Downsizing a BTK PROTAC yields a G-loop independent molecular glue that blurs the boundary between degrader classes

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Supplementary Information

Supplementary Table 1. Cryo-EM data collection, refinement, and validation statistics.

CRBN–PS-10–BTK kinase domain ternary complex (EMDB: EMD-73459; PDB: 9YTE).

Sample preparation	
Buffer	10 mM HEPES/NaOH, 240 mM NaCl, 3 mM TCEP, pH 7.0
Complex concentration (mg mL^{-1})	5
Grid	Gold, 300 mesh, R1.2/1.3
Vitrification	FEI Vitrobot Mark IV; ethane; 100% humidity; 277 K

Data collection and processing	
Microscope	TFS Titan Krios (Cs 2.7 mm, field emission gun)
Detector; mode	Gatan K3 BioQuantum (6k × 4k); counting
Illumination / imaging mode	Flood beam / bright field
Voltage (kV)	300
Nominal magnification	165,000×
Pixel size (Å)	1.0226 (0.5113 super-resolution)
Electron exposure (e^{-} Å^{-2})	65
Exposure time (s) / frames per movie	2 / 40
Defocus range (μm)	0.5–2.5
Acquisition software	SerialEM (fringe-free imaging; 6 × 6 beam-shift; 4 exposures per hole)
Micrographs collected (no.)	14,201
Symmetry imposed	C1
Initial particle images (no.)	13,381,946
Final particle images (no.)	321,907
Map resolution (Å)[a]	3.42
FSC threshold	0.143
Map resolution range (Å)	3.42 (FSC 0.143) – 3.94 (FSC 0.5)
Map sharpening B factor (Å²)	−50
Map box size (px) / dimension (Å)	280 × 280 × 280 / 286.3
Recommended contour level	0.007
Processing software	SCIPION 3.0.9; Topaz (particle picking); CTFFIND4; RELION (2D/3D classification, refinement, Bayesian polishing)
Refinement	
Initial models used	Internal closed-conformation CRBN coordinates (2.5 Å cryo-EM); BTK, PDB 8S93; PS-10 from molecular docking
Model building	Coot
Model refinement	Molecular dynamics flexible fitting (real space, flexible fit)
Model composition	
Non-hydrogen atoms	5,247 (protein 5,198; ligand 49)
Protein residues	652
BTK kinase domain (chain A)	268 (residues 392–659)
CRBN (chain B)	384 (residues 42–425)
Ligands	1 (A1CY2 / PS-10; $C_{35}H_{31}N_7O_7$)
B factors (Å²), protein: min / max / mean	12.6 / 292.6 / 136.3
chain A (BTK kinase domain), mean	198.9
chain B (CRBN), mean	89.0
R.m.s. deviations[b]	
Bond lengths (RMSZ)	0.12 (0 outliers)

Bond angles (RMSZ)	0.37 (0 outliers)
Validation	
MolProbity score[c]	1.49
Clashscore	5.92
Poor rotamers (%)	0.18 (1 of 564)
Chirality / planarity outliers	0 / 0
Q-score (all / BTK / CRBN)	0.182 / 0.102 / 0.240
Atom inclusion, all atoms / backbone	0.619 / 0.702
Ramachandran plot	
Favoured (%)	97.1
Allowed (%)	2.9
Outliers (%)	0.0

[a]Resolution determined from the Fourier shell correlation between the two independently refined half-maps at the 0.143 criterion, as calculated by the wwPDB validation pipeline. The author-provided FSC curve gives 3.44 Å at the same criterion.

[b]Values are root-mean-square Z scores (RMSZ) of bond lengths and bond angles reported by the wwPDB validation pipeline; no bond-length or bond-angle outlier (|Z| > 5) was detected.

