (s, 9H), 1.18 (qd, J = 12.4, 4.4 Hz, 2H). UPLC–MS (ESI$^+$): calculated for $C_{22}H_{29}N_3O_4$ $[M + H]^+$ 400.22; found 400.20.

*2.3. tert-Butyl 4-(((2-(3-(chloromethyl)phenyl)pyrimidin-5-yl)oxy)methyl)piperidine-1-carboxylate (**4**)*

A mixture of 4-(2-(3-(Hydroxymethyl)phenyl)pyrimidin-5-yl)oxy)methyl) piperidine-1-carboxylate tert-butyl ester (17.00g, 42.62mmol), PPh_3 (17.12g, 63.93 mmol) and CCl_4 (10.11g, 63.93 mmol) were dissolved in dichloromethane (100 mL) and stirred at 45 °C under N_2 protection for 6 h. After the completion of the reaction, the mixture was concentrated and purify by column chromatography to obtain a light yellow solid **4** (16.22g, yield 90.32%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.65 (s, 2H), 8.38 (s, 1H), 8.26 (dt, J = 7.3, 1.8 Hz, 1H), 7.55 – 7.52 (m, 1H), 7.50 (d, J = 7.5 Hz, 1H), 4.87 (s, 2H), 4.07 (d, J = 6.4 Hz, 2H), 3.98 (s, 2H), 2.76 (s, 2H), 1.98 (ddtt, J = 13.1, 9.5, 6.6, 3.5 Hz, 1H), 1.77 (d, J = 13.6 Hz, 2H), 1.41 (s, 9H), 1.19 (dd, J = 12.3, 4.2 Hz, 2H). UPLC–MS (ESI$^+$): calculated for $C_{22}H_{28}ClN_3O_3$ $[M + Na]^+$ 440.17; found 440.13.

*2.4. tert-Butyl 4-(((2-(3-((3-(3-cyanophenyl)-6-oxopyridazin-1(6H)-yl)methyl)phenyl) pyrimidin-5-yl)oxy)methyl)piperidine-1-carboxylate (**5**)*

4-(2-(3-(Chloromethyl)phenyl)pyrimidin-5-yl)oxy)methyl)piperidine-1-carboxylate tert-butyl ester (12.81g, 30.62mmol), 3-(6-Carbonyl-1,6-dihydro-3-pyridazinyl)benzonitrile (6.02g, 30.62 mmol), K_2CO_3 (8.12g, 61.24mmol) was dissolved in N,N-dimethylformamide (DMF) (100 mL), and stirred at 80°C for 4h. After the reaction was completed, EA was added to dilute, saturated $NaHCO_3$ solution (50 mL×3) was added to wash. The EA phase was dried over anhydrous Na_2SO_4, filtered, concentrated. The residue was purified by column chromatography to obtain a light yellow solid **5** (16.12g, yield 90.33%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.64 (s, 2H), 8.38 (d, J = 8.4 Hz, 2H), 8.26 – 8.21 (m, 2H), 8.17 (d, J = 9.7 Hz, 1H), 7.93 (dt, J = 7.7, 1.4 Hz, 1H), 7.72 (t, J = 7.9 Hz, 1H), 7.48 (d, J = 6.9 Hz, 2H), 7.16 (d, J = 9.8

Hz, 1H), 5.44 (s, 2H), 4.06 (d, *J* = 6.4 Hz, 2H), 3.99 (s, 2H), 2.75 (s, 2H), 2.02 – 1.89 (m, 1H), 1.76 (d, *J* = 10.0 Hz, 2H), 1.40 (s, 9H), 1.17 (dd, *J* = 12.1, 4.3 Hz, 2H). UPLC–MS (ESI^+): calculated for $C_{33}H_{34}N_6O_4$ $[M+Na]^+$ 601.25; found 601.24.

*2.5. 3-(6-Oxo-1-(3-(5-(piperidin-4-ylmethoxy)pyrimidin-2-yl)benzyl)-1,6-dihydropyridazin-3-yl)benzonitrile (**6**)*

Adding Trifluoroacetic acid (TFA) (8.21g, 71.57mmol) to a solution of tert-Butyl 4-((2-(3-((3-(3-cyanophenyl)-6-oxopyridin-1(6H)-yl)methyl)phenyl)pyrimidin-5-yl) Oxy)methyl)piperidine-1-carboxylate (9.81g, 17.23mmol) in Dichloromethane (DCM) (100 mL), the mixture was stirred at room temperature overnight. After completion of the reaction, the mixture was concentrated and purify by column chromatography to obtain a light yellow solid **6** (8.22g, yield 98.35%). ^1H NMR (600 MHz, DMSO-d_6) δ 8.65 (s, 2H), 8.38 (t, *J* = 1.8 Hz, 2H), 8.27 – 8.22 (m, 2H), 8.18 (d, *J* = 9.8 Hz, 1H), 7.94 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.72 (t, *J* = 7.9 Hz, 1H), 7.49 (d, *J* = 6.2 Hz, 2H), 7.17 (d, *J* = 9.8 Hz, 1H), 5.45 (s, 2H), 4.08 (d, *J* = 6.3 Hz, 2H), 3.24 (d, *J* = 12.5 Hz, 2H), 2.82 (t, *J* = 11.2 Hz, 2H), 2.39 (p, *J* = 1.9 Hz, 1H), 2.11 – 2.02 (m, 1H), 1.88 (d, *J* = 12.6 Hz, 2H), 1.44 – 1.40 (m, 2H). UPLC–MS (ESI^+): calculated for $C_{28}H_{26}N_6O_2$ $[M+H]^+$ 479.22; found 479.21.

2.6. tert-Butyl 2-(4-(((2-(3-((3-(3-cyanophenyl)-6-oxopyridazin-1(6H)-yl)methyl) phenyl)pyrimidin-5-yl)oxy)methyl)piperidin-1-yl)acetate (7)

3-(6-oxo-1-(3-(5-(piperidin-4-ylmethoxy)pyrimidin-2-yl)benzyl)-1,6-dihydropyridazin-3-yl) benzonitrile (1.00g, 2.17mmol), tert-Butyl bromoacetate (0.48g, 2.52mmol) and K_2CO_3 (0.42g, 3.17mmol) was dissolved in DMF (15mL). After stirred for 8h, EA was added to dilute, saturated NaHCO3 solution (50 mL×3) was added to wash. The EA phase was dried over anhydrous Na_2SO_4, filtered, concentrated. The residue was purified by column chromatography to obtain a light yellow solid to obtain a white solid **7** (1.22g, yield 81.35%). ^1H NMR (600 MHz, DMSO-d_6) δ 8.65 (s, 2H), 8.41 – 8.35 (m, 2H), 8.26 – 8.22 (m, 2H), 8.18 (d, *J* = 9.7 Hz, 1H), 7.93 (dt, *J* = 7.6, 1.4 Hz, 1H), 7.72 (t, *J* = 7.8 Hz, 1H), 7.52 – 7.45 (m, 2H), 7.16 (d, *J* = 9.7 Hz, 1H), 5.45 (s, 2H), 4.08 (s, 2H), 3.07 – 2.74 (m, 2H), 2.24 (d, *J* = 7.2 Hz, 2H), 1.88 (s, 2H), 1.54 – 1.46 (m, 4H), 1.40 (s, 9H), 1.23 (s, 1H). UPLC–MS (ESI^+): calculated for $C_{34}H_{36}N_6O_4$ $[M+H]^+$ 593.29; found 593.30.

*2.7. 2-(4-(((2-(3-((3-(3-cyanophenyl)-6-oxopyridazin-1(6H)-yl)methyl)phenyl) pyrimidin-5-yl)oxy)methyl)piperidin-1-yl)acetic acid (**8**)*

Adding TFA (1.22g, 11.23mmol) to a solution of tert-Butyl acetate 2-(4-(2-(3-((3-(3-

cyanophenyl)-6-oxopyridin-1(6H)-yl)methyl)phenylpyrimidine-5-(yl)oxy)methyl)piperidin-1-yl (1.24 g, 2.33mmol) in DCM (20 mL), the mixture was stirred at room temperature overnight. After completion of the reaction, the mixture was concentrated and purify by column chromatography to obtain a white solid **8** (1.23g, yield 91.33%). UPLC–MS (ESI^+): calculated for $C_{30}H_{28}N_6O_4$ $[M + H]^+$ 537.23; found 537.25.

*2.8. 3-Bromo-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)propanamide (**11a**)*

A mixture of Pomalidomide (1.22g, 3.72mmol) and 3-bromopropionyl chloride (1.22g, 7.44mmol) were dissolved in anhydrous THF (20 mL), and the mixture was refluxed at 65 °C for 6 h. After completion of the reaction, the mixture was concentrated and purify by column chromatography to obtain a white solid **11a** (1.21g, yield 81.23%). ^1H NMR (600 MHz, DMSO-d_6) δ 11.15 (s, 1H), 9.89 (s, 1H), 8.43 (d, J = 8.4 Hz, 1H), 7.91 – 7.83 (m, 1H), 7.65 (d, J = 7.1 Hz, 1H), 5.15 (dd, J = 12.9, 5.4 Hz, 1H), 3.74 (t, J = 6.4 Hz, 2H), 3.13 (t, J = 6.4 Hz, 2H), 2.90 (ddd, J = 17.2, 14.0, 5.4 Hz, 1H), 2.61 (dd, J = 14.4, 3.1 Hz, 1H), 2.58 – 2.51 (m, 1H), 2.11 – 2.04 (m, 1H). UPLC–MS (ESI^+): calculated for $C_{16}H_{14}BrN_3O_5$ $[M + H]^+$ 408.02; found 408.03.

Compound **11b-h** and 13 were synthesized in a similar way to **11a**, and obtained light yellow solid.

*2.9. tert-Butyl (2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)amino) ethoxy)ethyl)carbamate (**16a**)*

A mixture of 2-(2,6-Dioxypiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (2.11g, 7.22mmol), tert-butyl (2-(2-aminoethyl)Oxy)ethyl)carbamate (1.22g, 4.81mmol), DIPEA (1.21g, 7.22mmol) was dissolved in DMF (30 mL), and the solution was stirred for 8h. After the reaction was completed, EA was added to dilute, saturated $NaHCO_3$ solution (50 mL×3) was added to wash. The EA phase was dried over anhydrous Na_2SO_4, filtered, concentrated. The residue was purified by column chromatography to obtain a yellow-green oil **16a** (0.58g, yield 26.12%). ^1H NMR (600 MHz, DMSO-d_6) δ 11.06 (s, 1H), 7.57 (d, J = 8.3 Hz, 1H), 7.14 (t, J = 5.5 Hz, 1H), 7.01 (d, J = 2.2 Hz, 1H), 6.90 (dd, J = 8.4, 2.2 Hz, 1H), 6.79 (q, J = 7.5, 6.6 Hz, 1H), 5.77 (d, J = 5.4 Hz, 1H), 5.03 (dd, J = 12.8, 5.4 Hz, 1H), 3.57 (t, J = 5.4 Hz, 2H), 3.43 – 3.40 (m, 3H), 3.12 – 3.03 (m, 3H), 2.87 (ddd, J = 17.0, 13.9, 5.5 Hz, 1H), 2.58 (ddd, J = 17.0, 5.2, 2.5 Hz, 1H), 2.00 (dddd, J = 12.9, 11.1, 5.7, 3.0 Hz, 2H), 1.37 (s, 9H). UPLC–MS (ESI^+): calculated for $C_{22}H_{28}N_4O_7$ $[M + Na]^+$ 483.19; found 483.17. Adding TFA (1.22g, 8.26

mmol) to the solution in which compound **16a** is dissolved in DCM (20 mL), the mixture was stirred at room temperature overnight. After the reaction was completed, the mixture was concentrated and purify by column chromatography to obtain a yellow-green oil **17a** (0.43g, yield 95.23%).

Compound **16b**–**c** and **19** were synthesized in a similar way to **16a**, and obtained yellow oil. Compound **17b** is synthesized in a similar way to **17a**, and obtained yellow oil.

*2.10. tert-Butyl 4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl) amino) ethyl)piperazine-1-carboxylate (**22**)*

A mixture of 2-(2,6-Dioxypiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (5.00g, 18.13mmol), 4-(2-aminoethyl)piperazine tert-Butyl-1-carboxylate (4.12g, 18.13mmol), DIPEA (2.32g) was dissolved in DMF (50 mL), and the mixture was stirred and heated at 80 ℃ for 8h. After the reaction was completed, EA was added to dilute, saturated $NaHCO_3$ solution (50 mL×3) was added to wash. The EA phase was dried over anhydrous Na_2SO_4, filtered, concentrated. The residue was purified by column chromatography to obtain a yellow-green oil **22** (0.48 g, yield 6.34%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.06 (s, 1H), 7.57 (d, J = 8.3 Hz, 1H), 7.03 (s, 1H), 7.01 (s, 1H), 6.89 (d, J = 8.3 Hz, 1H), 5.03 (dd, J = 12.9, 5.4 Hz, 1H), 2.92 – 2.82 (m, 1H), 2.63 – 2.57 (m, 1H), 2.57 – 2.47 (m, 9H), 2.40 (s, 4H), 2.00 (dtd, J = 13.0, 5.4, 2.3 Hz, 1H), 1.40 (s, 9H). UPLC–MS (ESI$^+$): calculated for $C_{24}H_{31}N_5O_6$ $[M + Na]^+$ 508.22; found 508.03. Adding TFA (1.11g, 8.26 mmol) to the solution in which compound **22** is dissolved in DCM (20 mL), the mixture was stirred at room temperature overnight. After the reaction was completed, the mixture was concentrated and purify by column chromatography to obtain a yellow-green oil **23**. (0.38 g, yield 92.24%).

*2.11. tert-Butyl 4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)piperazine-1-carboxylate (**24a**)*

Compound **24a** is synthesized in a similar way to **22** by using compound **14a** and **24**, and obtained a yellow solid (0.32 g, yield 30.22%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.08 (s, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.35 (d, J = 2.3 Hz, 1H), 7.24 (d, J = 2.4 Hz, 1H), 5.08 (dd, J = 12.8, 5.4 Hz, 1H), 3.47 (s, 8H), 2.88 (ddd, J = 16.9, 13.9, 5.5 Hz, 1H), 2.65 – 2.53 (m, 2H), 2.02 (dtt, J = 12.7, 6.9, 3.5 Hz, 1H), 1.43 (s, 9H). UPLC–MS (ESI$^+$): calculated for $C_{22}H_{26}N_4O_6$ $[M + H]^+$ 465.18; found 465.17. Compound **25a** is synthesized in a similar way to **23**, and obtained a yellow oil (0.23g, yield 95.17%).

4.2.12. tert-Butyl 4-(2-(1-methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)

*piperazine-1-carboxylate (**24b**)*

Compound **24b** is synthesized in a similar way to **22** by using compound **14b** and **24**, and obtained a yellow solid (0.38g, yield 33.21%). Compound **25b** is synthesized in a similar way to **23**, and obtained a yellow oil (0.28 g, yield 95.32%).

*2.13. 3-(4-(((2-(3-((3-(3-cyanophenyl)-6-oxopyridazin-1(6H)-yl)methyl)phenyl) pyrimidin-5-yl)oxy)methyl)piperidin-1-yl)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)propanamide (**D1**)*

To a solution of intermediate **6** (0.30 g, 0, 63 mmol) and intermediate **11a** (0.51g, 1.25mmol) dissolving in DMF (10mL) add Triethylamine (TEA) (0.22g, 1.25mmol), and the mixture was stirred for 48h. After the reaction was completed, EA was added to dilute, saturated $NaHCO_3$ solution (50 mL×3) was added to wash. The EA phase was dried over anhydrous Na_2SO_4, filtered, concentrated. The residue was purified by column chromatography to obtain a white solid **D1** (0.25 g, yield 50.31%). ^{1}H NMR (600 MHz, DMSO-d_6) δ11.16 (s, 1H), 10.51 (s, 1H), 8.64 (s, 2H), 8.57 (d, J = 8.4 Hz, 1H), 8.38 (dd, J = 7.8, 1.9 Hz, 2H), 8.27 – 8.20 (m, 2H), 8.17 (d, J = 9.7 Hz, 1H), 7.93 (dt, J = 7.8, 1.4 Hz, 1H), 7.83 (dd, J = 8.4, 7.3 Hz, 1H), 7.72 (t, J = 7.9 Hz, 1H), 7.61 (d, J = 7.3 Hz, 1H), 7.50 – 7.45 (m, 2H), 7.16 (d, J = 9.7 Hz, 1H), 5.45 (s, 2H), 5.14 (dd, J = 12.8, 5.5 Hz, 1H), 4.13 – 3.88 (m, 2H), 3.11 – 2.98 (m, 2H), 2.89 (ddd, J = 16.8, 13.8, 5.5 Hz, 1H), 2.68 – 2.59 (m, 4H), 2.08 (dtd, J = 13.0, 5.2, 2.2 Hz, 1H), 1.99 (s, 2H), 1.76 (d, J = 12.4 Hz, 3H), 1.43 (pd, J = 11.9, 6.0 Hz, 2H), 1.23 (s, 2H). ^{13}C NMR (151 MHz, DMSO) δ 171.3, 170.1, 168.4, 166.0, 165.3, 157.4, 154.3, 150.2, 142.7, 140.6, 135.9, 135.5, 135.1, 134.6, 134.0, 131.4, 130.2, 129.4, 129.0, 128.8, 128.7, 128.2, 128.0, 127.5, 125.5, 125.0, 125.0, 117.1, 116.8, 115.3, 110.7, 71.6, 53.2, 52.1, 51.2, 51.1, 47.4, 33.9, 32.6, 29.5, 26.4, 26.4, 20.6, 12.5. ESI–HRMS: calculated for $C_{44}H_{39}N_9O_7$ $[M + H]^+$ 806.3051; found 806.3045. Purity is 98.791%, which was determined by HPLC.

*2.14. 4-(4-(((2-(3-((3-(3-Cyanophenyl)-6-oxopyridazin-1(6H)-yl)methyl) phenyl) pyrimidin-5-yl)oxy)methyl)piperidin-1-yl)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)butanamide (**D2**)*

Compound **D2** was synthesized from intermediate **6** and **11b** by a similar route to compound **D1**. Compound **D2** was obtained as a light yellow solid (0.21g, yield 43.36 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.17 (s, 1H), 9.86 (s, 1H), 8.66 (s, 2H), 8.42 (d, J = 8.4 Hz, 1H), 8.38 (d, J = 9.3 Hz, 2H), 8.24 (t, J = 7.2 Hz, 2H), 8.18 (d, J =

9.8 Hz, 1H), 7.93 (d, *J* = 7.7 Hz, 1H), 7.85 (t, *J* = 7.8 Hz, 1H), 7.72 (t, *J* = 7.9 Hz, 1H), 7.65 (d, *J* = 7.3 Hz, 1H), 7.49 (d, *J* = 6.2 Hz, 2H), 7.17 (d, *J* = 9.7 Hz, 1H), 5.45 (s, 2H), 5.16 (dd, *J* = 12.9, 5.5 Hz, 1H), 4.09 (d, *J* = 6.2 Hz, 2H), 3.55 (d, *J* = 11.6 Hz, 2H), 3.13 – 3.04 (m, 2H), 3.00 – 2.90 (m, 2H), 2.68 – 2.59 (m, 3H), 2.55 (s, 1H), 2.15 – 2.03 (m, 4H), 2.03 – 1.95 (m, 2H), 1.79 – 1.66 (m, 2H), 1.27 – 1.19 (m, 1H). ^{13}C NMR (151 MHz, DMSO) δ 171.3, 169.5, 168.3, 166.0, 165.2, 157.3, 154.5, 150.0, 142.8, 140.5, 135.8, 135.5, 134.7, 134.6, 134.0, 131.4, 130.1, 129.4, 128.9, 128.8, 128.7, 128.2, 128.0, 127.5, 125.5, 125.3, 124.9, 117.2, 117.0, 116.1, 110.7, 70.5, 53.9, 53.1, 49.7, 47.4, 40.6, 38.9, 31.7, 31.5, 29.5, 24.1, 21.0, 20.5, 17.5. ESI–HRMS (ESI$^+$): calculated for $C_{45}H_{41}N_9O_7$ $[M + H]^+$ 820.3207; found 820.3202. Purity is 98.101%, which was determined by HPLC.

*2.15. 5-(4-(((2-(3-((3-(3-Cyanophenyl)-6-oxopyridazin-1(6H)-yl)methyl)phenyl) pyrimidin-5-yl)oxy)methyl)piperidin-1-yl)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)pentanamide (**D3**)*

Compound **D3** was synthesized from intermediate **6** and **11c** by a similar route to compound **D1**. Compound **D3** was obtained as a light yellow solid (0.22g, yield 45.76%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.17 (s, 1H), 9.69 (s, 1H), 8.63 (s, 2H), 8.48 (d, *J* = 8.5 Hz, 1H), 8.38 (d, *J* = 12.8 Hz, 2H), 8.24 (d, *J* = 8.3 Hz, 2H), 8.17 (d, *J* = 9.8 Hz, 1H), 7.92 (d, *J* = 7.6 Hz, 1H), 7.83 (t, *J* = 7.9 Hz, 1H), 7.71 (t, *J* = 7.9 Hz, 1H), 7.61 (d, *J* = 7.3 Hz, 1H), 7.48 (s, 2H), 7.16 (d, *J* = 9.8 Hz, 1H), 5.44 (s, 2H), 5.16 (dd, *J* = 12.6, 5.5 Hz, 1H), 4.09 – 3.91 (m, 2H), 3.02 – 2.79 (m, 3H), 2.62 (d, *J* = 17.3 Hz, 2H), 2.29 (s, 2H), 2.09 (s, 2H), 1.95 – 1.81 (m, 2H), 1.74 (d, *J* = 11.7 Hz, 3H), 1.68 – 1.58 (m, 2H), 1.57 – 1.44 (m, 2H), 1.29 (q, *J* = 12.7 Hz, 2H), 1.24 – 1.13 (m, 1H). ^{13}C NMR (151 MHz, DMSO) δ 171.1, 170.4, 168.1, 166.1, 165.0, 157.1, 154.1, 150.0, 142.5, 140.3, 135.7, 135.3, 134.9, 134.5, 133.8, 131.2, 129.8, 129.2, 128.7, 128.6, 128.5, 128.0, 127.8, 127.3, 125.3, 124.7, 124.6, 116.9, 116.6, 115.3, 110.5, 71.3, 58.1, 56.1, 53.0, 51.2, 47.3, 34.7, 33.7, 29.3, 26.7, 24.2, 21.1, 20.3, 19.1, 12.4. ESI–HRMS: calculated for $C_{46}H_{43}N_9O_7$ $[M + H]^+$ 834.336; found 834.3358. Purity is 98.896%, which was determined by HPLC.