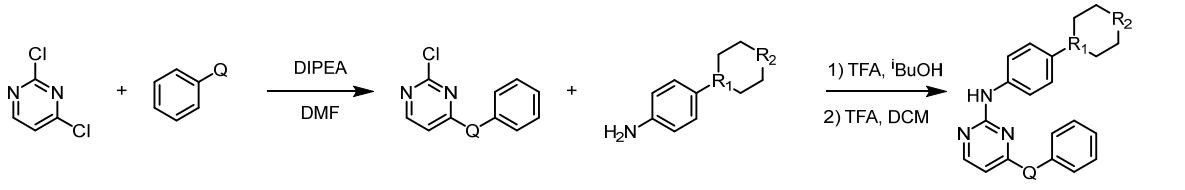
[c]Calculated from the clashscore, poor-rotamer and Ramachandran statistics reported by the wwPDB validation pipeline.

Chemistry

General Information: ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian (Palo Alto, CA) 400-MR spectrometer and are calibrated using residual undertreated solvent. Chemical shifts (δ) are reported in ppm and coupling constants (*J*) are in Hertz (Hz). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Flash chromatography was performed on a Smart Flash EPCLC W-Prep 2XY. ESI mass spectrometry was measured on an Agilent Mass Spectrometer.

Materials: All reagents were used as received from commercial sources without further purification, unless specified otherwise, or prepared as described in the literature.

Compound Synthesis and Characterization



1

2: Q=O
3: Q=NH

4: Q=O
5: Q=NH

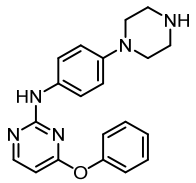
6: R1=N, R2=NBoc
7: R1=CH, R2=NBoc
8: R1=N, R2=NMe

9: Q=O, R1=N, R2=NH
10: Q=NH, R1=N, R2=NH
11: Q=O, R1=CH, R2=NH

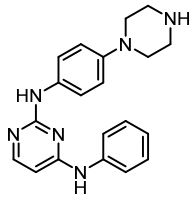
2-chloro-4-phenoxypyrimidine (4)[1] and **2-chloro-N-phenylpyrimidin-4-amine (5)**[2] were prepared as described in the literature.

General procedure for the synthesis of compounds 9, 10, 11 and PS-W

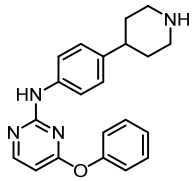
Compound **4** or **5** (2mmol) and aniline derivatives **6-8** (2.2 mmol) were dissolved in 2-Methyl-1-propanol (10 mL), 1-2 drops of trifluoroacetic acid was added. The mixture was heated to 90°C. After 12 hours, the mixture was cooled to room temperature and concentrated under reduced. the residue was purified with silica gel chromatography (DCM/Methanol = 20:1) to give crude product. The crude product was stirred in 10% triacetic acid DCM solution for 30 minutes to remove Boc to obtain product **9-11.**



9 **4-phenoxy-N-(4-(piperazin-1-yl)phenyl)pyrimidin-2-amine (9)** The product (633 mg, 71%) was purified as a light brown solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.37 (br, 1H), 8.72 (br, 1H), 8.29 (d, *J* = 5.6 Hz, 1H), 7.53 – 7.44 (m, 2H), 7.37 (d, *J* = 8.4 Hz, 2H), 7.34 – 7.29 (m, 1H), 7.25 – 7.19 (m, 2H), 6.76 (d, *J* = 8.5 Hz, 2H), 6.34 (d, *J* = 5.6 Hz, 1H), 3.21 (q, *J* = 6.1, 5.6 Hz, 8H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.51, 159.99, 159.82, 152.55, 145.04, 133.50, 129.90, 125.57, 122.03, 120.23, 116.60, 46.49, 42.99. MS (ESI): m/z (%) 348.3 (M+H.

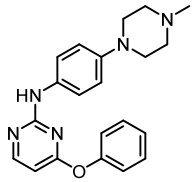


10 **N4-phenyl-N2-(4-(piperazin-1-yl)phenyl)pyrimidine-2,4-diamine (10)** The product (615 mg, 69%) was purified as a dark green solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.71 (br, 2H), 9.01 (br, 2H), 7.96 (br, 1H), 7.69 – 7.56 (m, 2H), 7.39 (d, *J* = 8.5 Hz, 2H), 7.34 (t, *J* = 7.6 Hz, 2H), 7.16 (t, *J* = 7.4 Hz, 1H), 7.08 – 6.99 (m, 2H), 6.40 (d, *J* = 7.1 Hz, 1H), 3.27 (br, 8H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.93, 169.03, 168.81, 163.06, 157.67, 147.63, 138.77, 138.69, 134.68, 132.78, 131.55, 129.94, 127.80, 126.36, 125.82, 55.80, 52.68. MS (ESI): m/z (%) 347.3 ($M+H^+$).



11

***4-phenoxy-N-(4-(piperidin-4-yl)phenyl)pyrimidin-2-amine* (11)** The product (562 mg, 63%) was purified as a yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.59 (br, 1H), 8.87 (br, 1H), 8.64 (br, 1H), 8.34 (d, *J* = 5.6 Hz, 1H), 7.49 (t, *J* = 7.8 Hz, 2H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.32 (t, *J* = 7.4 Hz, 1H), 7.25 (d, *J* = 7.8 Hz, 2H), 6.94 (d, *J* = 8.1 Hz, 2H), 6.42 (d, *J* = 5.5 Hz, 1H), 3.33 (m, 2H), 2.96 (q, *J* = 12.2 Hz, 2H), 2.71 (tt, *J* = 12.2, 3.7 Hz, 1H), 1.85 (m, 2H), 1.75 (qd, *J* = 13.2, 3.7 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.93, 160.21, 160.02, 152.94, 138.94, 138.15, 130.26, 126.67, 125.94, 122.50, 119.56, 44.05, 38.72, 30.00. MS (ESI): m/z (%) 347.2 ($M+H^+$).

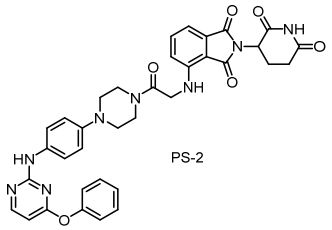


PS-W

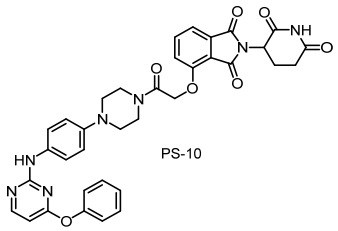
N-(4-(4-methylpiperazin-1-yl)phenyl)-4-phenoxypyrimidin-2-amine (PS-W) The product (506 mg, 70% yield) was purified as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.35 (br, 1H), 8.29 (d, *J* = 5.5 Hz, 1H), 7.52 – 7.45 (m, 2H), 7.36 (m, 2H), 7.31 (t, *J* = 7.4 Hz, 1H), 7.25 – 7.20 (m, 2H), 6.74 (d, *J* = 8.5 Hz, 2H), 6.33 (d, *J* = 5.5 Hz, 1H), 3.14 (br, 4H), 2.96 (br, 4H), 2.54 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.85, 160.39, 160.29, 152.96, 130.25, 125.89, 122.42, 120.58, 116.54, 40.93, 40.55. MS (ESI): m/z (%) 362.2 ($M+H^+$).

General procedure for the synthesis of compounds PS2, PS10, PS10Me, PS16, PS22, PS29, PS31, PS32

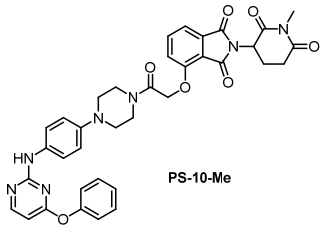
In a 25 mL flask was added compound **9-11** (4.5 mg, 0.01 mmol), then corresponding carboxylic acid (0.015 mol), HATU (5.7 mg, 0.015 mmol) and DIPEA (6.4 mg, 0.05 mmol) and DMF (2 mL) was added. The mixture was stirred at room temperature for 4h. Then the reaction mixture was concentrated in vacuo and the residue was purified by prepHPLC with a reverse phase C18 column to afford the product.