*2.16. 6-(4-(((2-(3-((3-(3-Cyanophenyl)-6-oxopyridazin-1(6H)-yl)methyl)phenyl) pyrimidin-5-yl)oxy)methyl)piperidin-1-yl)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)hexanamide (**D4**)*

Compound **D4** was synthesized from intermediate **6** and **11d** by a similar route to compound **D1**. Compound **D4** was obtained as a yellow solid (0.20g, yield 41.94%).^{1}H

NMR (600 MHz, DMSO-d_6) δ 11.17 (s, 1H), 9.70 (s, 1H), 8.63 (s, 2H), 8.48 (d, J = 8.4 Hz, 1H), 8.42 – 8.32 (m, 2H), 8.28 – 8.20 (m, 2H), 8.17 (d, J = 9.7 Hz, 1H), 7.93 (d, J = 7.6 Hz, 1H), 7.83 (t, J = 7.9 Hz, 1H), 7.72 (t, J = 7.9 Hz, 1H), 7.61 (d, J = 7.3 Hz, 1H), 7.53 – 7.43 (m, 2H), 7.16 (d, J = 9.8 Hz, 1H), 5.44 (s, 2H), 5.16 (dd, J = 12.9, 5.5 Hz, 1H), 4.02 (d, J = 6.1 Hz, 2H), 3.00 – 2.82 (m, 3H), 2.65 – 2.59 (m, 1H), 2.55 (td, J = 13.3, 4.6 Hz, 2H), 2.47 (t, J = 7.4 Hz, 2H), 2.37 – 2.20 (m, 2H), 2.13 – 2.02 (m, 1H), 1.89 (s, 1H), 1.81 – 1.70 (m, 3H), 1.64 (p, J = 7.5 Hz, 2H), 1.47 (dd, J = 14.4, 6.5 Hz, 2H), 1.32 (td, J = 15.6, 8.2 Hz, 4H). ^{13}C NMR (151 MHz, DMSO) δ 173.3, 172.5, 170.3, 168.2, 167.1, 159.3, 156.3, 152.1, 144.6, 142.5, 137.8, 137.4, 137.0, 136.6, 135.9, 133.3, 131.9, 131.3, 130.9, 130.7, 130.6, 130.1, 129.9, 129.5, 127.4, 126.9, 126.7, 119.0, 118.8, 117.4, 112.6, 73.4, 58.4, 55.4, 55.1, 53.3, 49.4, 36.9, 35.8, 31.4, 28.7, 26.9, 26.6, 25.2, 22.5. ESI–HRMS: calculated for $C_{47}H_{45}N_9O_7$ $[M + H]^+$ 848.3520; found 848.3515. Purity is 98.663%, which was determined by HPLC.

*2.17. 7-(4-(((2-(3-((3-(3-Cyanophenyl)-6-oxopyridazin-1(6H)-yl)methyl)phenyl) pyrimidin-5-yl)oxy)methyl)piperidin-1-yl)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)heptanamide (**D5**)*

Compound **D5** was synthesized from intermediate **6** and **11e** by a similar route to compound **D1**. Compound **D5** was obtained as a light yellow solid (0.17g, yield 42.62%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.17 (s, 1H), 9.69 (s, 1H), 8.62 (s, 2H), 8.48 (d, J = 8.4 Hz, 1H), 8.38 (dd, J = 12.4, 1.9 Hz, 2H), 8.27 – 8.19 (m, 2H), 8.17 (d, J = 9.8 Hz, 1H), 7.92 (dt, J = 7.7, 1.4 Hz, 1H), 7.89 – 7.78 (m, 1H), 7.71 (t, J = 7.9 Hz, 1H), 7.61 (d, J = 7.3 Hz, 1H), 7.53 – 7.40 (m, 2H), 7.16 (d, J = 9.8 Hz, 1H), 5.44 (s, 2H), 5.16 (dd, J = 12.9, 5.4 Hz, 1H), 4.01 (d, J = 5.9 Hz, 2H), 2.97 – 2.80 (m, 3H), 2.62 (ddd, J = 17.1, 4.4, 2.5 Hz, 1H), 2.55 (s, 1H), 2.46 (t, J = 7.4 Hz, 2H), 2.23 (t, J = 7.3 Hz, 2H), 2.12 – 2.03 (m, 1H), 1.84 (t, J = 11.2 Hz, 2H), 1.72 (dd, J = 10.9, 4.0 Hz, 3H), 1.63 (p, J = 7.4 Hz, 2H), 1.42 (p, J = 7.2 Hz, 2H), 1.31 (ddt, J = 26.3, 9.9, 5.3 Hz, 6H). ^{13}C NMR (151 MHz, DMSO) δ 173.2, 172.5, 170.3, 168.2, 167.1, 159.2, 156.3, 152.1, 144.6, 142.4, 137.8, 137.4, 137.0, 136.6, 135.9, 133.3, 131.9, 131.3, 130.8, 130.7, 130.6, 130.1, 129.9, 129.4, 127.4, 126.9, 126.7, 119.0, 118.7, 117.4, 112.6, 73.4, 58.6, 55.1, 53.4, 49.4, 40.9, 37.0, 35.9, 31.4, 28.9, 28.9, 27.2, 26.8, 25.2, 22.5. ESI–HRMS: calculated for $C_{48}H_{47}N_9O_7$ $[M + H]^+$ 862.3677; found 862.3672. Purity is 98.142%, which was determined by HPLC.

2.18. 8-(4-(((2-(3-((3-(3-Cyanophenyl)-6-oxopyridazin-1(6H)-yl)methyl)phenyl) pyrimidin-5-yl)oxy)methyl)piperidin-1-yl)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-

*dioxoisoindolin-4-yl)octanamide (**D6**)*

Compound **D6** was synthesized from intermediate **6** and **11f** by a similar route to compound **D1**. Compound **D6** was obtained as a yellow solid (0.16g, yield 39.93%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.18 (s, 1H), 9.71 (s, 1H), 8.66 (s, 2H), 8.47 (d, J = 8.4 Hz, 1H), 8.38 (d, J = 8.6 Hz, 2H), 8.24 (t, J = 7.6 Hz, 2H), 8.18 (d, J = 9.8 Hz, 1H), 7.93 (d, J = 7.7 Hz, 1H), 7.84 (t, J = 7.9 Hz, 1H), 7.72 (t, J = 7.8 Hz, 1H), 7.62 (d, J = 7.3 Hz, 1H), 7.49 (d, J = 6.2 Hz, 2H), 7.17 (d, J = 9.7 Hz, 1H), 5.45 (s, 2H), 5.16 (dd, J = 12.8, 5.5 Hz, 1H), 4.09 (s, 2H), 3.46 (d, J = 35.5 Hz, 2H), 3.02 – 2.74 (m, 4H), 2.63 (dt, J = 17.0, 3.4 Hz, 1H), 2.59 – 2.52 (m, 2H), 2.48 (t, J = 7.4 Hz, 2H), 2.14 – 2.02 (m, 2H), 1.94 (d, J = 12.8 Hz, 2H), 1.75 – 1.52 (m, 6H), 1.43 – 1.25 (m, 6H). ^{13}C NMR (151 MHz, DMSO) δ 173.3, 172.5, 170.3, 168.2, 167.1, 159.3, 156.4, 152.0, 144.7, 142.5, 137.8, 137.4, 137.0, 136.6, 135.9, 133.3, 131.9, 131.3, 130.9, 130.7, 130.6, 130.2, 129.9, 129.5, 127.4, 126.9, 126.8, 119.0, 118.8, 117.5, 112.6, 72.6, 60.2, 56.7, 55.1, 51.8, 49.4, 40.9, 36.9, 33.8, 31.4, 28.7, 26.6, 25.1, 24.0, 22.5, 21.2, 14.6. ESI–HRMS: calculated for $C_{49}H_{49}N_9O_7$ $[M + H]^+$ 876.3833; found 876.3828. Purity is 98.554%, which was determined by HPLC.

*2.19. 9-(4-(((2-(3-((3-(3-Cyanophenyl)-6-oxopyridazin-1(6H)-yl)methyl)phenyl) pyrimidin-5-yl)oxy)methyl)piperidin-1-yl)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)nonanamide (**D7**)*

Compound **D7** was synthesized from intermediate **6** and **11g** by a similar route to compound **D1**. Compound **D7** was obtained as a light yellow solid (0.18g, yield 38.62 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.18 (s, 1H), 9.69 (s, 1H), 8.63 (s, 2H), 8.48 (d, J = 8.4 Hz, 1H), 8.38 (dd, J = 11.5, 1.9 Hz, 2H), 8.23 (td, J = 8.8, 8.2, 2.0 Hz, 2H), 8.17 (d, J = 9.7 Hz, 1H), 7.93 (d, J = 7.7 Hz, 1H), 7.82 (t, J = 7.9 Hz, 1H), 7.71 (t, J = 7.8 Hz, 1H), 7.61 (d, J = 7.3 Hz, 1H), 7.51 – 7.43 (m, 2H), 7.16 (d, J = 9.8 Hz, 1H), 5.44 (s, 2H), 5.16 (dd, J = 12.9, 5.4 Hz, 1H), 4.02 (d, J = 5.9 Hz, 2H), 2.91 (ddd, J = 17.1, 13.8, 5.5 Hz, 3H), 2.62 (ddd, J = 17.2, 4.5, 2.5 Hz, 1H), 2.59 – 2.52 (m, 1H), 2.46 (t, J = 7.5 Hz, 2H), 2.28 (s, 2H), 2.08 (dtd, J = 13.1, 5.3, 2.3 Hz, 1H), 1.90 (s, 2H), 1.75 (d, J = 12.4 Hz, 3H), 1.62 (p, J = 7.2 Hz, 2H), 1.48 – 1.37 (m, 2H), 1.36 – 1.21 (m, 11H). ^{13}C NMR (151 MHz, DMSO) δ 171.4, 170.6, 168.4, 166.3, 165.3, 157.4, 154.4, 150.3, 142.8, 140.6, 136.0, 135.6, 135.2, 134.7, 134.1, 131.5, 130.1, 129.4, 129.0, 128.9, 128.8, 128.3, 128.1, 127.6, 125.6, 125.0, 124.8, 117.1, 116.9, 115.5, 110.8, 71.5, 56.7, 53.2, 51.3, 47.5, 39.1, 35.2, 33.8, 29.6, 27.4, 27.3, 27.1, 25.5, 24.9, 23.4, 20.6.ESI–HRMS: calculated for $C_{50}H_{51}N_9O_7$ $[M + H]^+$ 890.3990; found 890.3982. Purity is

97.632%, which was determined by HPLC.

*2.20. 10-(4-(((2-(3-((3-(3-Cyanophenyl)-6-oxopyridazin-1(6H)-yl)methyl)phenyl) pyrimidin-5-yl)oxy)methyl)piperidin-1-yl)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)decanamide (**D8**)*

Compound **D8** was synthesized from intermediate **6** and **11h** by a similar route to compound **D1**. Compound **D8** was obtained as a yellow solid (0.18g, yield 37.33%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.19 (s, 1H), 9.68 (s, 1H), 8.62 (s, 2H), 8.48 (d, J = 8.5 Hz, 1H), 8.38 (dt, J = 14.0, 1.8 Hz, 2H), 8.26 – 8.20 (m, 2H), 8.17 (d, J = 9.8 Hz, 1H), 7.92 (dt, J = 7.7, 1.3 Hz, 1H), 7.82 (dd, J = 8.4, 7.3 Hz, 1H), 7.71 (t, J = 7.9 Hz, 1H), 7.60 (d, J = 7.3 Hz, 1H), 7.51 – 7.45 (m, 2H), 7.16 (d, J = 9.7 Hz, 1H), 5.44 (s, 2H), 5.16 (dd, J = 12.9, 5.5 Hz, 1H), 4.01 (d, J = 5.9 Hz, 2H), 2.98 – 2.78 (m, 3H), 2.62 (ddd, J = 17.2, 4.5, 2.5 Hz, 1H), 2.56 (d, J = 6.5 Hz, 1H), 2.45 (t, J = 7.4 Hz, 2H), 2.21 (t, J = 7.3 Hz, 2H), 2.08 (ddq, J = 10.6, 5.5, 2.7 Hz, 1H), 1.83 (t, J = 11.4 Hz, 2H), 1.77 – 1.68 (m, 3H), 1.62 (p, J = 7.4 Hz, 2H), 1.38 (p, J = 7.0 Hz, 2H), 1.34 – 1.21 (m, 12H). ^{13}C NMR (151 MHz, DMSO) δ 173.2, 172.5, 170.3, 168.2, 167.1, 159.2, 156.3, 152.1, 144.6, 142.4, 137.8, 137.4, 137.1, 136.6, 135.9, 133.3, 131.9, 131.3, 130.8, 130.7, 130.6, 130.1, 129.9, 129.4, 127.4, 126.9, 126.7, 119.0, 118.7, 117.3, 112.6, 73.4, 58.7, 55.1, 53.3, 49.4, 40.9, 37.0, 35.9, 31.4, 29.4, 29.3, 29.2, 28.9, 28.9, 27.5, 27.0, 25.2, 22.5. ESI–HRMS: calculated for $C_{51}H_{53}N_9O_7$ $[M + H]^+$ 904.4146; found 904.4141. Purity is 98.027%, which was determined by HPLC.

*2.21. 5-(4-(((2-(3-((3-(3-cyanophenyl)-6-oxopyridazin-1(6H)-yl)methyl)phenyl) pyrimidin-5-yl)oxy)methyl)piperidin-1-yl)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)pentanamide (**D9**)*

Compound **D9** was synthesized from intermediate **6** and **13** by a similar route to compound **D1**. Compound **D9** was obtained as a yellow solid (0.22g, yield 45.71%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.13 (s, 1H), 11.10 – 10.94 (m, 1H), 8.66 (s, 2H), 8.38 (d, J = 7.0 Hz, 2H), 8.33 (s, 1H), 8.27 – 8.22 (m, 2H), 8.18 (d, J = 9.8 Hz, 1H), 8.02 (ddt, J = 7.8, 5.1, 2.7 Hz, 1H), 7.93 (d, J = 7.7 Hz, 1H), 7.87 (d, J = 8.2 Hz, 1H), 7.72 (t, J = 7.9 Hz, 1H), 7.49 (q, J = 4.4, 3.1 Hz, 2H), 7.16 (d, J = 9.7 Hz, 1H), 5.45 (s, 2H), 5.13 (dd, J = 12.9, 5.4 Hz, 1H), 4.08 (s, 2H), 3.01 (s, 2H), 2.90 (ddd, J = 17.1, 13.9, 5.4 Hz, 2H), 2.61 (dt, J = 17.3, 3.3 Hz, 1H), 2.12 – 1.99 (m, 2H), 1.99 – 1.90 (m, 2H), 1.80 – 1.59 (m, 6H), 1.30 (s, 1H), 1.26 (s, 1H), 1.23 (d, J = 8.1 Hz, 3H), 0.85 (t, J = 6.9 Hz, 1H). ^{13}C NMR (151 MHz, DMSO) δ 173.3, 172.5, 170.4, 167.6, 167.3, 159.3, 156.4, 152.0, 145.7, 144.7, 142.5, 137.8, 137.5, 135.9, 133.4, 133.2, 131.3, 130.9, 130.8,

130.6, 130.2, 129.9, 129.5, 127.4, 126.9, 125.2, 125.1, 124.0, 119.0, 113.4, 112.6, 72.6, 56.3, 55.1, 51.8, 49.4, 36.3, 31.8, 31.6, 31.4, 30.9, 30.3, 29.5, 22.6, 22.5, 14.4. ESI–HRMS: calculated for $C_{46}H_{43}N_9O_7$ $[M + H]^+$ 834.336; found 834.3357. Purity is 97.630%, which was determined by HPLC.

*2.22. 2-(4-(((2-(3-((3-(3-Cyanophenyl)-6-oxopyridazin-1(6H)-yl)methyl)phenyl) pyrimidin-5-yl)oxy)methyl)piperidin-1-yl)-N-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)amino)ethoxy)ethyl)acetamide (**D10**)*

To a solution of intermediate **8** (0.30g, 0.56 mmol) and intermediate **17a** (0.21 g, 0.56 mmol) dissolving in DMF (30 mL) slowly add HATU (0.32 g, 0.83 mmol), DIPEA (0.11g, 0.83 mmol) at 0 °C, the mixture was stirred for 48 h. After the reaction was completed, EA was added to dilute, saturated $NaHCO_3$ solution (50 mL×3) was added to wash. The EA phase was dried over anhydrous Na_2SO_4, filtered, concentrated. The residue was purified by column chromatography to obtain a yellow-green solid (0.14 g, yield 28.32%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.06 (s, 1H), 8.62 (s, 2H), 8.45 – 8.31 (m, 2H), 8.25 (dt, J = 8.0, 1.4 Hz, 1H), 8.23 – 8.21 (m, 1H), 8.19 (d, J = 9.8 Hz, 1H), 7.93 (dt, J = 7.9, 1.4 Hz, 1H), 7.72 (t, J = 7.9 Hz, 1H), 7.55 (d, J = 8.3 Hz, 1H), 7.51 – 7.46 (m, 2H), 7.32 (s, 1H), 7.16 (d, J = 9.7 Hz, 1H), 7.04 (s, 1H), 6.96 – 6.87 (m, 1H), 5.45 (s, 2H), 5.02 (dd, J = 12.8, 5.5 Hz, 1H), 4.02 (d, J = 6.0 Hz, 2H), 3.61 (t, J = 5.4 Hz, 2H), 3.49 (t, J = 5.7 Hz, 2H), 3.36 – 3.32 (m, 3H), 3.30 (q, J = 5.8 Hz, 2H), 2.86 (ddd, J = 16.6, 13.6, 5.4 Hz, 5H), 2.58 – 2.52 (m, 1H), 2.50 – 2.46 (m, 1H), 2.11 (d, J = 19.6 Hz, 2H), 2.01 – 1.93 (m, 1H), 1.75 (d, J = 12.6 Hz, 3H), 1.50 – 1.30 (m, 2H). ^{13}C NMR (151 MHz, DMSO) δ 173.3, 170.6, 168.2, 167.6, 159.3, 156.3, 155.0, 152.1, 144.6, 142.5, 137.8, 137.4, 135.9, 134.6, 133.4, 131.4, 130.9, 130.8, 130.6, 130.1, 129.9, 129.5, 127.4, 126.9, 125.5, 119.0, 116.5, 112.6, 73.3, 69.4, 69.0, 68.8, 56.3, 55.4, 55.1, 53.3, 49.1, 43.0, 38.5, 32.6, 31.4, 30.1, 22.7. ESI–HRMS: calculated for $C_{47}H_{46}N_{10}O_8$ $[M + H]^+$ 879.3578; found 879.3573. Purity is 98.171%, which was determined by HPLC.

*2.23. 2-(4-(((2-(3-((3-(3-Cyanophenyl)-6-oxopyridazin-1(6H)-yl)methyl)phenyl) pyrimidin-5-yl)oxy)methyl)piperidin-1-yl)-N-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)amino)ethoxy)ethoxy)ethyl)acetamide (**D11**)*

Compound **D11** was synthesized from intermediate **8** and **17b** by a similar route to compound **D10**. Compound **D11** was obtained as a yellow solid (0.12g, yield 25.42%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.06 (s, 1H), 8.62 (s, 2H), 8.38 (dt, J = 9.6, 1.8 Hz, 2H), 8.23 (ddt, J = 10.7, 6.6, 1.8 Hz, 2H), 8.17 (d, J = 9.7 Hz, 1H), 7.92 (dt, J = 7.7, 1.4

Hz, 1H), 7.71 (t, *J* = 7.9 Hz, 2H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.51 – 7.45 (m, 2H), 7.19 – 7.12 (m, 2H), 7.00 (d, *J* = 2.1 Hz, 1H), 6.88 (dd, *J* = 8.4, 2.1 Hz, 1H), 5.44 (s, 2H), 5.03 (dd, *J* = 12.8, 5.5 Hz, 1H), 4.03 (d, *J* = 5.9 Hz, 2H), 3.59 (t, *J* = 5.5 Hz, 2H), 3.58 – 3.51 (m, 4H), 3.44 (t, *J* = 5.8 Hz, 2H), 3.27 (q, *J* = 5.9 Hz, 2H), 3.18 (d, *J* = 4.9 Hz, 1H), 2.98 – 2.75 (m, 5H), 2.57 (dt, *J* = 16.8, 3.2 Hz, 1H), 2.07 (d, *J* = 20.9 Hz, 2H), 2.01 – 1.95 (m, 1H), 1.75 (d, *J* = 12.1 Hz, 3H), 1.44 – 1.35 (m, 2H), 1.22 (s, 2H). ^{13}C NMR (151 MHz, DMSO) *δ* 173.2, 171.5, 168.9, 166.4, 165.8, 157.5, 154.5, 153.1, 150.3, 142.8, 140.7, 136.0, 135.6, 134.1, 132.8, 131.5, 129.5, 129.1, 128.9, 128.8, 128.3, 128.1, 127.6, 125.6, 125.1, 123.7, 117.2, 114.8, 110.8, 71.5, 68.5, 68.2, 67.7, 67.4, 53.6, 53.3, 51.6, 47.3, 41.1, 36.7, 32.3, 29.7, 27.7, 23.2, 20.9, 20.8, 12.6. ESI–HRMS: calculated for $C_{49}H_{50}N_{10}O_9$ $[M + H]^+$ 923.3840; found 923.3834. Purity is 97.768%, which was determined by HPLC.