2-(2,6-dioxopiperidin-3-yl)-4-((2-oxo-2-(4-(4-((4-phenoxypyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethyl)amino)isoindoline-1,3-dione (PS-2). The product (4.72mg, 71%) was purified with prepHPLC with a reverse phase C18 column as a yellow solid. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.62 (s, 1H), 8.24 (d, *J* = 5.6 Hz, 1H), 7.51 (t, *J* = 7.8 Hz, 1H), 7.44 (t, *J* = 7.9 Hz, 2H), 7.35 – 7.27 (m, 3H), 7.21 – 7.12 (m, 5H), 6.79 (dd, *J* = 12.6, 8.4 Hz, 3H), 6.26 (d, *J* = 5.6 Hz, 1H), 4.93 (dd, *J* = 12.4, 5.4 Hz, 1H), 4.08 (d, *J* = 4.4 Hz, 2H), 3.84 (t, *J* = 5.2 Hz, 2H), 3.66 – 3.56 (m, 2H), 3.11 (dt, *J* = 21.2, 5.2 Hz, 4H), 2.93 – 2.68 (m, 3H), 2.19 – 2.10 (m, 1H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 172.81, 170.16, 169.65, 168.06, 165.95, 159.87, 159.26, 152.67, 146.34, 145.45, 136.06, 133.07, 132.50, 129.60, 125.50, 122.02, 120.57, 117.74, 110.93, 98.30, 53.12, 52.79, 51.76, 50.49, 50.13, 44.48, 43.96, 42.19, 41.39, 30.88, 29.68, 24.34, 18.20. MS (ESI): m/z (%) 661.2 ($M+H^+$).

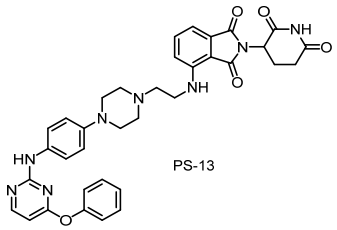


2-(2,6-dioxopiperidin-3-yl)-4-(2-oxo-2-(4-(4-((4-phenoxypyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethoxy)isoindoline-1,3-dione (PS-10). The product (4.5 mg, 68%) was purified with prepHPLC with a reverse phase C18 column as a white solid. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.75 (br, 1H), 8.23 (d, *J* = 5.6 Hz, 1H), 7.52 (d, *J* = 7.3 Hz, 1H), 7.47 – 7.40 (m, 2H), 7.35 (d, *J* = 8.5 Hz, 1H), 7.31 – 7.27 (m, 3H), 7.20 – 7.13 (m, 3H), 6.75 (d, *J* = 8.5 Hz, 2H), 6.24 (d, *J* = 5.7 Hz, 1H), 5.02 (s, 2H), 4.95 (dd, *J* = 12.5, 5.3 Hz, 1H), 3.81 – 3.70 (m, 4H), 3.16 – 2.99 (m, 4H), 2.94 – 2.68 (m, 3H), 2.13 (dtd, *J* = 13.5, 6.0, 5.5, 2.7 Hz, 1H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 171.08, 170.18, 168.08, 166.76, 165.51, 165.24, 159.89, 159.30, 155.27, 152.68, 146.41, 136.69, 133.86, 132.91, 129.60, 125.50, 122.03, 120.56, 120.17, 117.66, 117.56, 117.11, 98.26, 68.56, 50.75, 50.07, 49.24, 45.40, 42.21, 31.41, 22.60. MS (ESI): m/z (%) 662.2 ($M+H^+$).