*2.24. 2-(4-(((2-(3-((3-(3-Cyanophenyl)-6-oxopyridazin-1(6H)-yl)methyl)phenyl) pyrimidin-5-yl)oxy)methyl)piperidin-1-yl)-N-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)acetamide (**D12**)*

Compound **D12** was synthesized from intermediate **8** and **17c** by a similar route to compound **D10**. Compound **D12** was obtained as a yellow solid (0.11g, yield 22.86%). ^{1}H NMR (600 MHz, DMSO-d_6) *δ* 11.07 (s, 1H), 8.64 (s, 2H), 8.43 – 8.34 (m, 2H), 8.27 – 8.20 (m, 2H), 8.17 (d, *J* = 9.7 Hz, 1H), 7.93 (dt, J = 7.7, 1.4 Hz, 1H), 7.71 (t, *J* = 7.9 Hz, 2H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.51 – 7.45 (m, 2H), 7.20 (t, *J* = 5.6 Hz, 1H), 7.16 (d, *J* = 9.7 Hz, 1H), 7.00 (d, *J* = 2.2 Hz, 1H), 6.88 (dd, *J* = 8.4, 2.1 Hz, 1H), 5.44 (s, 2H), 5.04 (dd, *J* = 12.8, 5.5 Hz, 1H), 4.04 (d, *J* = 5.9 Hz, 2H), 3.58 (t, *J* = 5.5 Hz, 2H), 3.56 – 3.48 (m, 8H), 3.43 (d, *J* = 11.6 Hz, 2H), 3.34 (d, *J* = 5.6 Hz, 1H), 3.26 (q, *J* = 5.8 Hz, 2H), 2.88 (ddt, *J* = 23.4, 11.8, 5.8 Hz, 5H), 2.58 (dt, *J* = 19.9, 4.5 Hz, 1H), 2.11 (d, *J* = 20.3 Hz, 3H), 2.00 (dtt, *J* = 12.8, 5.4, 2.9 Hz, 1H), 1.83 – 1.71 (m, 3H), 1.39 (q, *J* = 12.3 Hz, 2H), 1.15 (s, 1H). ^{13}C NMR (151 MHz, DMSO) *δ* 207.8, 205.9, 172.2, 169.6, 167.1, 166.5, 158.2, 155.2, 153.8, 151.0, 143.5, 141.4, 136.7, 136.3, 134.8, 133.5, 132.2, 130.2, 129.8, 129.6, 129.5, 129.0, 128.8, 128.3, 126.3, 125.8, 124.4, 117.9, 115.4, 111.5, 72.2, 69.2, 69.1, 68.9, 68.4, 68.1, 67.9, 55.2, 54.0, 52.3, 48.0, 41.8, 37.4, 34.1, 31.5, 30.4, 30.1, 29.0, 27.6, 21.6. ESI–HRMS: calculated for $C_{51}H_{54}N_{10}O_{10}$ $[M + H]^+$ 967.4103; found 967.4098. Purity is 98.099%, which was determined by HPLC.

*2.25. 2-(4-(((2-(3-((3-(3-Cyanophenyl)-6-oxopyridazin-1(6H)-yl)methyl)phenyl) pyrimidin-5-yl)oxy)methyl)piperidin-1-yl)-N-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethyl)acetamide (**D13**)*

Compound **D13** was synthesized from intermediate **8** and **20** by a similar route to compound **D10**. Compound **D13** was obtained as a yellow solid (0.18g, yield 38.32%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.11 (s, 1H), 8.61 (s, 2H), 8.40 – 8.34 (m, 2H), 8.26 – 8.20 (m, 2H), 8.17 (d, J = 9.8 Hz, 1H), 7.92 (dt, J = 7.7, 1.4 Hz, 1H), 7.74 – 7.66 (m, 2H), 7.56 (dd, J = 8.5, 7.1 Hz, 1H), 7.51 – 7.45 (m, 2H), 7.14 (dd, J = 20.6, 9.1 Hz, 2H), 7.01 (d, J = 7.0 Hz, 1H), 6.60 (t, J = 5.8 Hz, 1H), 5.45 (s, 2H), 5.06 (dd, J = 12.9, 5.5 Hz, 1H), 3.99 (d, J = 6.0 Hz, 2H), 3.62 (t, J = 5.5 Hz, 2H), 3.48 (dt, J = 16.6, 5.8 Hz, 4H), 3.30 (q, J = 5.9 Hz, 2H), 2.94 – 2.87 (m, 3H), 2.82 (d, J = 10.8 Hz, 2H), 2.59 (dt, J = 16.9, 3.3 Hz, 1H), 2.11 – 1.96 (m, 3H), 1.80 – 1.67 (m, 3H), 1.40 – 1.30 (m, 2H), 1.22 (d, J = 4.5 Hz, 1H). ^{13}C NMR (151 MHz, DMSO) δ 172.2, 169.4, 168.9, 168.4, 166.6, 158.2, 155.2, 151.0, 145.8, 143.5, 141.4, 136.8, 136.3, 135.6, 134.8, 132.2, 131.4, 130.2, 129.8, 129.6, 129.5, 129.0, 128.8, 128.4, 126.3, 125.8, 117.9, 116.8, 111.5, 110.1, 108.7, 72.3, 68.3, 68.0, 61.0, 54.3, 54.0, 52.3, 47.9, 41.1, 37.4, 34.2, 30.4, 27.6, 21.5. ESI–HRMS: calculated for $C_{47}H_{46}N_{10}O_8$ $[M + H]^+$ 879.3578; found 879.3570. Purity is 98.797%, which was determined by HPLC.

*2.26. 3-(1-(3-(5-((1-(2-(4-(2-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl) amino)ethyl)piperazin-1-yl)-2-oxoethyl)piperidin-4-yl)methoxy)pyrimidin-2-yl)benzyl)-6-oxo-1,6-dihydropyridazin-3-yl)benzonitrile (**D14**)*

To a solution of intermediate **8** (0.34g, 0.56 mmol) and intermediate **23** (0.21 g, 0.56 mmol) dissolving in DMF (30 mL) slowly add HATU (0.32 g, 0.83 mmol), DIPEA (0.12g, 0.83 mmol) at 0 °C, the mixture was stirred for 48 h. After the reaction was completed, EA was added to dilute, saturated $NaHCO_3$ solution (50 mL×3) was added to wash. The EA phase was dried over anhydrous Na_2SO_4, filtered, concentrated. The residue was purified by column chromatography to obtain a yellow-green solid (0.05 g, yield 10.03%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.08 (s, 1H), 8.64 (s, 2H), 8.38 (d, J = 13.4 Hz, 2H), 8.24 (t, J = 6.7 Hz, 2H), 8.17 (d, J = 9.8 Hz, 1H), 7.93 (d, J = 7.7 Hz, 1H), 7.71 (t, J = 7.9 Hz, 1H), 7.57 (d, J = 8.4 Hz, 1H), 7.48 (d, J = 6.4 Hz, 2H), 7.16 (d, J = 9.7 Hz, 1H), 7.06 (t, J = 5.4 Hz, 1H), 7.04 – 7.00 (m, 1H), 6.94 – 6.86 (m, 1H), 5.45 (s, 2H), 5.04 (dd, J = 12.8, 5.5 Hz, 1H), 4.04 (d, J = 5.9 Hz, 2H), 3.52 (d, J = 46.6 Hz, 4H), 3.32 (d, J = 6.2 Hz, 2H), 2.99 – 2.80 (m, 3H), 2.63 – 2.53 (m, 4H), 2.45 (d, J = 39.3 Hz, 4H), 2.01 (ddd, J = 10.8, 6.3, 3.6 Hz, 2H), 1.79 (d, J = 12.0 Hz, 3H), 1.44 – 1.17 (m, 5H). ^{13}C NMR (151 MHz, DMSO) δ 173.3, 170.7, 168.2, 167.6, 159.3, 156.3, 154.8, 152.1, 144.6, 142.5, 137.8, 137.4, 135.9, 134.7, 133.3, 131.3, 130.9, 130.7, 130.6, 130.1, 129.9, 129.4, 127.4, 126.9, 125.5, 119.0, 116.5, 112.6, 73.2, 56.6, 55.4, 55.1,

53.7, 53.1, 53.0, 49.1, 45.5, 41.7, 35.2, 31.5, 29.5, 29.2, 29.0, 22.7, 14.4. ESI–HRMS: calculated for $C_{49}H_{49}N_{11}O_7$ $[M + H]^+$ 904.3895; found 904.3885. Purity is 97.921%, which was determined by HPLC.

*2.27. 3-(1-(3-(5-((1-(2-(4-(2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl) piperazin-1-yl)-2-oxoethyl)piperidin-4-yl)methoxy) pyrimidin-2-yl)benzyl)-6-oxo-1,6-dihydropyridazin-3-yl)benzonitrile (**D15**)*

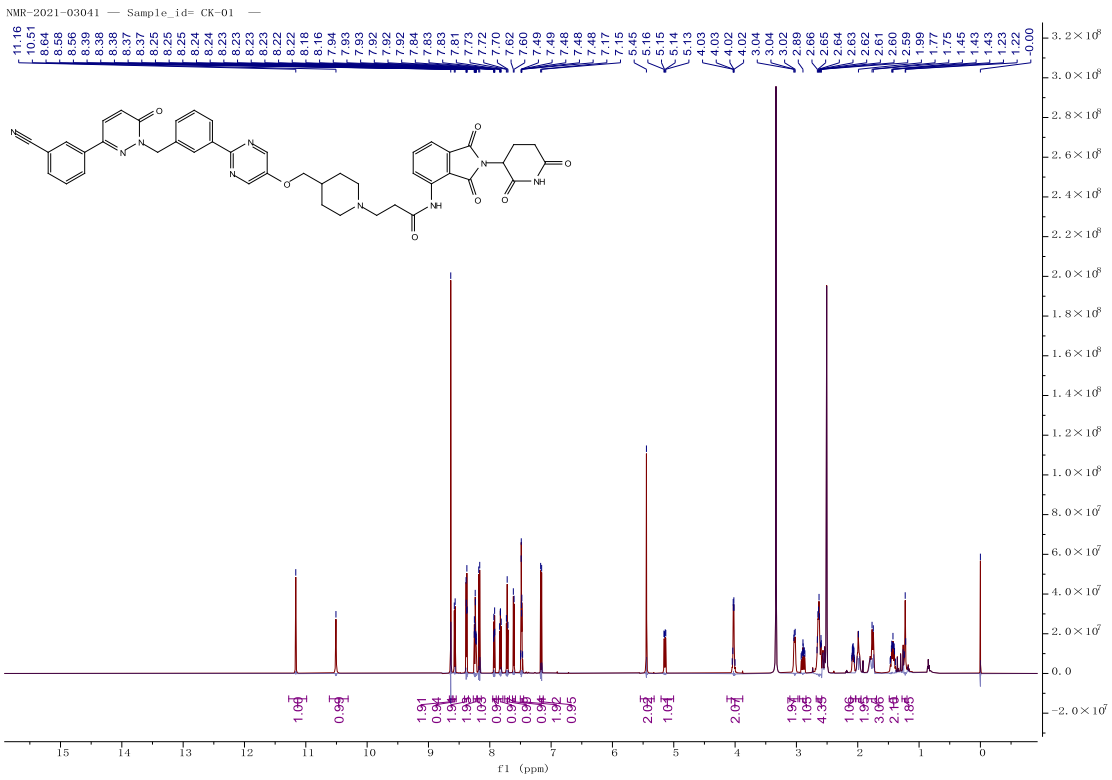
Compound **D15** was synthesized from intermediate **8** and **25a** by a similar route to compound **D14**. Compound **D15** was obtained as a yellow solid (0.07g, yield 15.26%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.10 (s, 1H), 8.64 (s, 2H), 8.38 (d, J = 4.6 Hz, 2H), 8.27 – 8.20 (m, 2H), 8.18 (d, J = 9.7 Hz, 1H), 7.93 (d, J = 7.6 Hz, 1H), 7.75 – 7.66 (m, 2H), 7.48 (d, J = 5.8 Hz, 2H), 7.40 – 7.35 (m, 1H), 7.27 (dd, J = 8.7, 2.3 Hz, 1H), 7.16 (d, J = 9.6 Hz, 1H), 5.44 (s, 2H), 5.08 (dd, J = 12.8, 5.5 Hz, 1H), 4.05 (d, J = 6.1 Hz, 2H), 3.73 (s, 2H), 3.63 – 3.45 (m, 6H), 3.21 (s, 1H), 3.00 – 2.80 (m, 3H), 2.64 – 2.56 (m, 1H), 2.10 – 1.97 (m, 2H), 1.79 (d, J = 11.5 Hz, 3H), 1.45 – 1.17 (m, 5H).^{13}C NMR (151 MHz, DMSO) δ 173.3, 170.6, 168.0, 167.5, 159.3, 156.3, 155.4, 152.1, 144.7, 142.5, 137.8, 137.4, 135.9, 134.3, 133.4, 131.3, 130.9, 130.8, 130.6, 130.1, 129.9, 129.5, 127.4, 126.9, 125.4, 119.0, 118.4, 112.6, 108.5, 73.3, 55.4, 55.1, 53.6, 53.0, 49.3, 47.6, 47.0, 44.8, 41.2, 35.3, 31.5, 28.8, 22.6, 18.4, 17.2, 12.5. ESI–HRMS: calculated for $C_{47}H_{44}N_{10}O_7$ $[M + H]^+$ 861.3473; found 861.3467. Purity is 99.820%, which was determined by HPLC.

*2.28. 3-(1-(3-(5-((1-(2-(4-(2-(1-Methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)piperazin-1-yl)-2-oxoethyl)piperidin-4-yl)methoxy)pyrimidin-2-yl)benzyl)-6-oxo-1,6-dihydropyridazin-3-yl)benzonitrile (**D16**)*

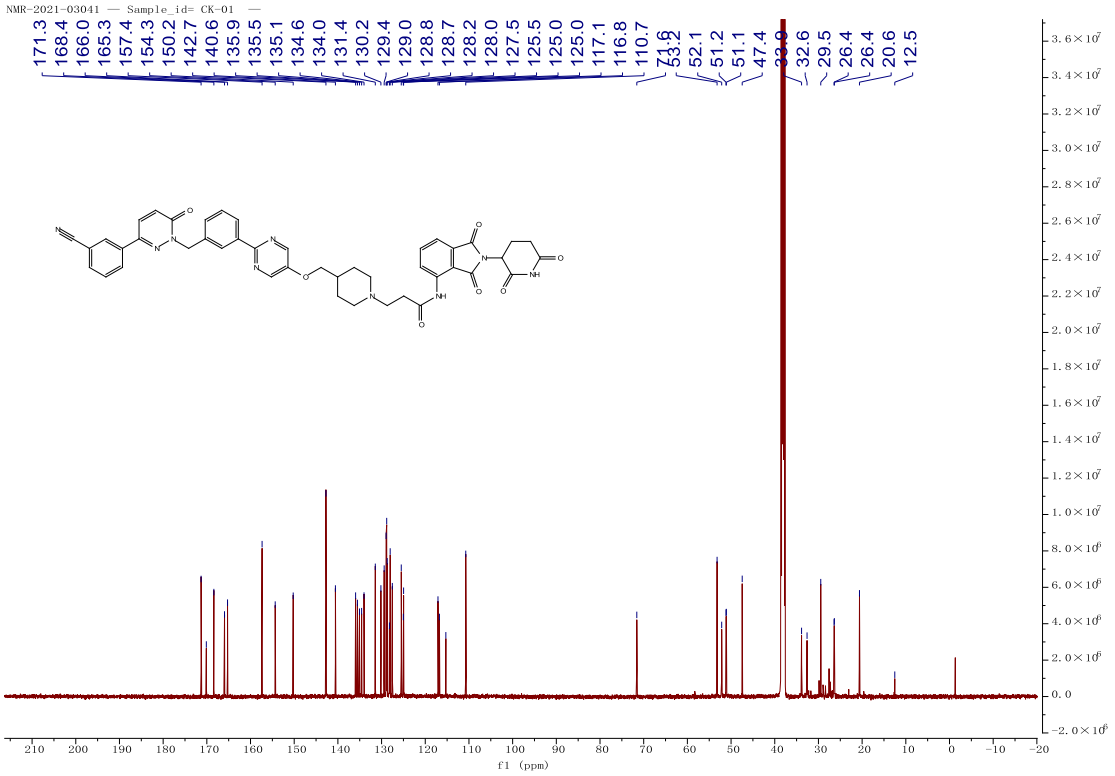
Compound **D16** was synthesized from intermediate **8** and **25b** by a similar route to compound **D14**. Compound **D16** was obtained as a yellow solid (0.10g, yield 21.37%) ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.64 (s, 2H), 8.38 (s, 2H), 8.24 (d, J = 8.4 Hz, 1H), 8.23 – 8.20 (m, 1H), 8.17 (d, J = 9.7 Hz, 1H), 7.93 (dt, J = 7.6, 1.4 Hz, 1H), 7.76 – 7.65 (m, 2H), 7.48 (d, J = 6.1 Hz, 2H), 7.37 (s, 1H), 7.27 (dd, J = 8.7, 2.4 Hz, 1H), 7.16 (d, J = 9.7 Hz, 1H), 5.44 (s, 2H), 5.15 (dd, J = 13.1, 5.3 Hz, 1H), 4.05 (d, J = 5.9 Hz, 2H), 3.74 (s, 2H), 3.64 – 3.41 (m, 6H), 3.19 (s, 2H), 3.01 (s, 3H), 2.98 – 2.91 (m, 1H), 2.88 (d, J = 11.5 Hz, 2H), 2.79 – 2.71 (m, 1H), 2.55 (dd, J = 13.4, 4.6 Hz, 1H), 2.08 – 1.98 (m, 3H), 1.77 (d, J = 11.5 Hz, 3H), 1.32 (d, J = 11.6 Hz, 2H). ^{13}C NMR (151 MHz, DMSO) δ 171.2, 169.2, 166.9, 166.4, 158.2, 155.2, 154.4, 151.1, 143.6, 141.4, 136.8, 136.4, 134.9, 133.2, 132.3, 130.3, 129.8, 129.7, 129.5, 129.1, 128.9, 128.4, 126.3, 125.8,

124.4, 117.9, 117.9, 117.3, 111.6, 107.4, 72.3, 60.6, 54.0, 52.0, 48.8, 48.0, 46.6, 46.0, 43.8, 40.1, 34.3, 30.5, 28.4, 27.8, 26.0, 20.8. ESI–HRMS: calculated for $C_{48}H_{46}N_{10}O_7$ $[M + H]^+$ 875.3629; found 875.3629. Purity is 98.746%, which was determined by HPLC.

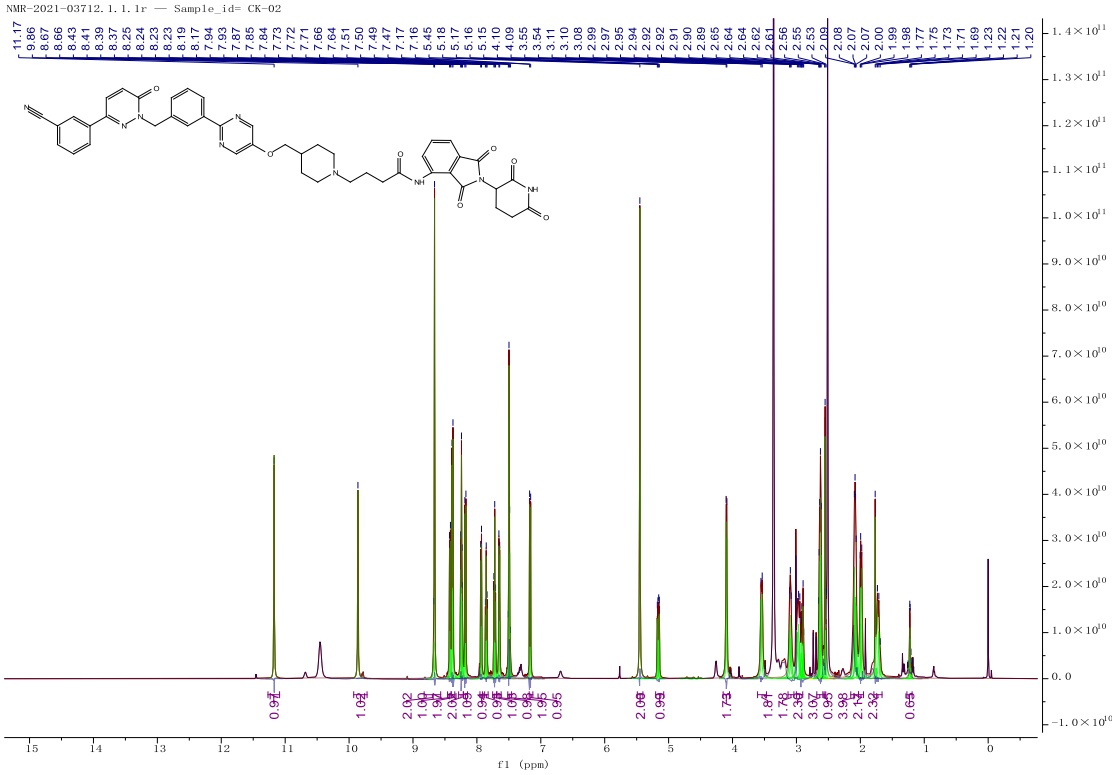
3. ^{1}H NMR and ^{13}C NMR of D1-D16



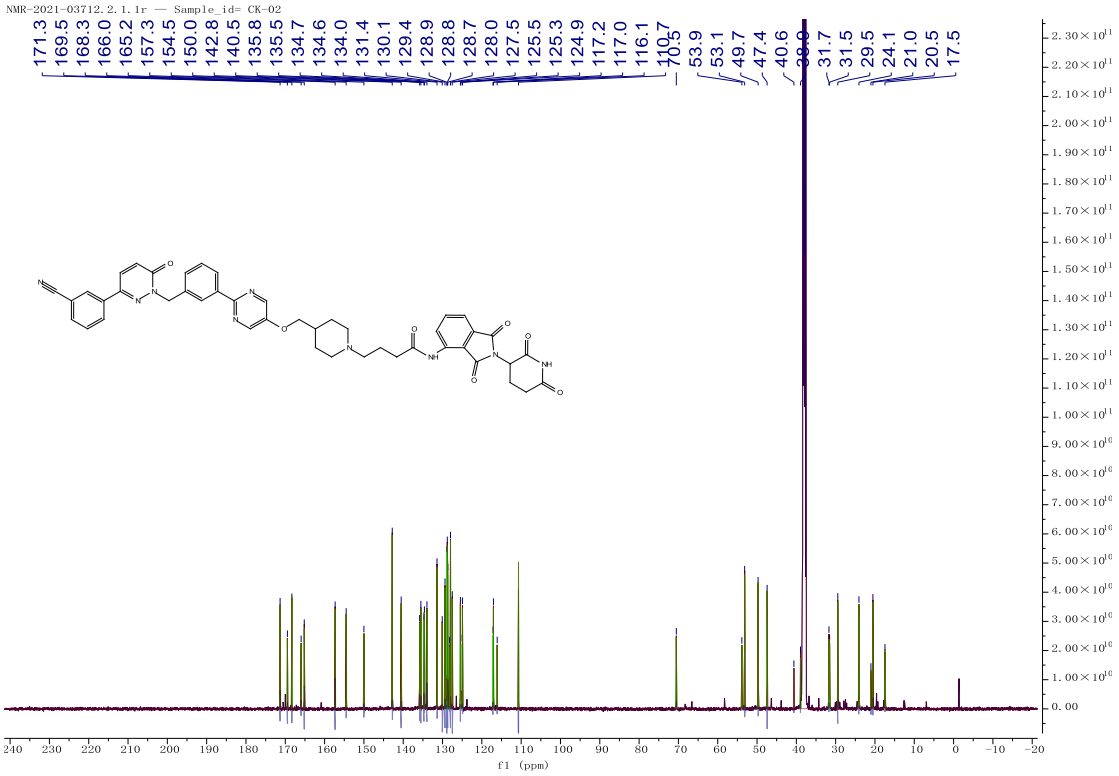
^{1}H NMR of **D1**



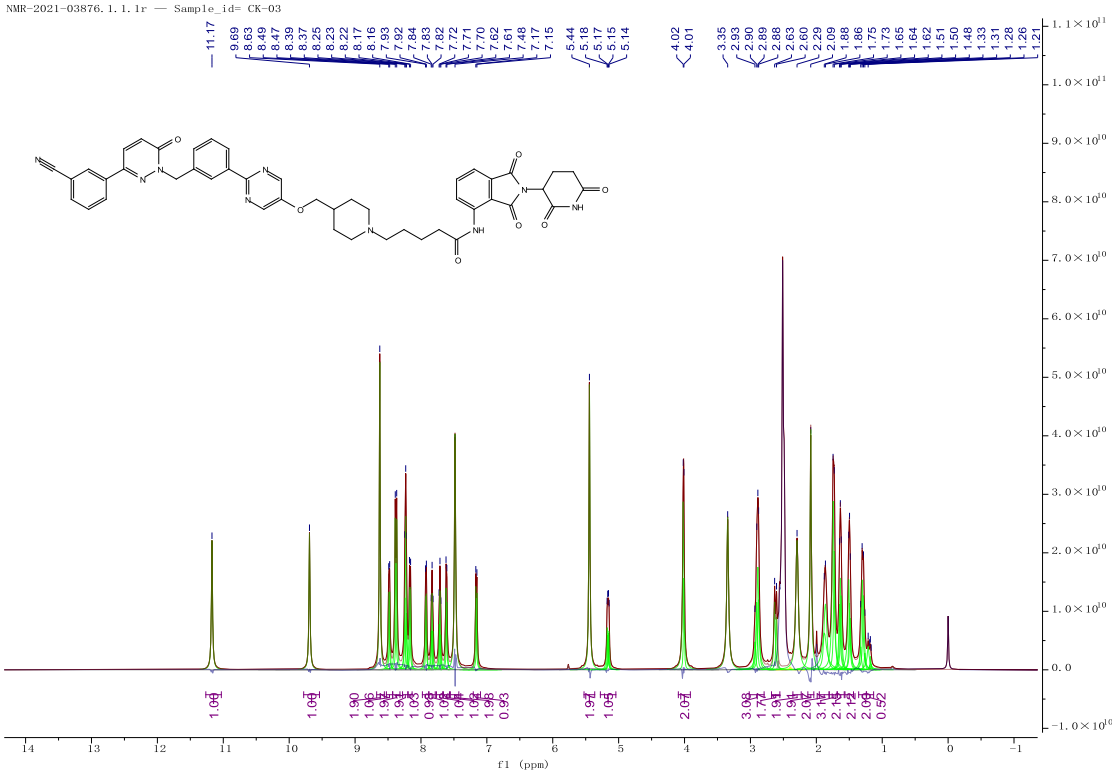
^{13}C NMR of **D1**



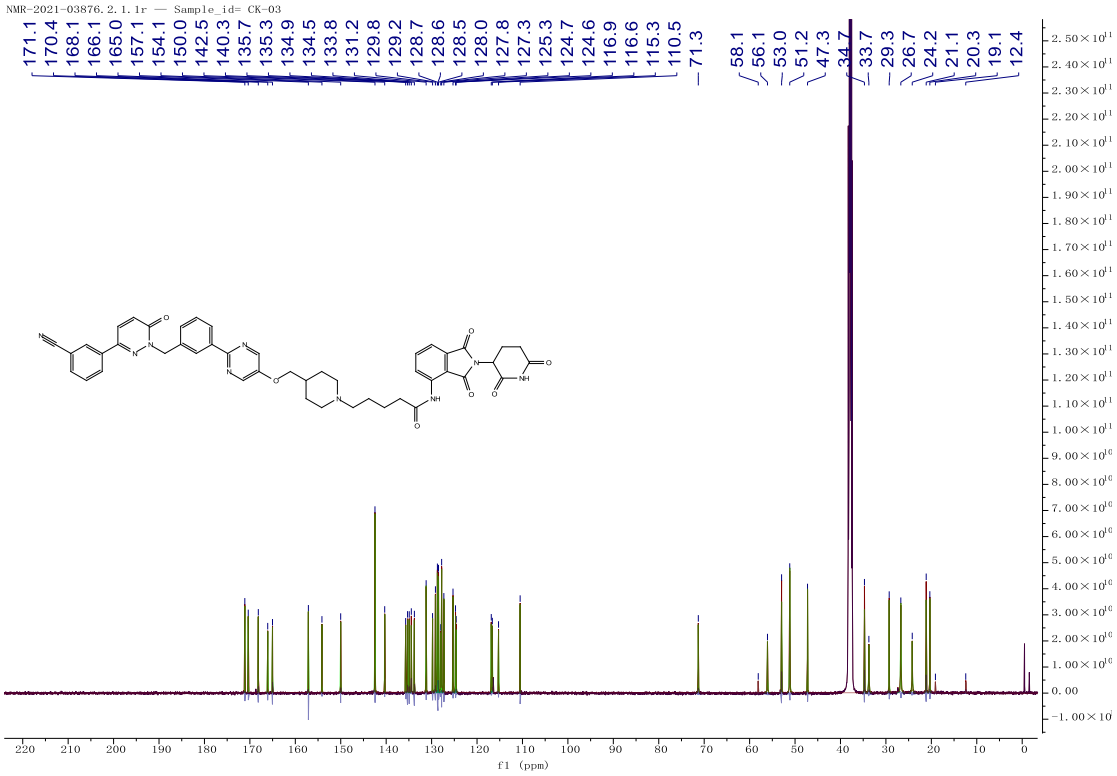
^{1}H NMR of **D2**



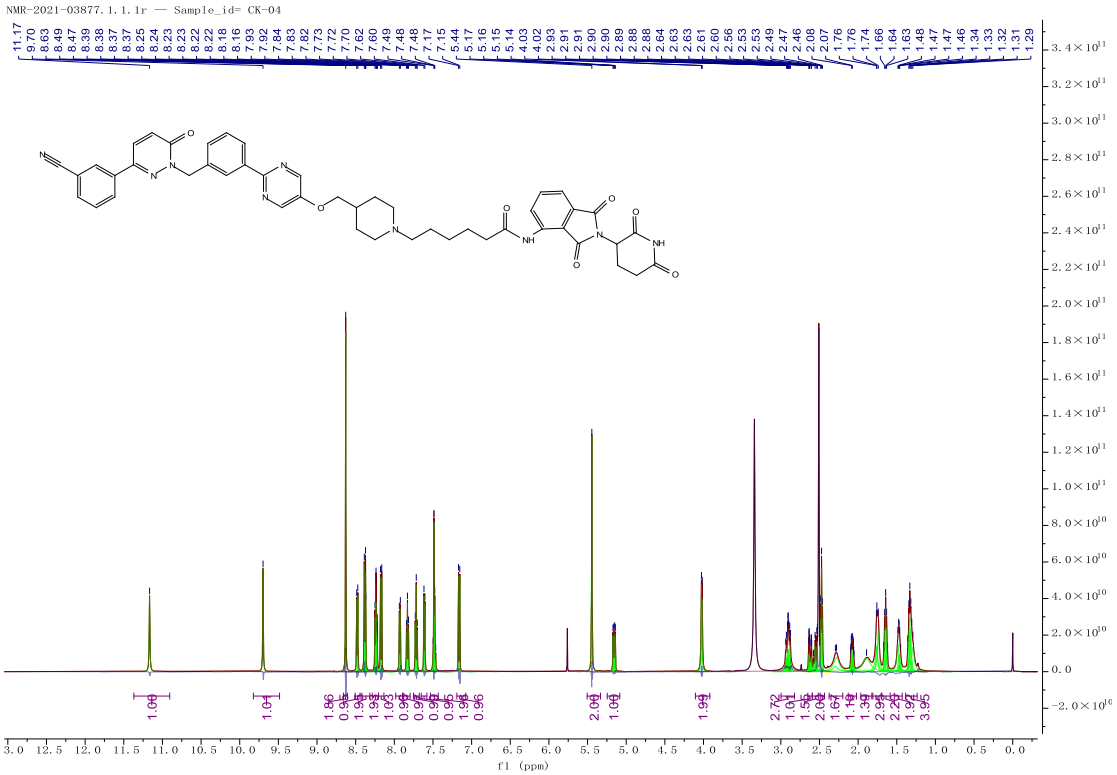
^{13}C NMR of **D2**



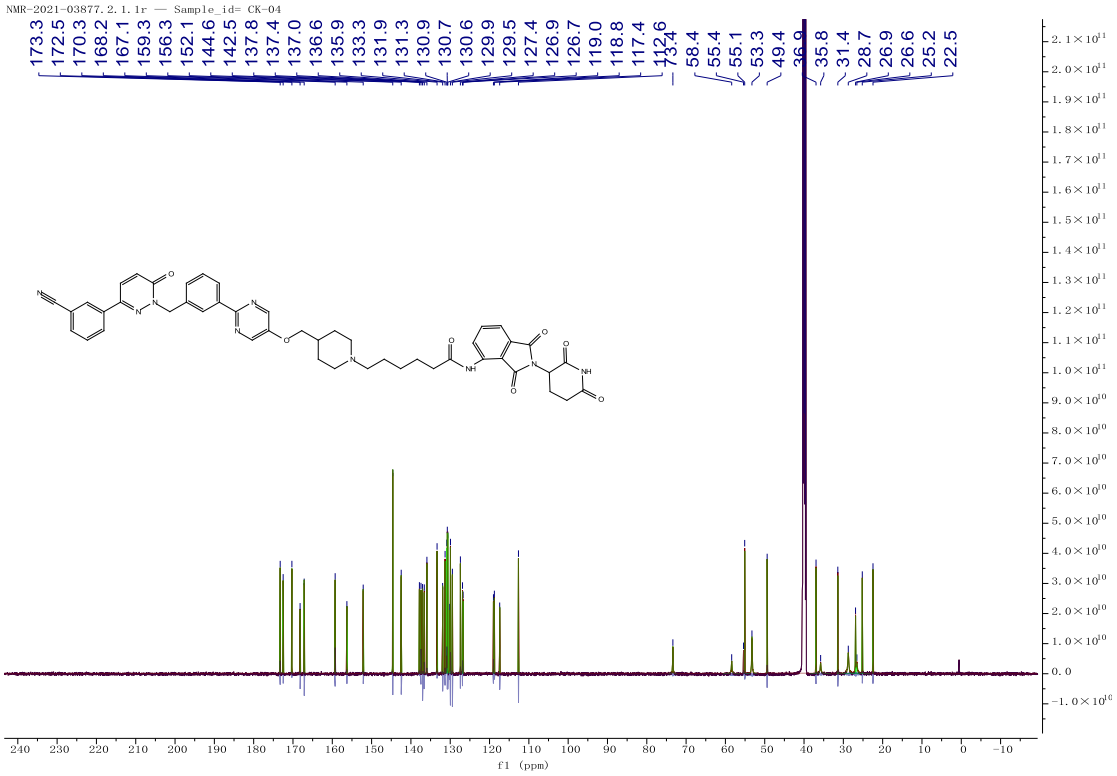
^{1}H NMR of **D3**



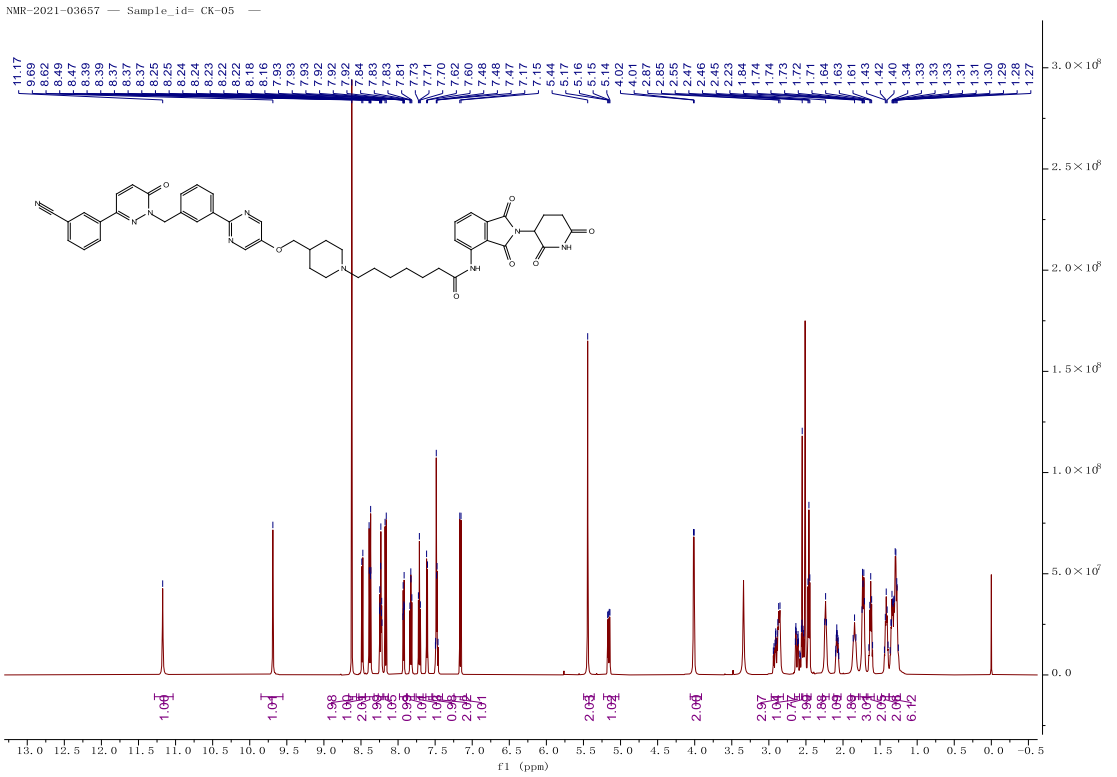
^{13}C NMR of **D3**



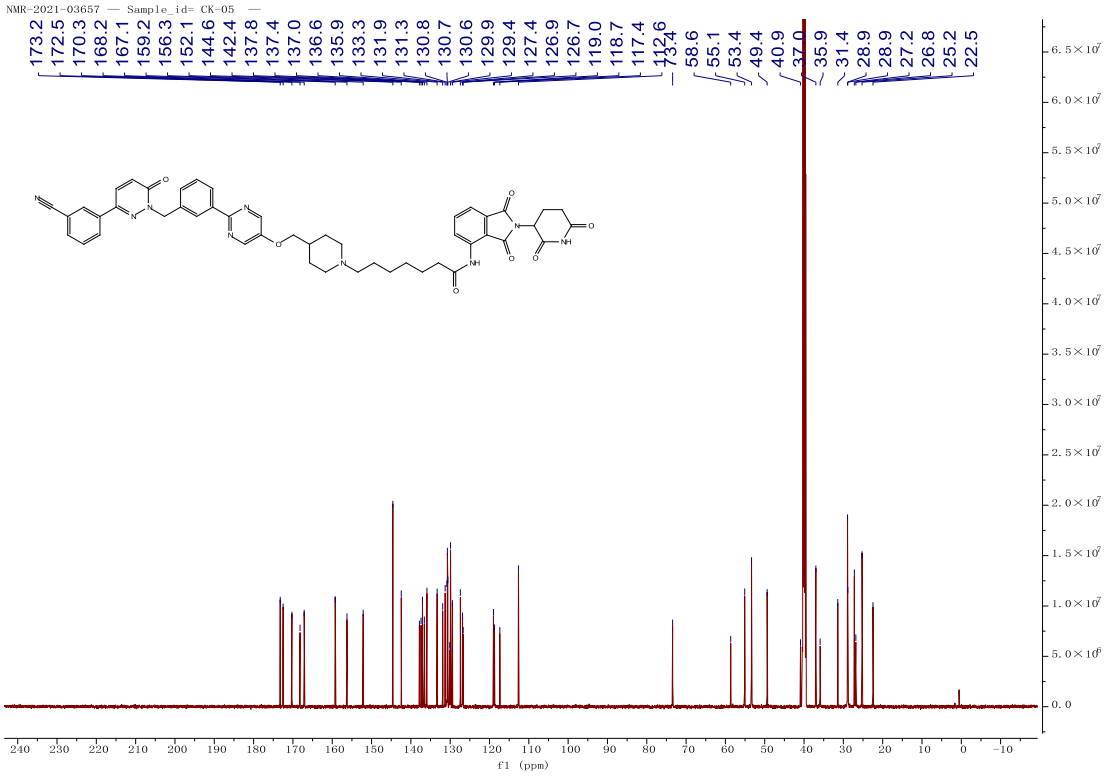
^{1}H NMR of **D4**



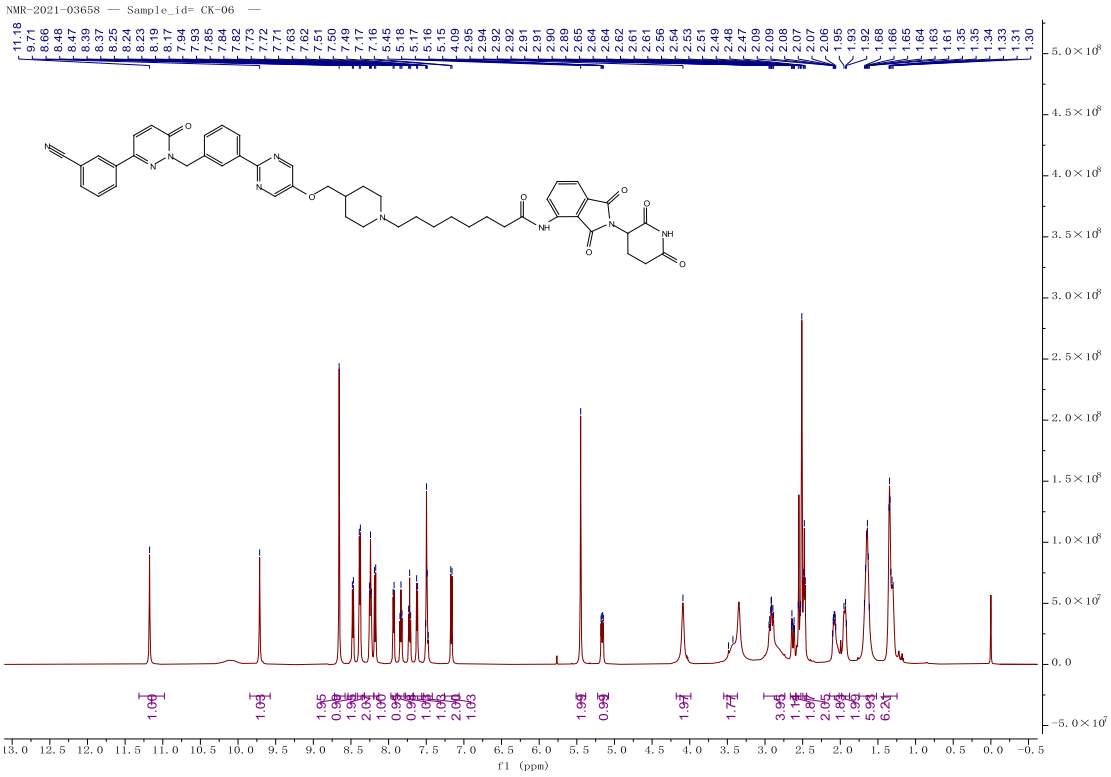
^{13}C NMR of **D4**



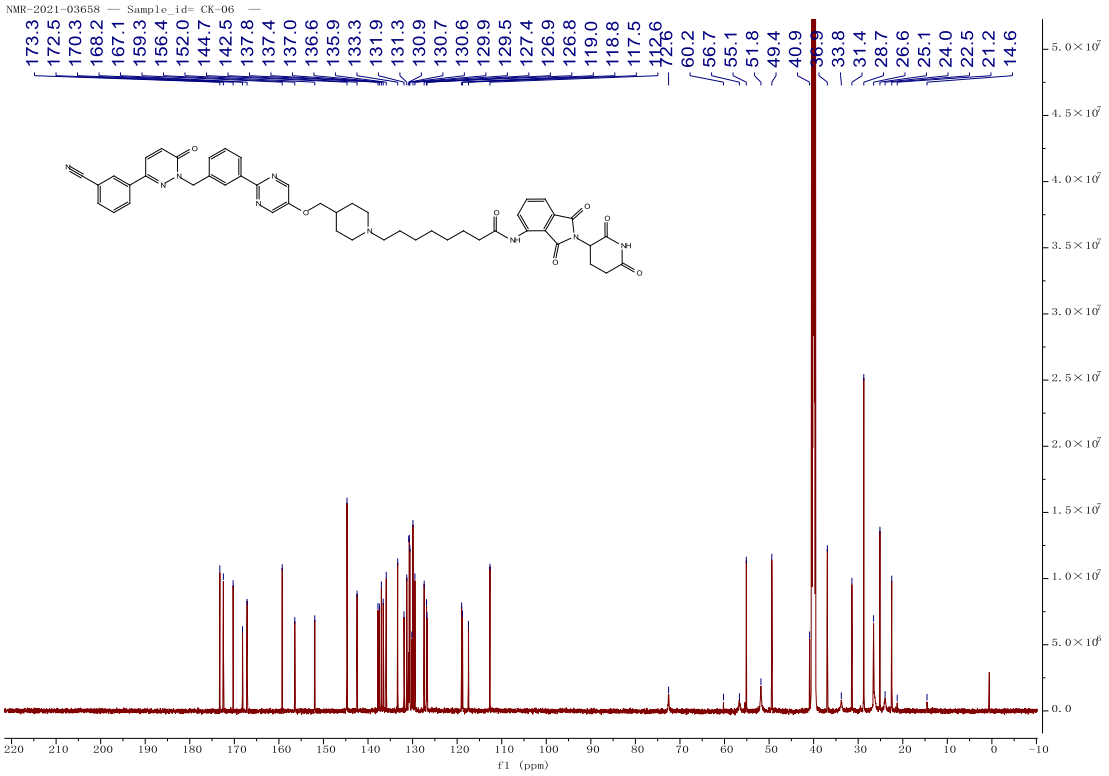
^{1}H NMR of **D5**



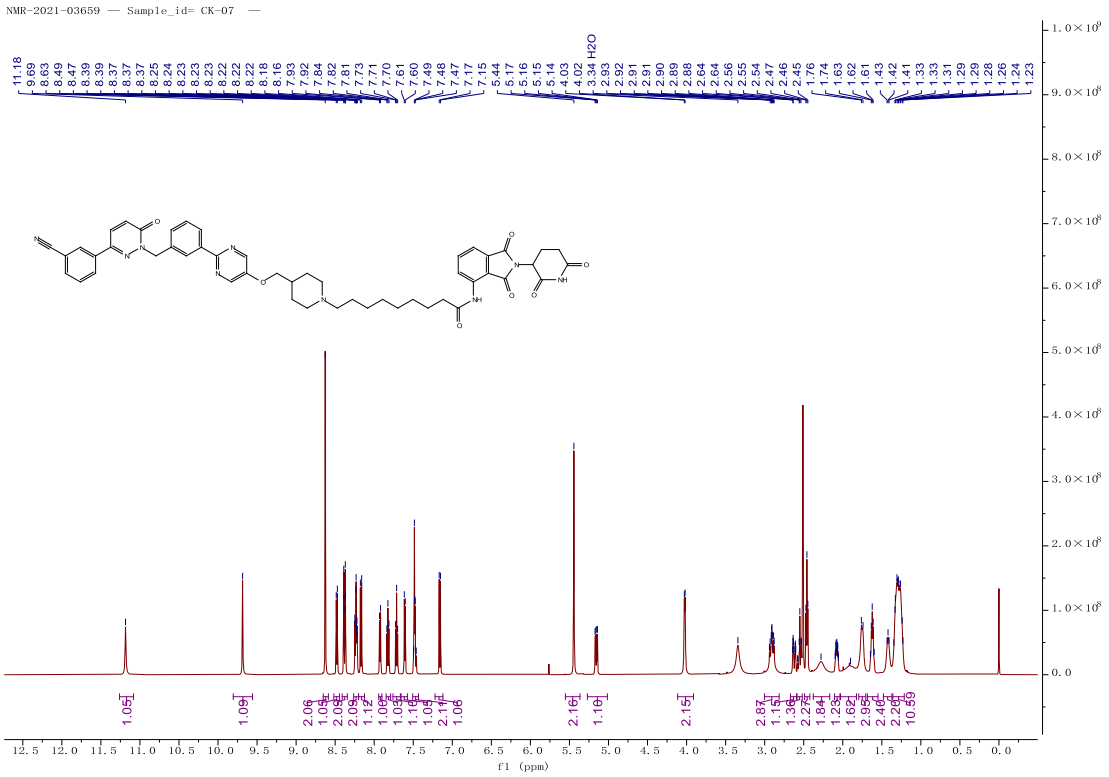
^{13}C NMR of **D5**



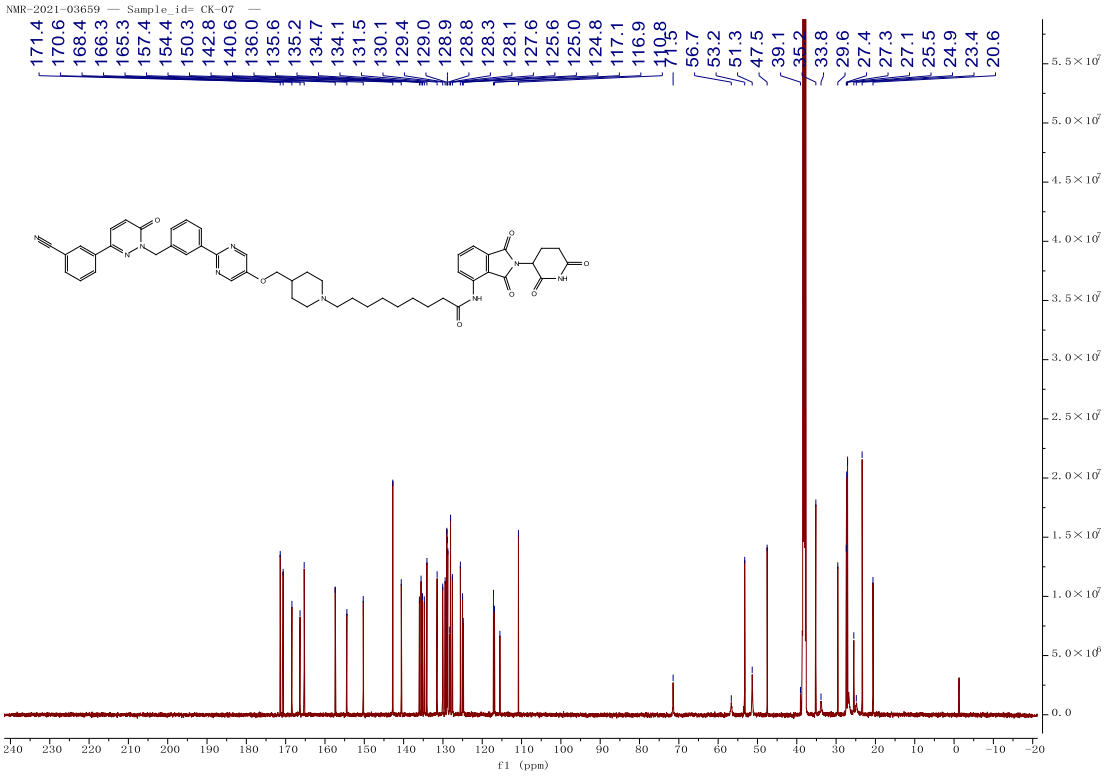
^{1}H NMR of **D6**



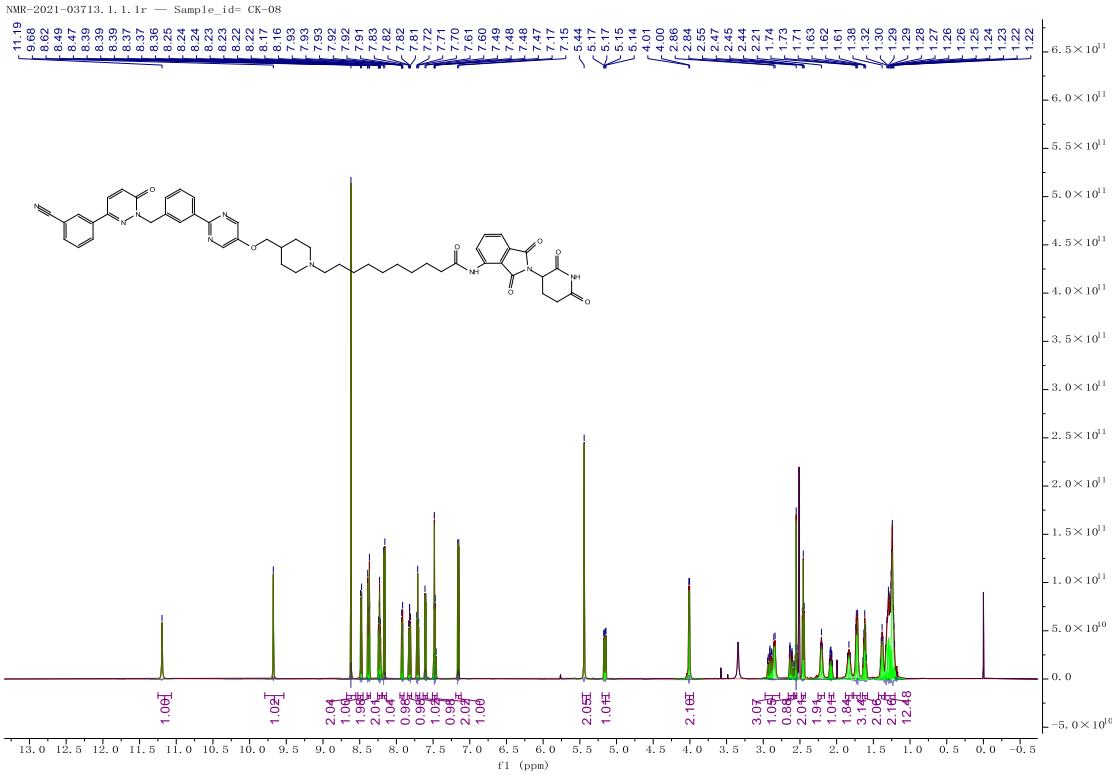
^{13}C NMR of **D6**



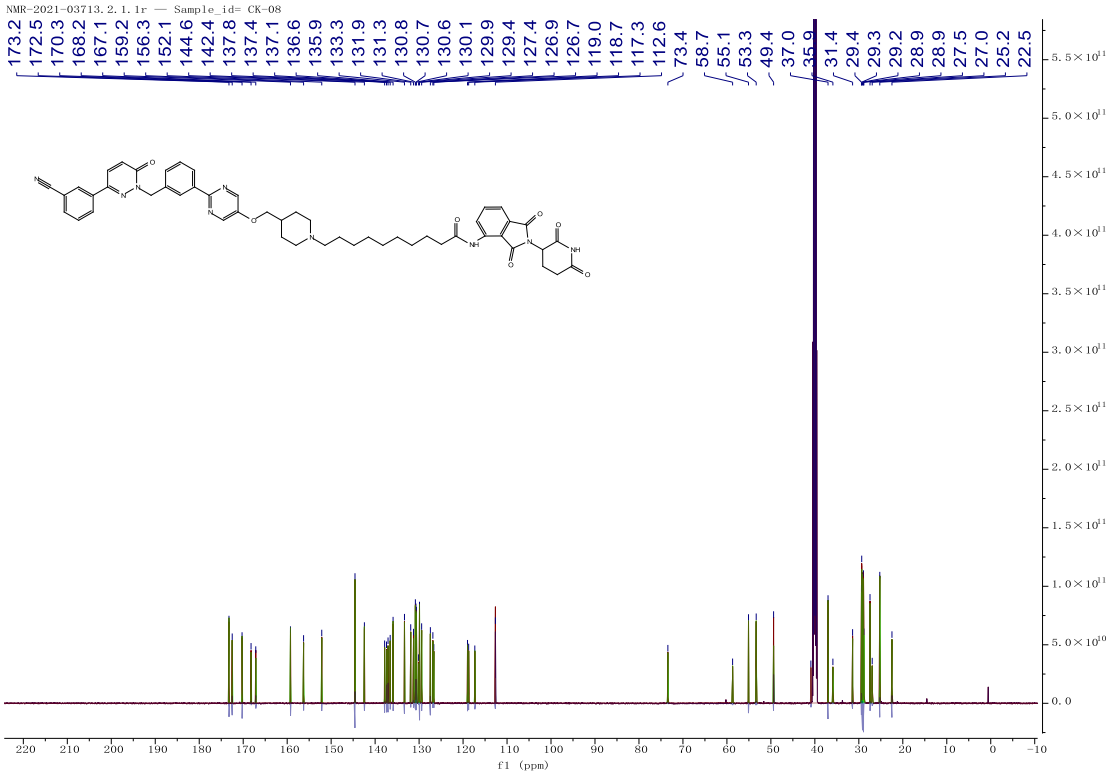
^{1}H NMR of **D7**



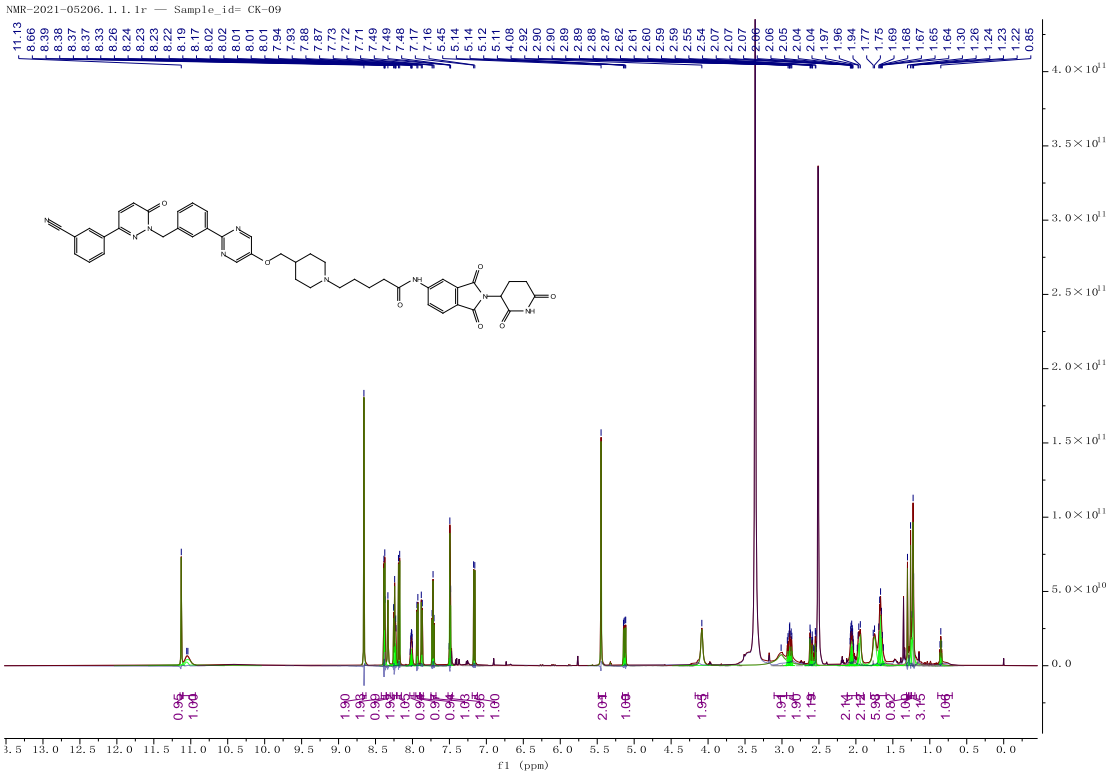
^{13}C NMR of **D7**



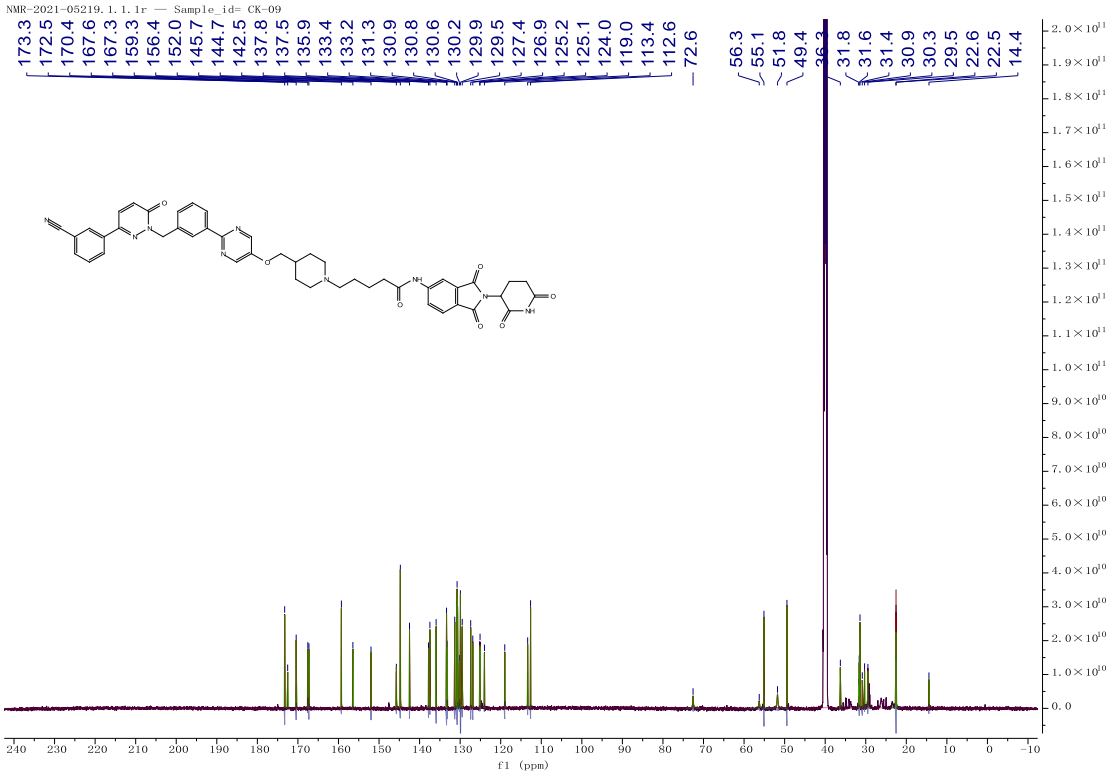
^{1}H NMR of **D8**



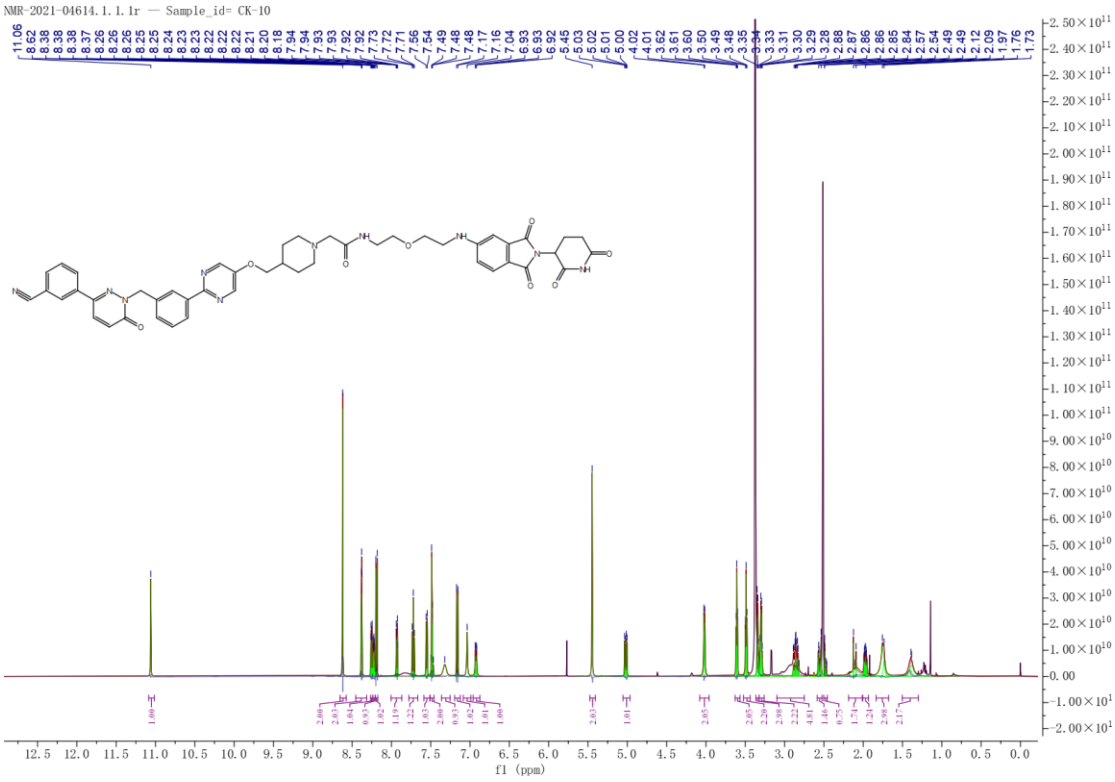
^{13}C NMR of **D8**



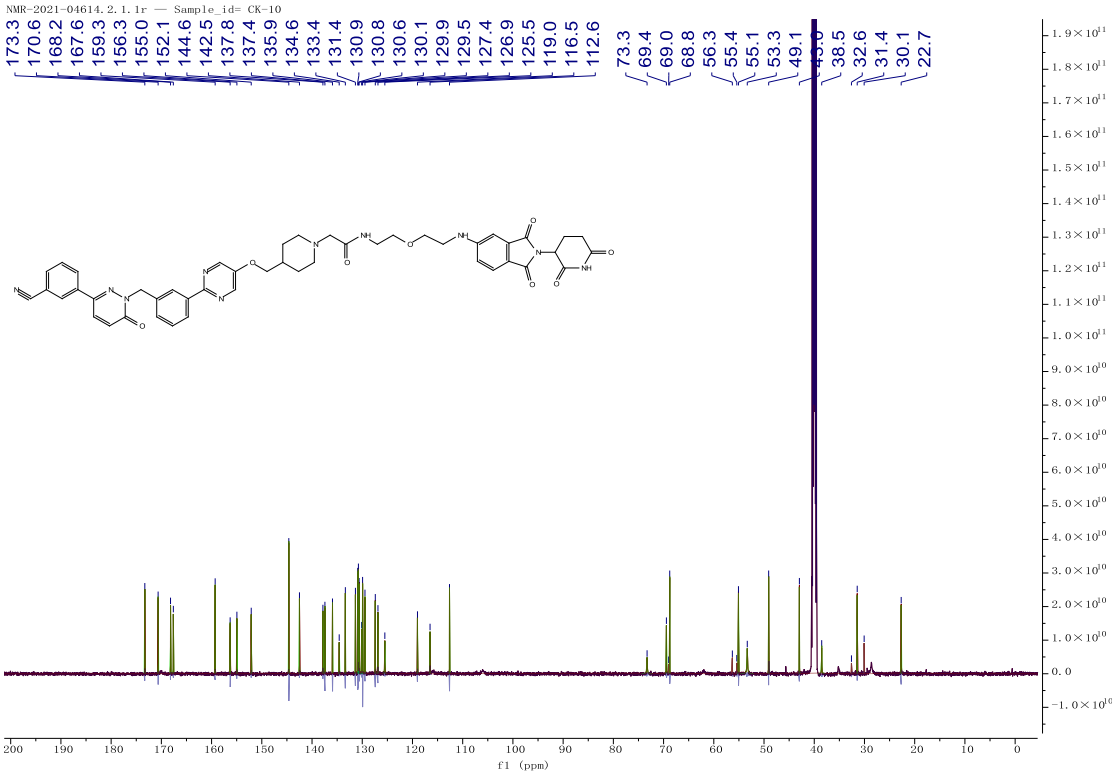
^{1}H NMR of **D9**



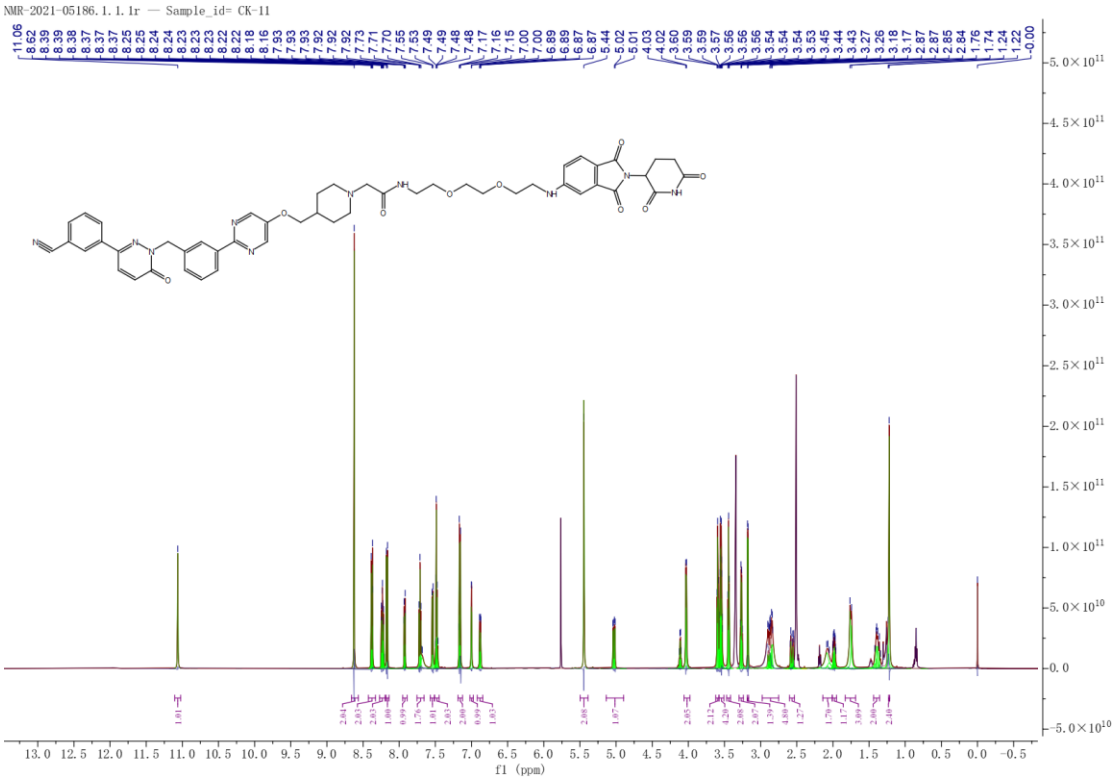
^{13}C NMR of **D9**



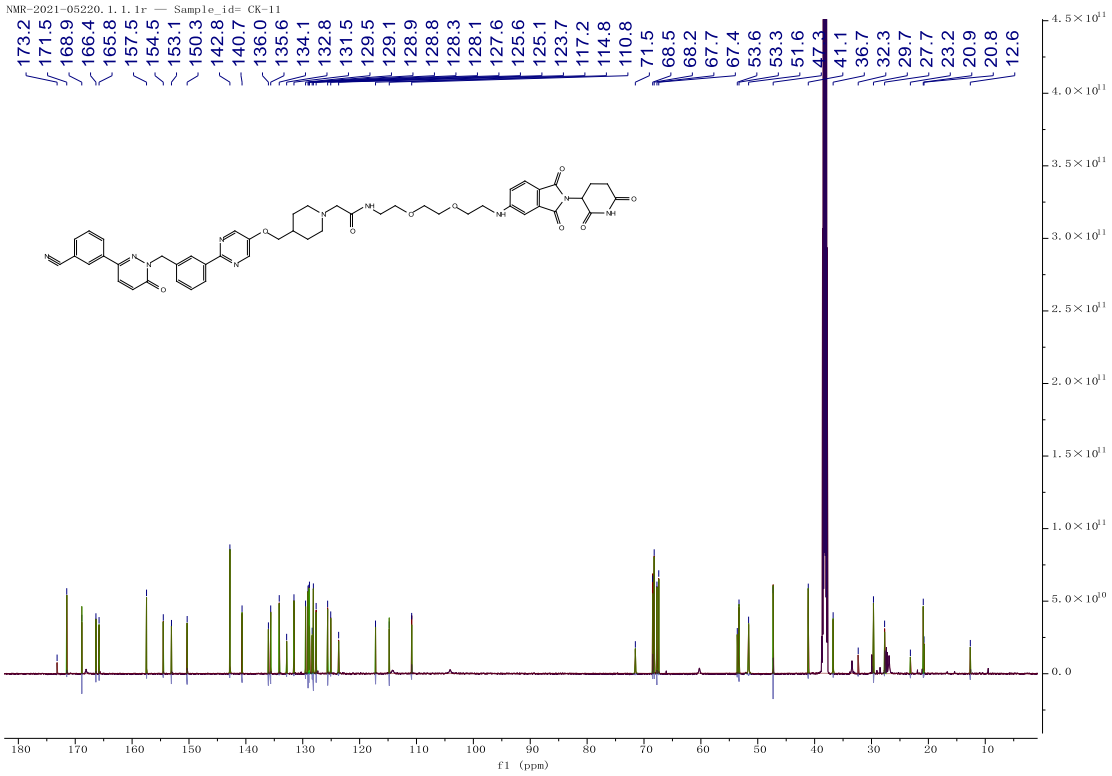
^{1}H NMR of **D10**



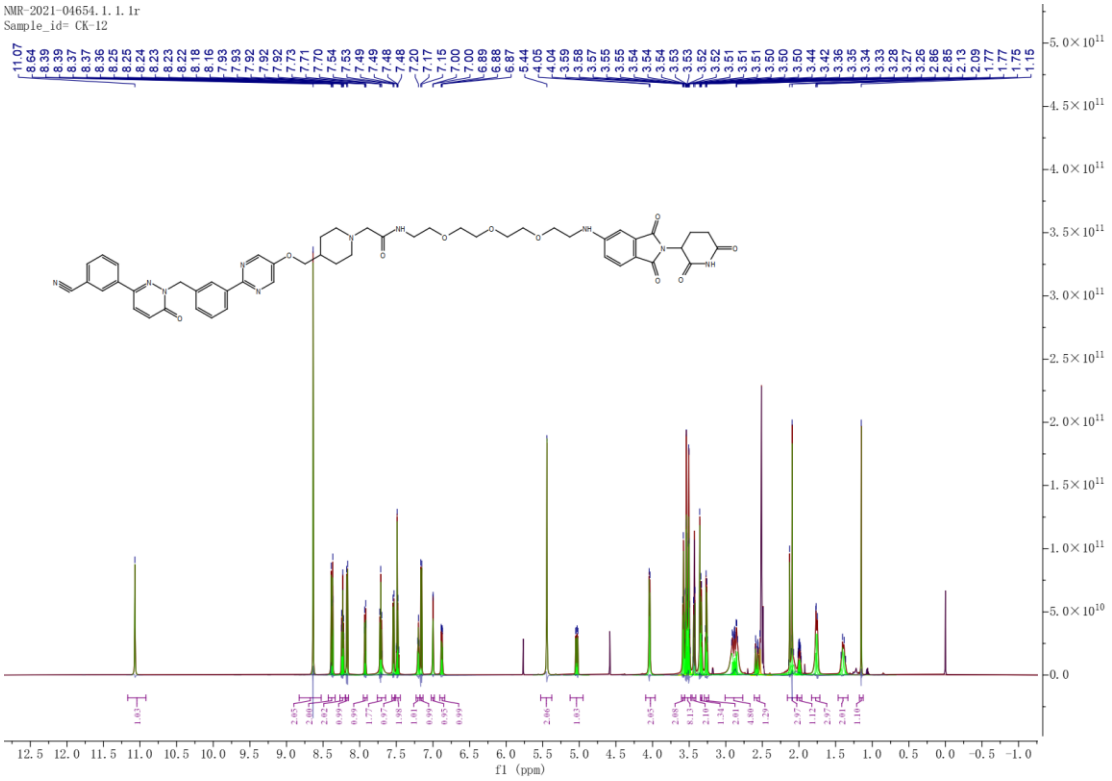