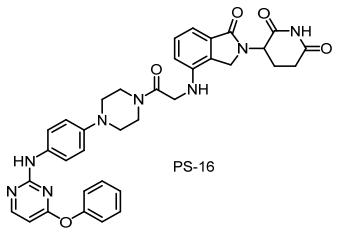
PS-10-Me

2-(1-methyl-2,6-dioxopiperidin-3-yl)-4-(2-oxo-2-(4-(4-((4-phenoxypyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethoxy)isoindoline-1,3-dione (PS-10Me). The product (4.3mg, 66%) was purified with prepHPLC with a reverse phase C18 column as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.35 (br, 1H), 8.29 (d, J = 5.5 Hz, 1H), 8.14 (s, 1H), 7.78 (t, J = 7.9 Hz, 1H), 7.48 (dt, J = 15.3, 7.5 Hz, 3H), 7.34 (dt, J = 27.9, 8.0 Hz, 4H), 7.23 (d, J = 7.8 Hz, 2H), 6.75 (d, J = 8.6 Hz, 2H), 6.34 (d, J = 5.6 Hz, 1H), 5.75 (s, 1H), 5.23 (s, 2H), 5.17 (dd, J = 13.1, 5.4 Hz, 1H), 3.59 (t, J = 5.1 Hz, 4H), 3.09 (t, J = 5.0 Hz, 2H), 3.05 – 2.87 (m, 6H), 2.76 (dt, J = 17.4, 3.8 Hz, 1H), 2.55 (td, J = 13.3, 4.5 Hz, 1H), 2.05 (dt, J = 12.0, 5.6 Hz, 1H). MS (ESI): m/z (%) 662.2 ($M+H^+$).



PS-13

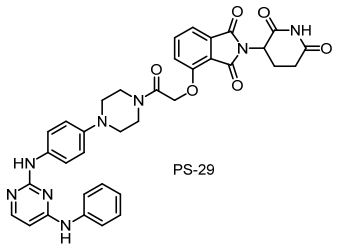
2-(2,6-dioxopiperidin-3-yl)-4-((2-(4-(4-((4-phenoxypyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethyl)amino)isoindoline-1,3-dione (PS-13). The product (4.4 mg) was purified with prepHPLC with a reverse phase C18 column as a white solid. ^{1}H NMR (600 MHz, Chloroform-d) δ 8.33 (s, 1H), 8.22 (d, J = 5.6 Hz, 1H), 7.51 (dd, J = 8.4, 7.2 Hz, 1H), 7.47 – 7.41 (m, 2H), 7.31 – 7.27 (m, 3H), 7.19 – 7.15 (m, 2H), 7.11 (d, J = 7.1 Hz, 1H), 6.92 (d, J = 8.5 Hz, 1H), 6.81 – 6.74 (m, 3H), 6.24 (d, J = 5.6 Hz, 1H), 4.91 (dd, J = 12.6, 5.4 Hz, 1H), 3.42 (s, 2H), 3.19 (s, 4H), 2.93 – 2.65 (m, 8H), 2.15 – 2.05 (m, 1H). ^{13}C NMR (151 MHz, Chloroform-d) δ 171.55, 171.01,170.17, 168.31,167.67, 132.60, 129.60, 125.48, 122.03, 116.97, 116.87, 98.15, 55.97, 52.82, 49.78, 48.89, 41.02, 31.44, 22.73. MS (ESI): m/z (%) 647.3 ($M+H^+$).