^{13}C NMR of **D10**



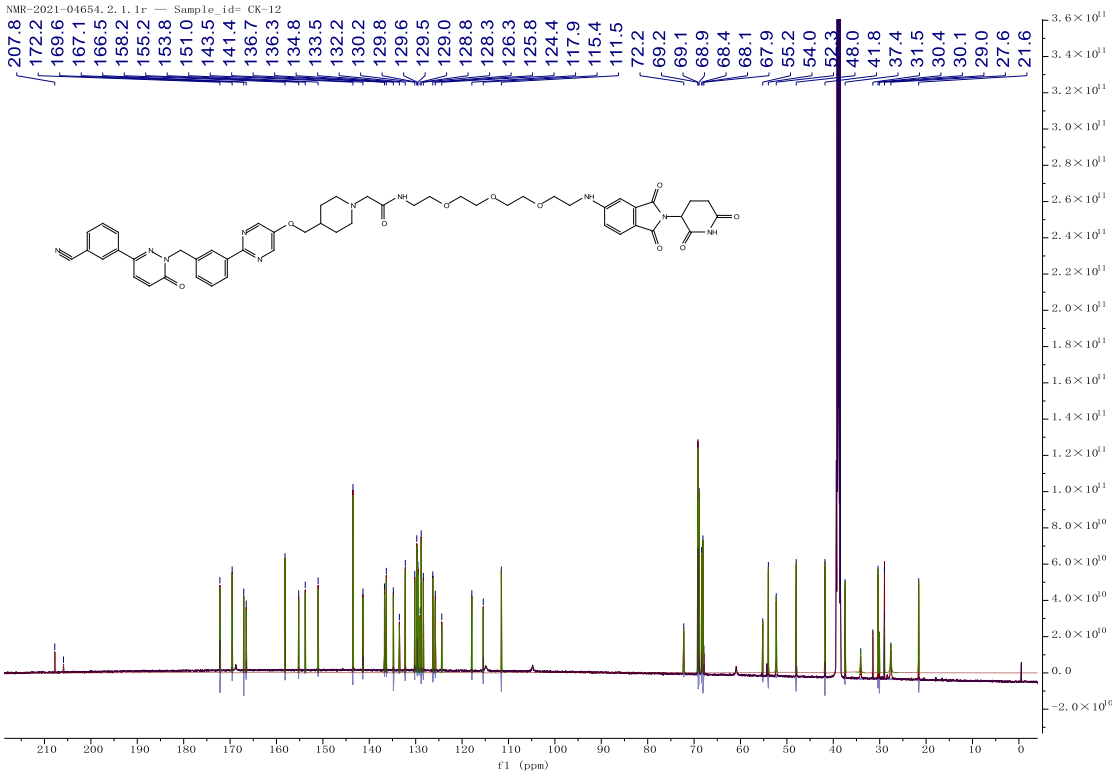
^{1}H NMR of **D11**



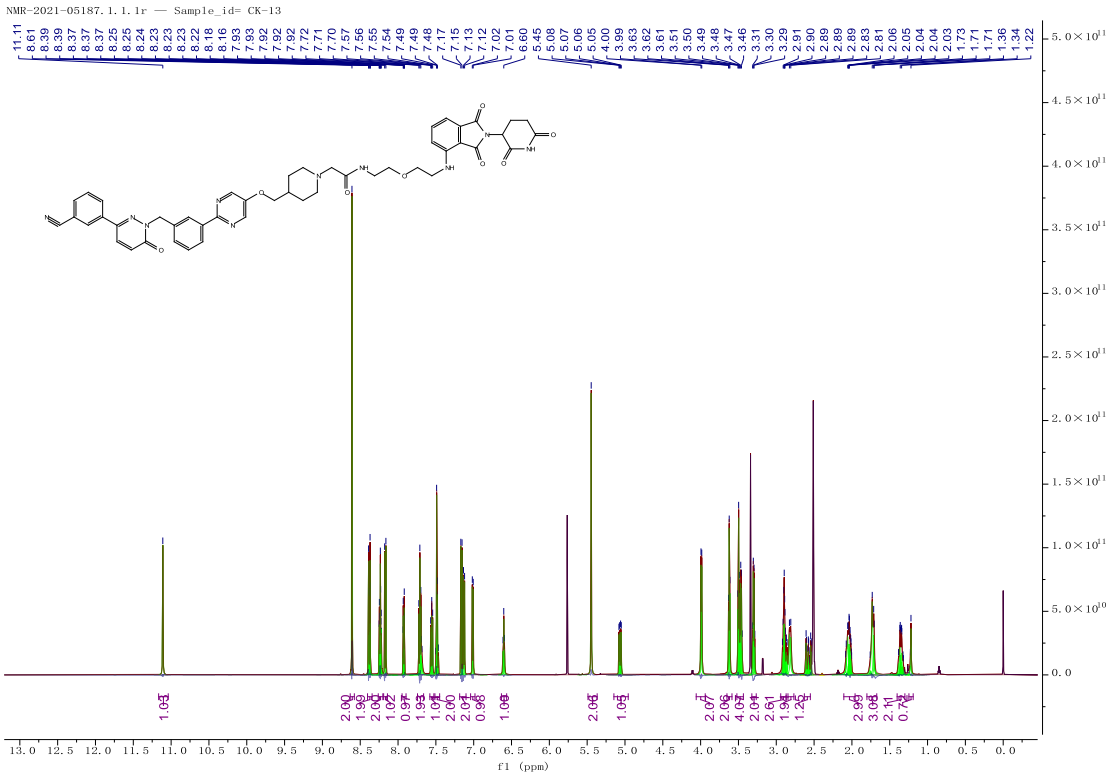
^{13}C NMR of **D11**



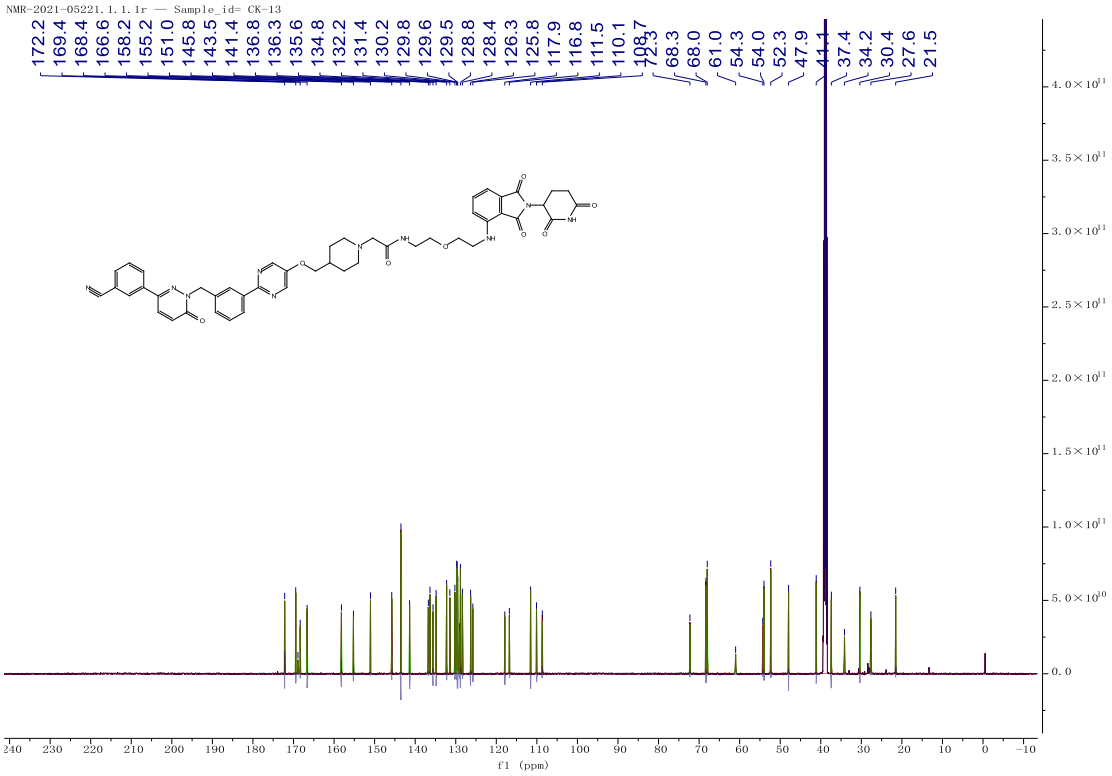
^{1}H NMR of **D12**



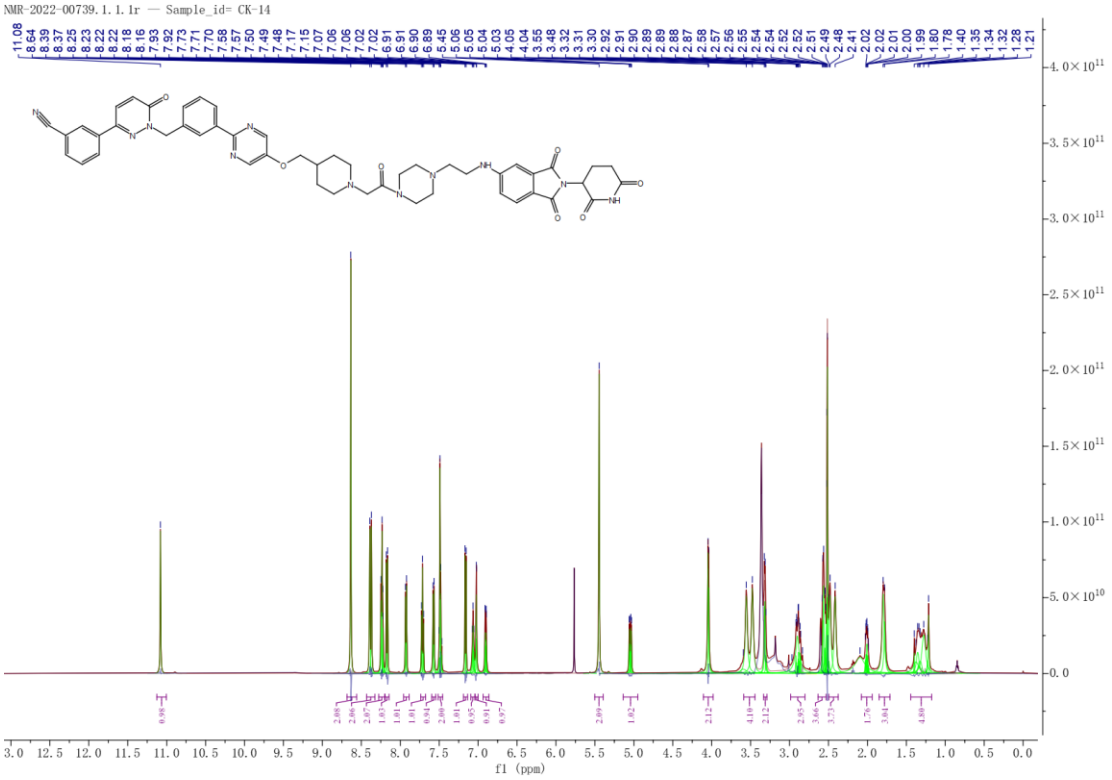
^{13}C NMR of **D12**



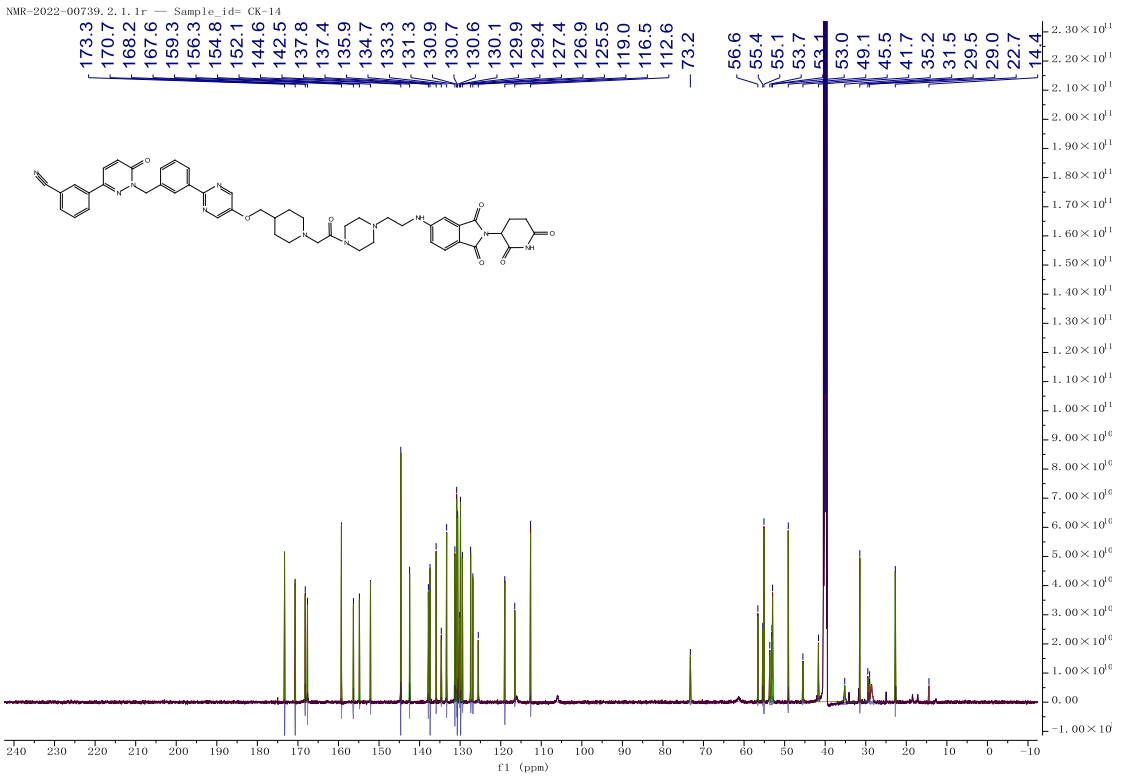
^{1}H NMR of **D13**



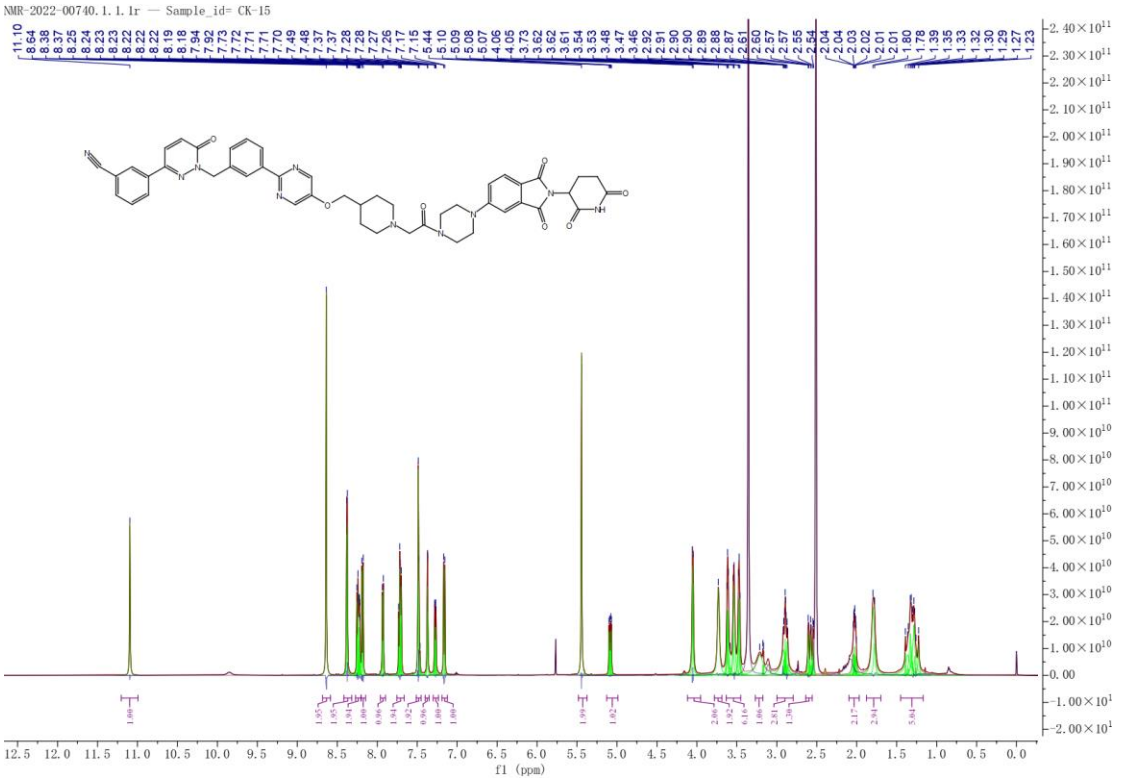
^{13}C NMR of **D13**



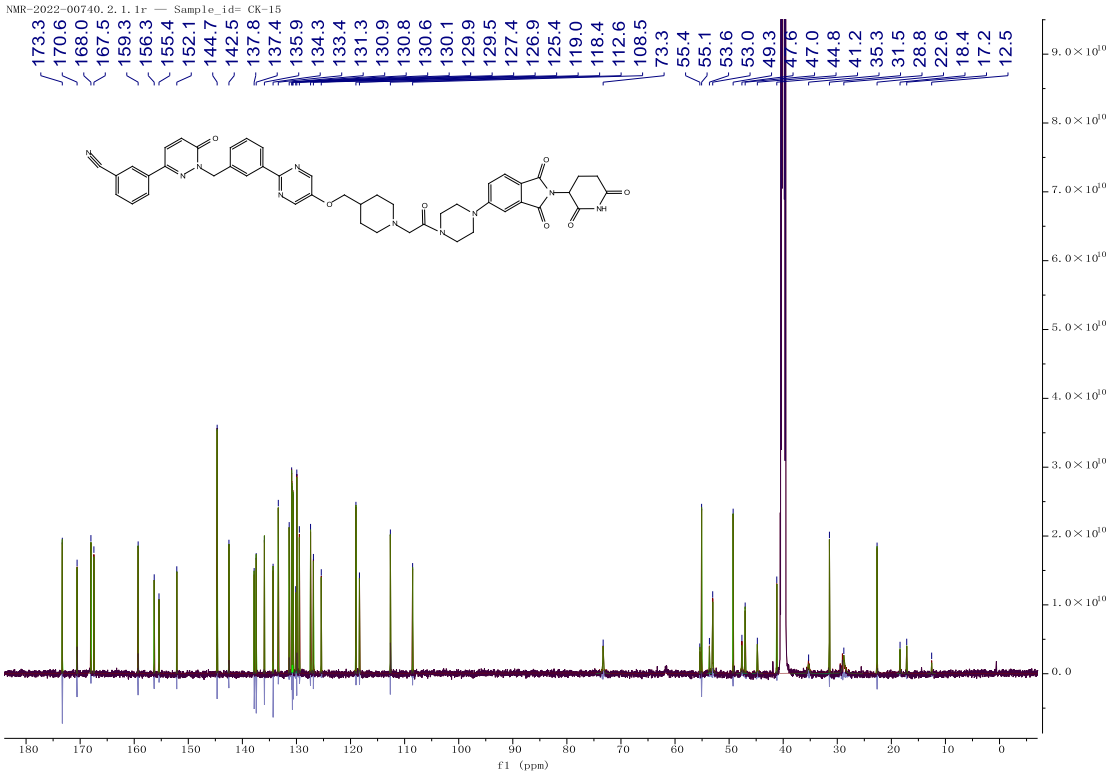
^{1}H NMR of **D14**



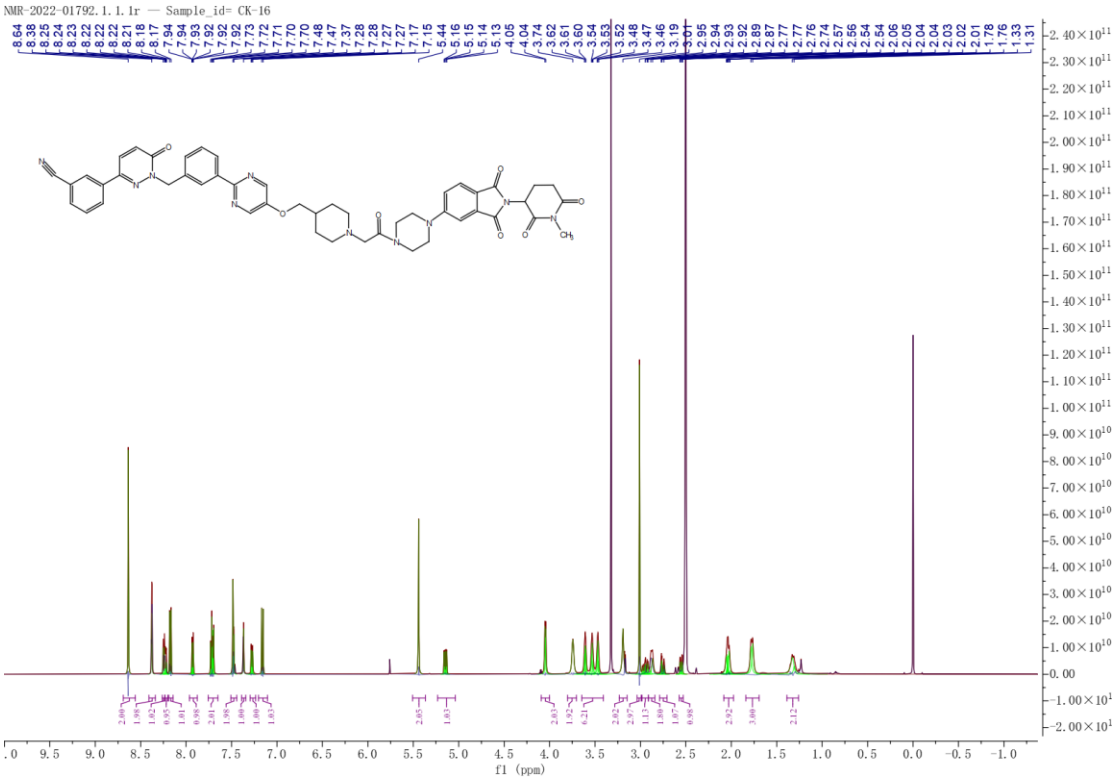
^{13}C NMR of **D14**



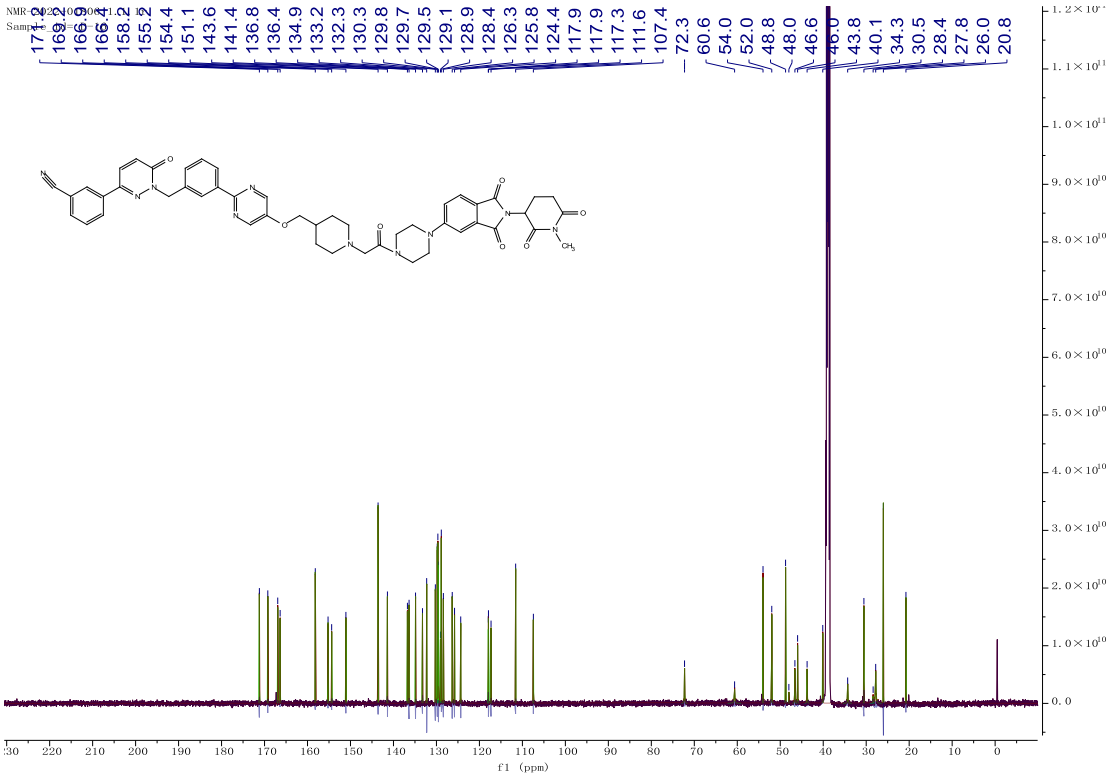
^{1}H NMR of **D15**



^{13}C NMR of **D15**

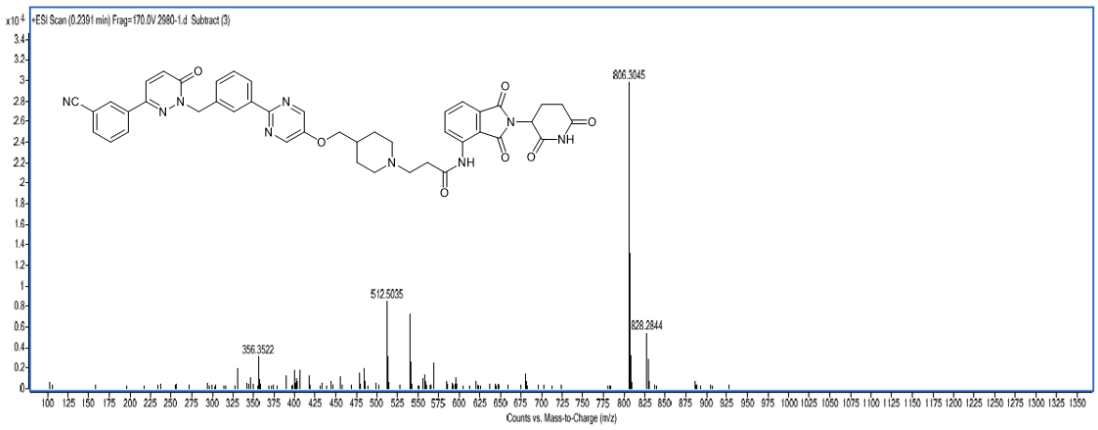


^{1}H NMR of **D16**

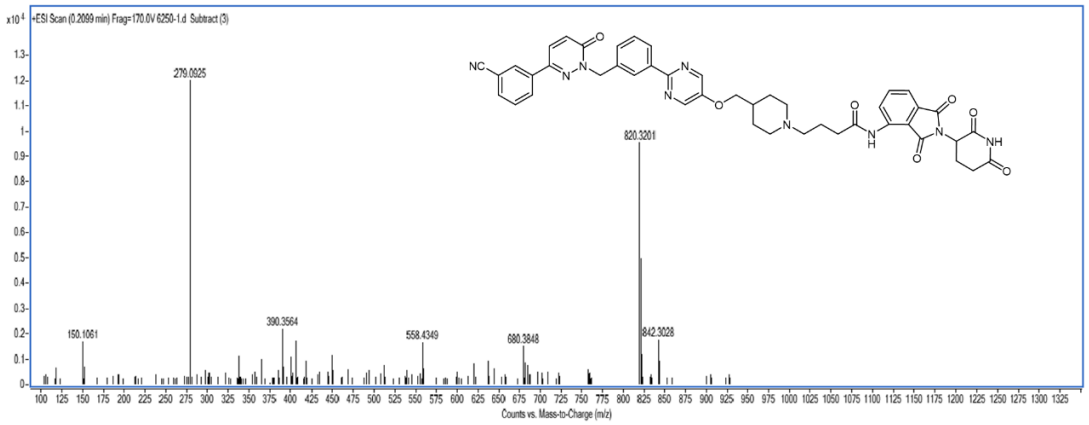


^{13}C NMR of **D16**

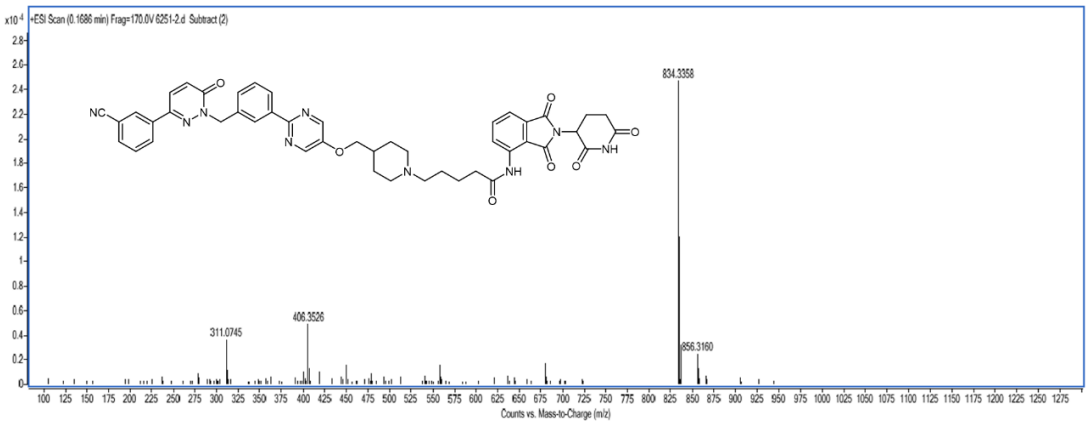
3. HRMS of D1-D16



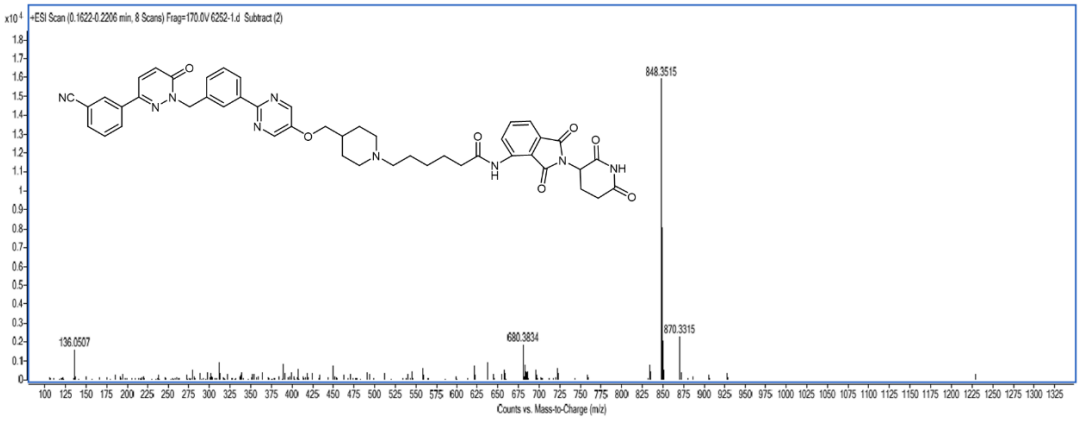
HRMS (ESI) of **D1**



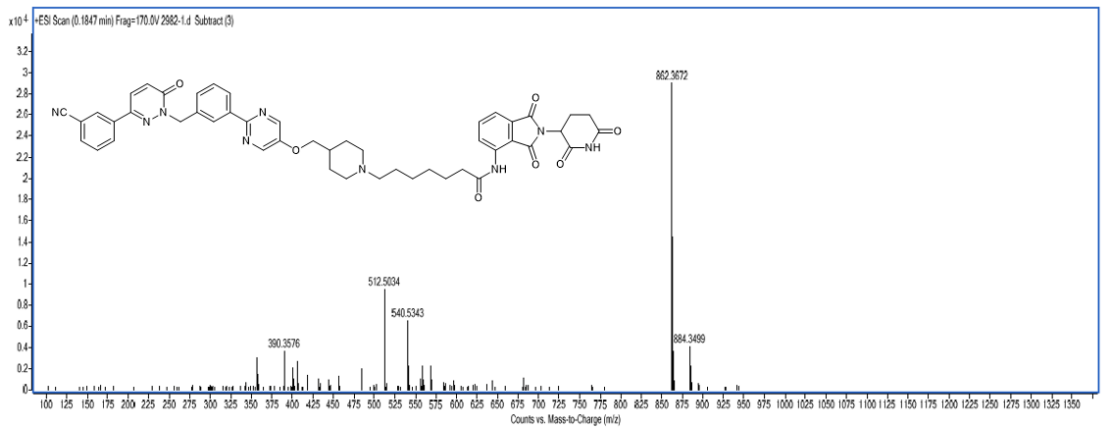
HRMS (ESI) of **D2**



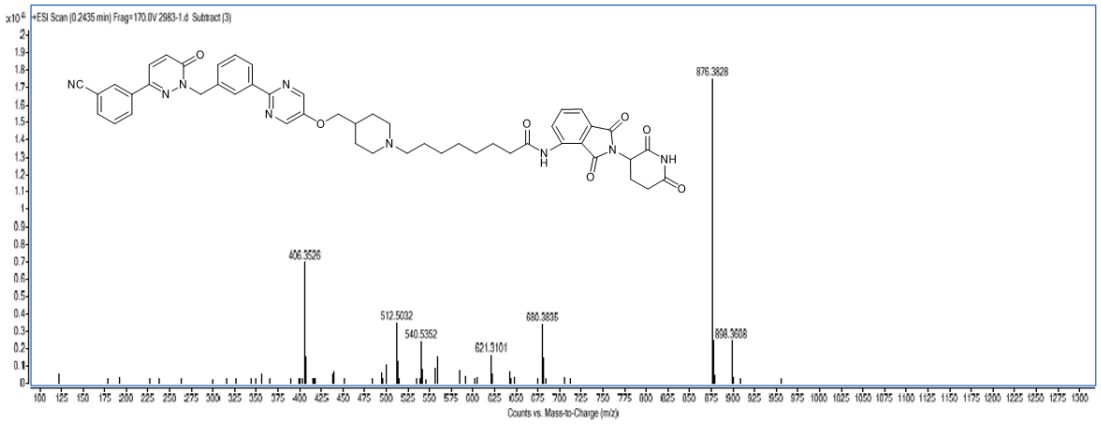
HRMS (ESI) of **D3**



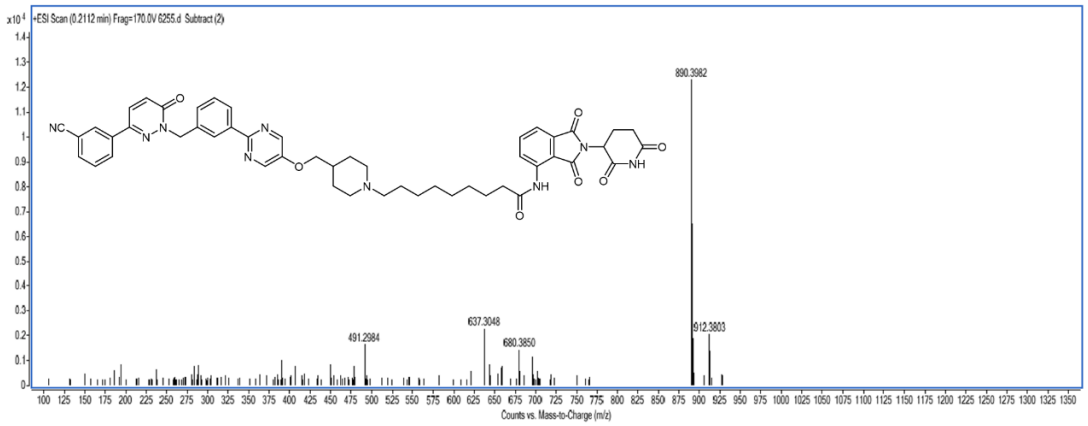
HRMS (ESI) of **D4**



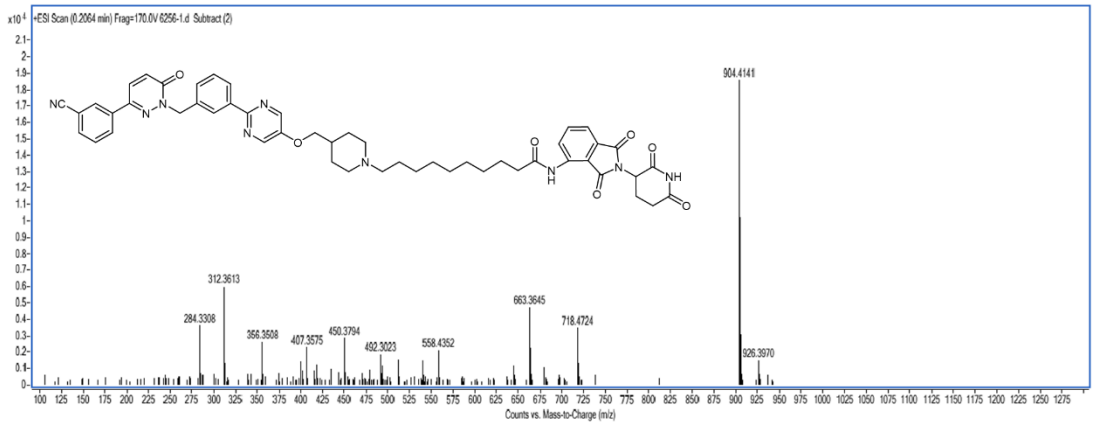
HRMS (ESI) of **D5**



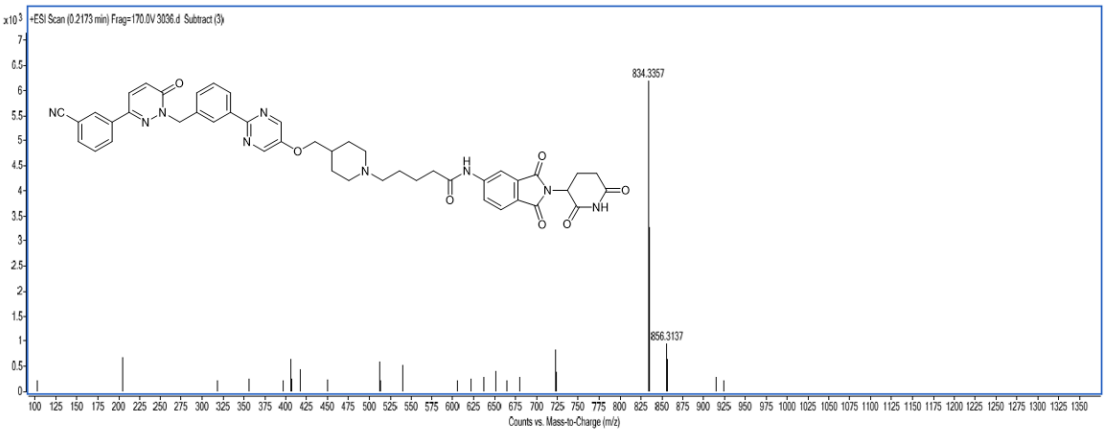
HRMS (ESI) of **D6**



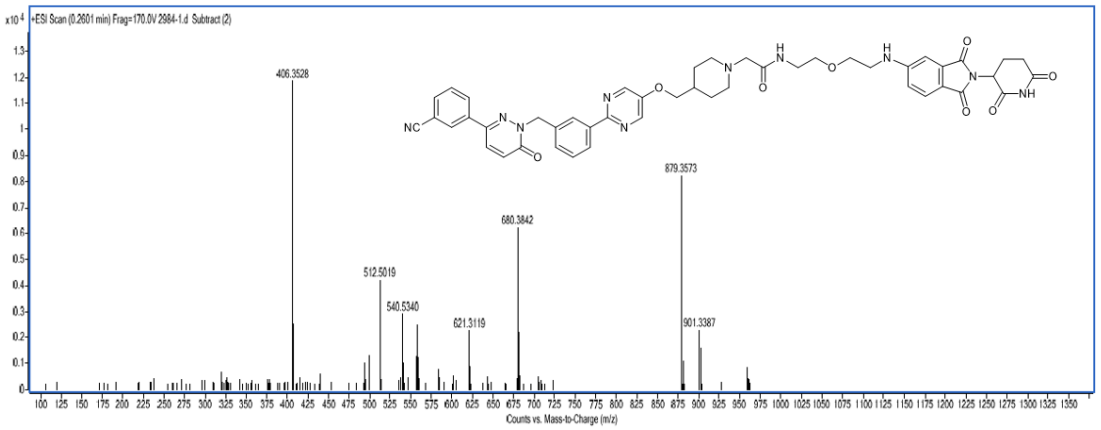
HRMS (ESI) of **D7**



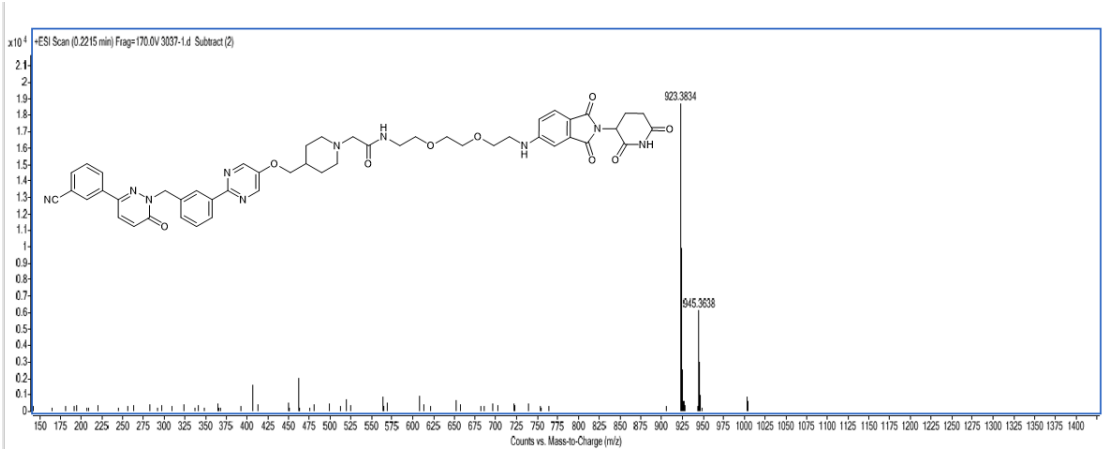
HRMS (ESI) of **D8**



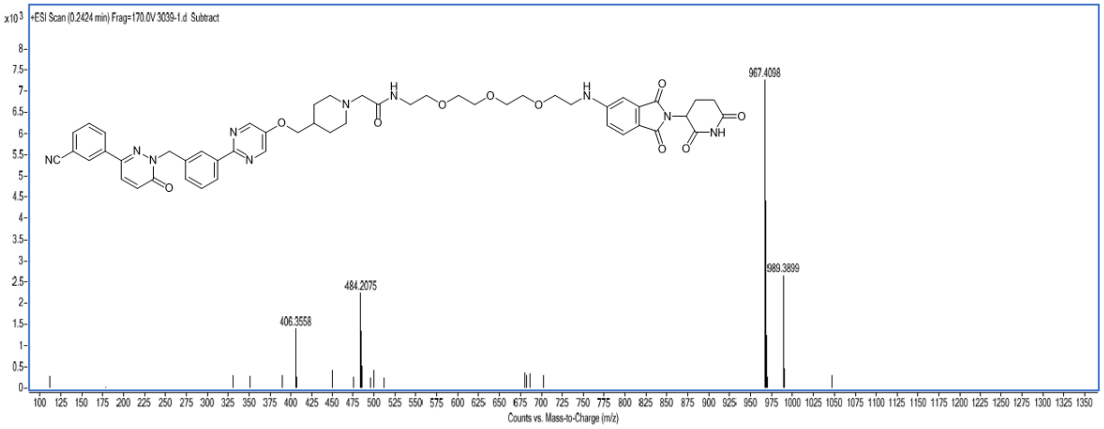
HRMS (ESI) of **D9**



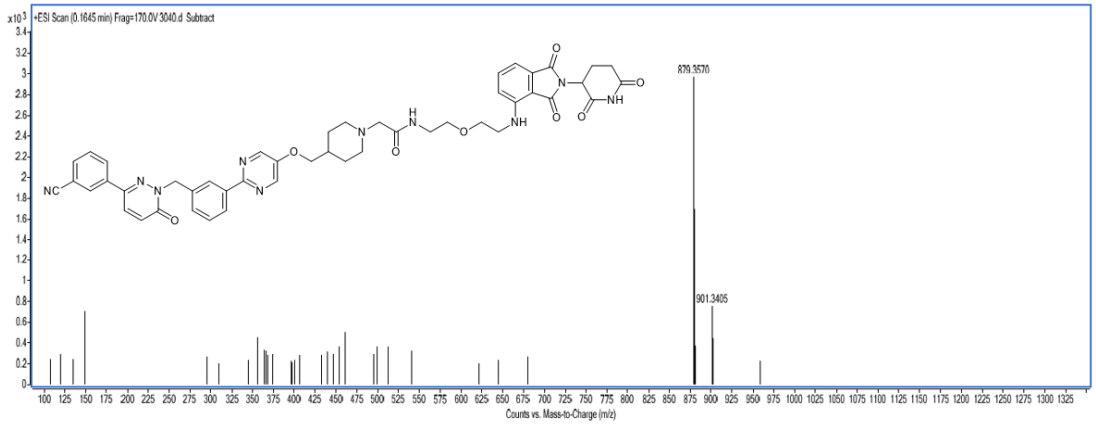
HRMS (ESI) of **D10**



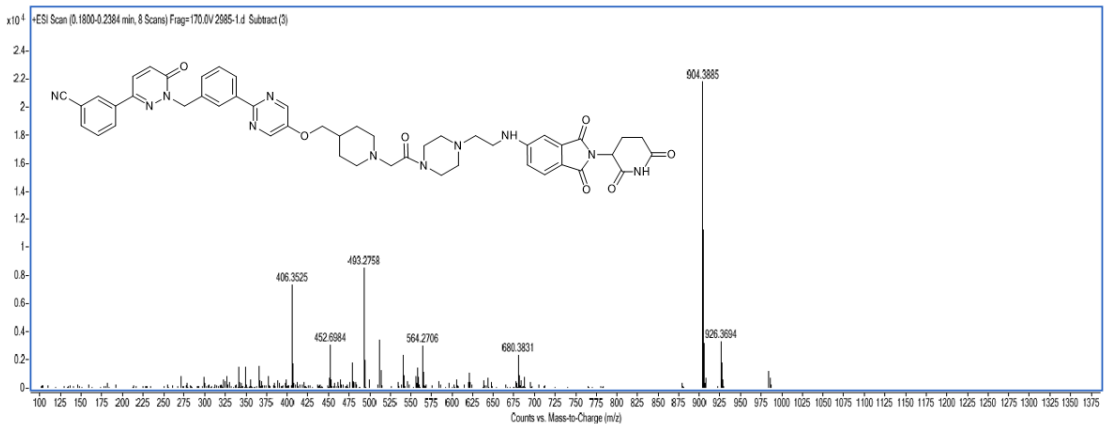
HRMS (ESI) of **D11**



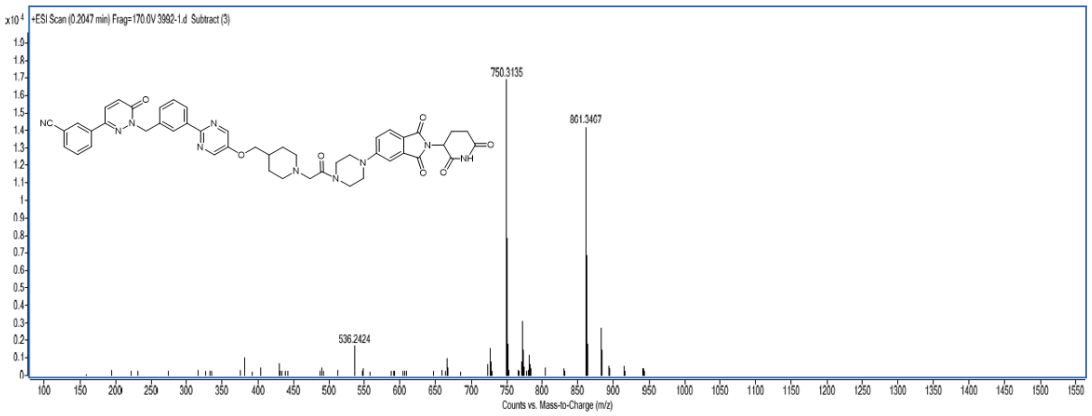
HRMS (ESI) of **D12**



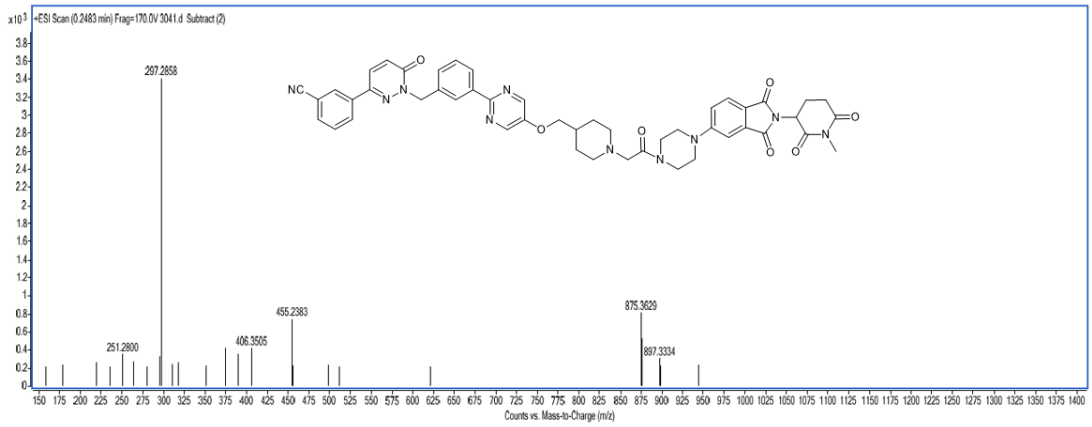
HRMS (ESI) of **D13**



HRMS (ESI) of **D14**

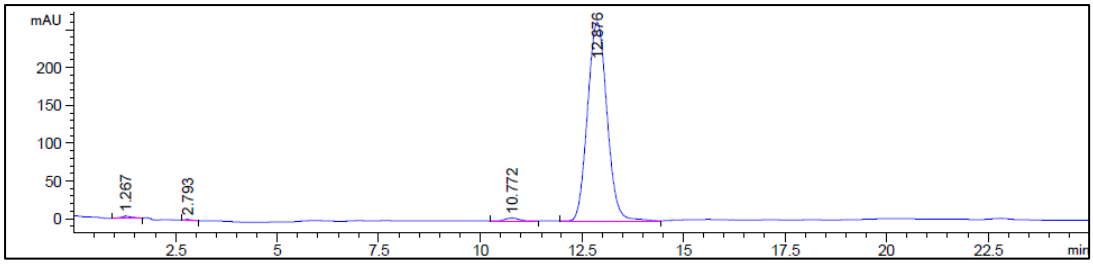


HRMS (ESI) of **D15**



HRMS (ESI) of **D16**

4、HPLC analysis of D10, D15

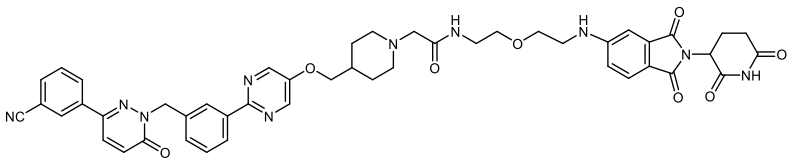


Gradient: 80% CH_3OH over 25 min at a flow rate of 1 mL/min.

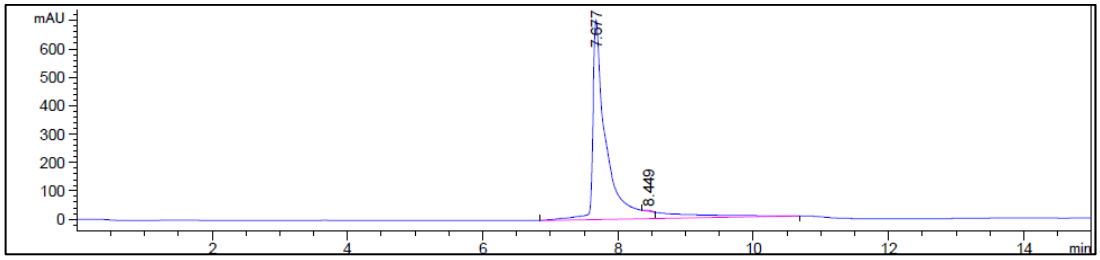
Wavelength: 254 nm

Purity: 98.171%

Peak	Ret. Time	Area	Area%
1	1.267	49.971	0.560
2	2.793	12.372	0.139
3	10.772	100.705	1.130
4	12.876	8751.672	98.171
Total		8914.720	100.000



HPLC data of D10

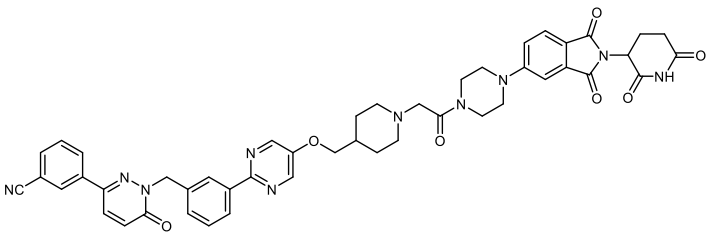


Gradient: 85% CH_3OH over 15 min at a flow rate of 1 mL/min.

Wavelength: 254 nm

Purity: 99.820%

Peak	Ret. Time	Area	Area%
1	7.677	1.043e4	99.820
2	8.449	18.828	0.180
Total		1.045e4	100.000



HPLC data of D15

5. Transcellular Transport Experiment with Caco-2 Cells

Caco-2 cells were cultured in DMEM supplemented with 10% fetal bovine serum, 1% antibiotic-antimycotic, and 1% nonessential amino acids maintained at 37 °C in 5% CO_2/95% air (Gao et al., 2020; Kono et al., 2021). The cells were plated onto Coring