PS-16

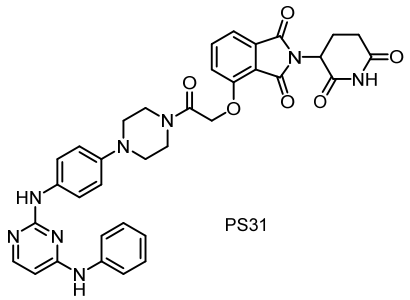
3-(1-oxo-4-((2-oxo-2-(4-(4-((4-phenoxypyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethyl)amino)isoindolin-2-yl)piperidine-2,6-dione (PS-16). The product (4.5mg, 71%) was purified with prepHPLC with a reverse phase C18 column as a yellow solid^{1}H NMR (600 MHz, Chloroform-d) δ 8.29 (s, 0H), 8.25 (s, 1H), 8.23 (d, J =

5.7 Hz, 1H), 7.44 (t, *J* = 7.7 Hz, 2H), 7.38 (t, *J* = 7.7 Hz, 1H), 7.30 (q, *J* = 10.0, 8.2 Hz, 3H), 7.17 (d, *J* = 7.9 Hz, 2H), 6.79 (d, *J* = 8.5 Hz, 2H), 6.71 (d, *J* = 7.9 Hz, 1H), 6.27 (d, *J* = 5.7 Hz, 1H), 5.23 (dd, *J* = 13.3, 5.1 Hz, 1H), 4.86 (s, 1H), 4.06 – 3.95 (m, 2H), 3.89 – 3.80 (m, 2H), 3.63 (d, *J* = 5.7 Hz, 2H), 3.21 – 3.07 (m, 3H), 2.97 – 2.77 (m, 2H), 2.37 (qd, *J* = 13.4, 4.8 Hz, 1H), 2.21 (d, *J* = 13.0 Hz, 1H).. MS (ESI): m/z (%) 646.2 ($M+H^+$).



PS-29

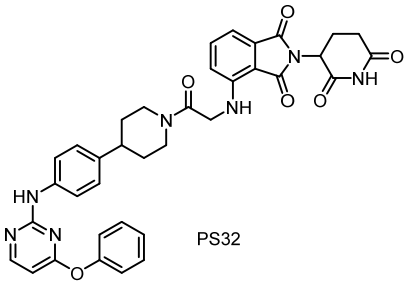
2-(2,6-dioxopiperidin-3-yl)-4-(2-oxo-2-(4-(4-((4-(phenylamino)pyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethoxy)isoindoline-1,3-dione (PS-29). The product (4.6mg, 71%) was purified with prepHPLC with a reverse phase C18 column as a yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.12 (s, 1H), 9.29 (s, 1H), 8.92 (s, 1H), 7.97 (d, *J* = 5.8 Hz, 1H), 7.71 (d, *J* = 8.0 Hz, 2H), 7.62 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.58 (d, *J* = 9.0 Hz, 1H), 7.33 – 7.26 (m, 2H), 7.17 – 7.05 (m, 3H), 6.99 (tt, *J* = 7.3, 1.2 Hz, 1H), 6.95 – 6.86 (m, 2H), 6.16 (d, *J* = 5.7 Hz, 1H), 4.26 (d, *J* = 4.6 Hz, 2H), 3.67 (dt, *J* = 19.0, 5.2 Hz, 4H), 3.47 – 3.33 (m, overlap with water peat), 3.17 – 3.12 (m, 2H), 3.07 (t, *J* = 5.3 Hz, 2H), 2.89 (ddd, *J* = 16.9, 13.8, 5.5 Hz, 1H), 2.66 – 2.37 (m, overlap with DMSO peat), 2.11 – 1.99 (m, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.91, 170.15, 168.88, 167.44, 166.49, 160.50, 159.83, 156.07, 145.73, 145.56, 140.23, 136.28, 133.75, 132.07, 128.67, 121.94, 120.78, 119.84, 118.33, 116.67, 110.93, 109.62, 98.28, 49.68, 49.37, 48.66, 43.82, 43.72, 41.61, 40.06, 31.05, 22.22. MS (ESI): m/z (%) 661.2 ($M+H^+$).