Transwell polycarbonate membrane cell culture inserts (0.4 μm pore size, 6.5 mm diameter, Corning, ME, USA). For the transport experiment at a density of 1.0×10^4 cells /mL. The culture medium was replaced on the day after seeding, after which it was refreshed every other day and on the day before the transport experiment. The cells were cultured for 20-22 days after seeding and then evaluated by determination of the transepithelial electrical resistance (TEER) (Millicell ERS®, Millipore Corporation) before experiments. Batches of Caco-2 cells were certified by measuring the TEER values along with the apparent permeability coefficient (P_{app}) of control compounds: atenolol (2 μM), metoprolol (2 μM), and P-gp substrate digoxin (2 μM). 0.1 mL of HBSS at pH 7.4 with 0.1% DMSO in the presence or absence of tariquidar (5 μM, P-gp inhibitor), Ko143 (10 μM, BCRP inhibitor), or GF129018 (10 μM, P-gp and BCRP inhibitor) was added to the apical (AP) side of the cell monolayer. Similarly, 0.6 mL of HBSS at pH 7.4 with 0.1% DMSO in the presence or absence of each inhibitor was added to the basal (BL) side of the cell monolayer. After preincubating for 30 min, either the AP or BL side of the cell monolayer was refreshed with HBSS at pH 7.4, containing **D15** (2 μM) with or without inhibitors. 