PS31

2-(2,6-dioxopiperidin-3-yl)-4-(2-oxo-2-(4-(4-((4-(phenylamino)pyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethoxy)isoindoline-1,3-dione (PS-31). The product (4.9mg, 74%) was purified with prepHPLC with a reverse phase C18 column as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.12 (s, 1H), 9.29 (s, 1H), 8.94 (s, 1H), 7.97 (d, *J* = 5.7 Hz, 1H), 7.79 (dd, *J* = 8.5, 7.2 Hz, 1H), 7.72 (d, *J* = 8.0 Hz, 2H), 7.59 (d, *J* = 8.9 Hz, 2H), 7.46 (d, *J* = 7.2 Hz, 1H), 7.38 (d, *J* = 8.6 Hz, 1H), 7.30 (t, *J* = 7.9 Hz, 2H), 7.02

– 6.97 (m, 1H), 6.92 (d, *J* = 9.0 Hz, 2H), 6.17 (d, *J* = 5.7 Hz, 1H), 5.26 (s, 2H), 5.12 (dd, *J* = 12.9, 5.5 Hz, 1H), 3.62 (t, *J* = 5.2 Hz, 4H), 3.16 (t, *J* = 5.0 Hz, 2H), 3.07 (t, *J* = 5.3 Hz, 2H), 2.90 (ddd, *J* = 16.9, 13.9, 5.5 Hz, 1H), 2.65 – 2.52 (m, 2H), 2.05 (dtd, *J* = 13.0, 5.3, 2.3 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.26, 170.40, 167.28, 165.74, 165.48, 160.91, 160.24, 156.48, 156.11, 146.11, 140.67, 137.06, 134.16, 133.58, 129.07, 122.32, 121.14, 120.71, 120.25, 117.02, 116.64, 115.95, 98.71, 66.60, 50.10, 49.77, 49.24, 44.50, 41.78, 31.42, 22.47.MS (ESI): m/z (%) 661.2 ($M+H^+$).