1% BSA was added to the receiver solution to avoid nonspecific binding. After incubation at 37℃ for 120min, 50 μL of the medium was collected from each compartment for measuring P_{app}. Harvested samples were precipitated by adding 6× volumes of acetonitrile containing IS and centrifuged. The supernatants were stored at -20℃ until analysis by LC-MS/MS.

Data analysis

For *in vitro* Caco-2 cell transport studies, the apparent permeability coefficient (P_{app}) was obtained according to Equation (1)

$$P_{app} = \Delta Q/\Delta t \times 1/ (A \times C_0) \quad (1)$$

where $\Delta Q/\Delta t$ is the mass transport rate of **D15**, A is the surface area of the porous membrane, and C_0 is the initial concentration of **D15** added to the donor side.

The efflux ratio (ER) was obtained according to Equation (2). An ER greater than 2 indicates net efflux.

$$ER = P_{app,BA}/P_{app,AB} \quad (2)$$

where $P_{app,AB}$ and $P_{app,BA}$ are the P_{app} values for AP-to-BL and BL-to-AP transport, respectively.

Reference:
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Table S2: Bidirectional P_{app} and efflux ratio (ER) of **D15** across Caco-2 cell lines in the presence and absence of efflux transporters (n=3)

	P_{app} (10^{-6} cm/s)		**ER**
	A-B	**B-A**	
D15	0.06	0.79	12.78
+Tariquidar	0.05 ± 0.02	0.04 ± 0.01	0.88
+ Ko143	0.07 ± 0.06	0.50 ± 0.05	6.90
+GF120918	0.00 ± 0.01	0.01 ± 0.01	2.09