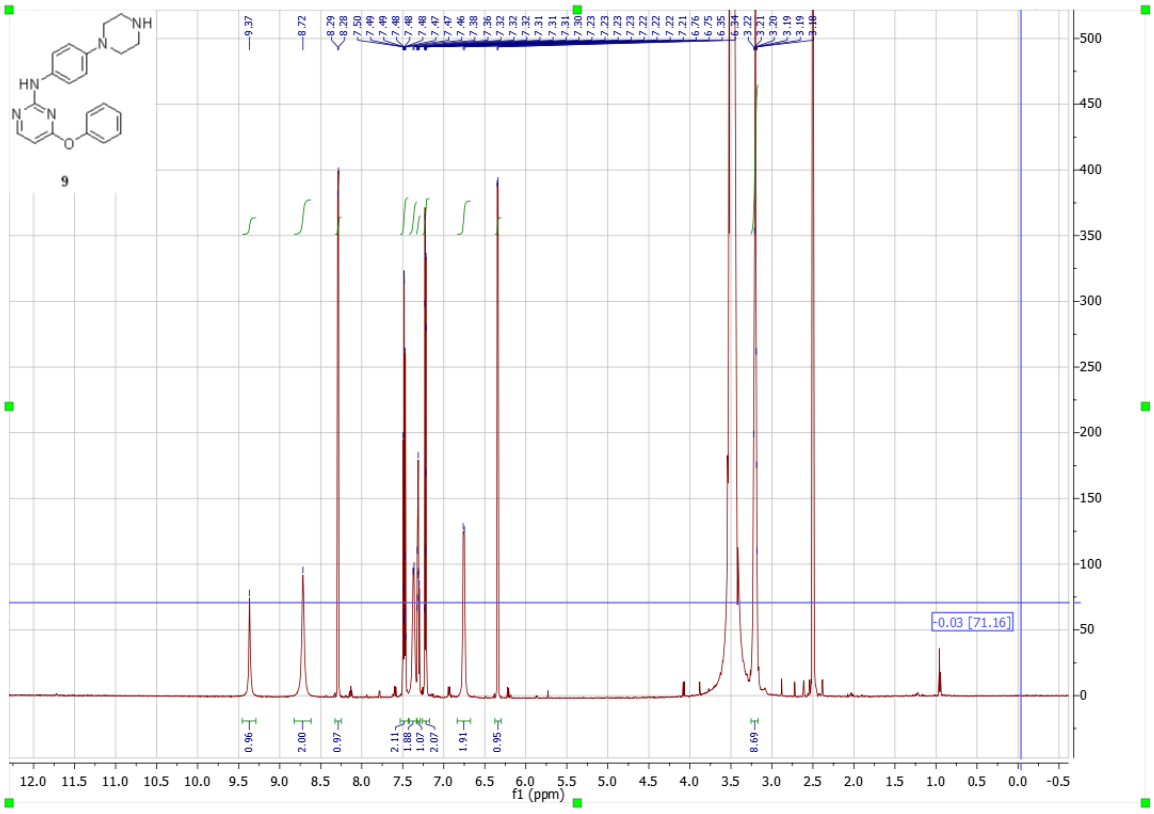


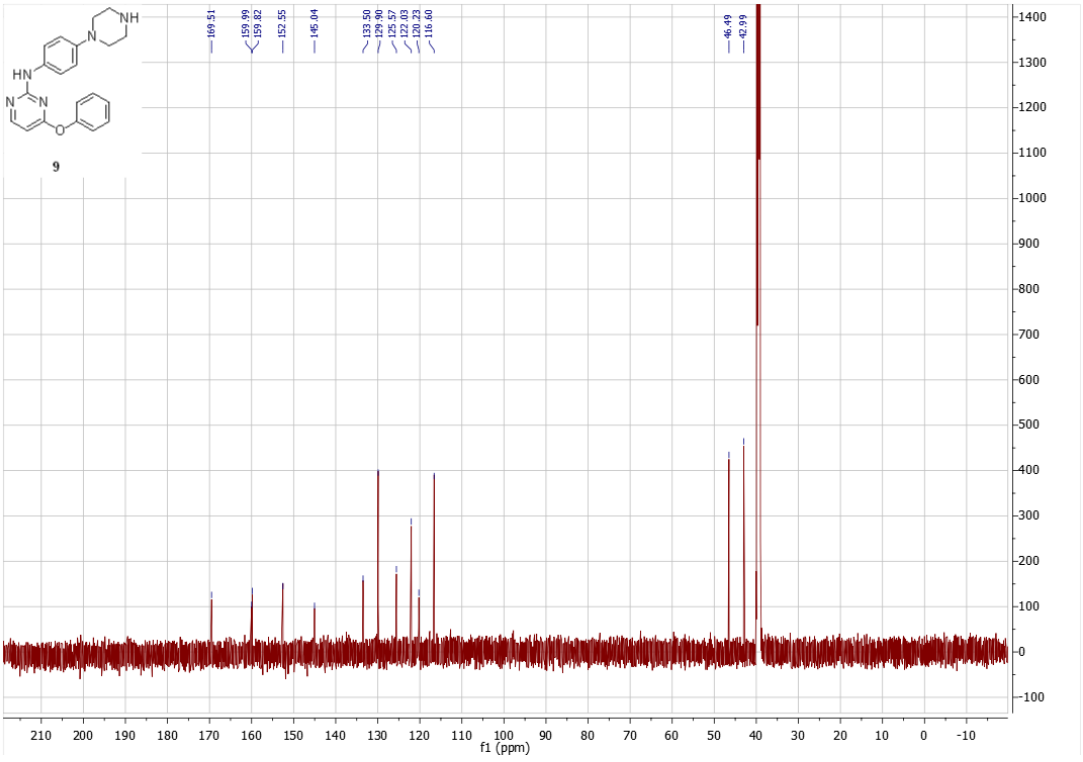
PS32

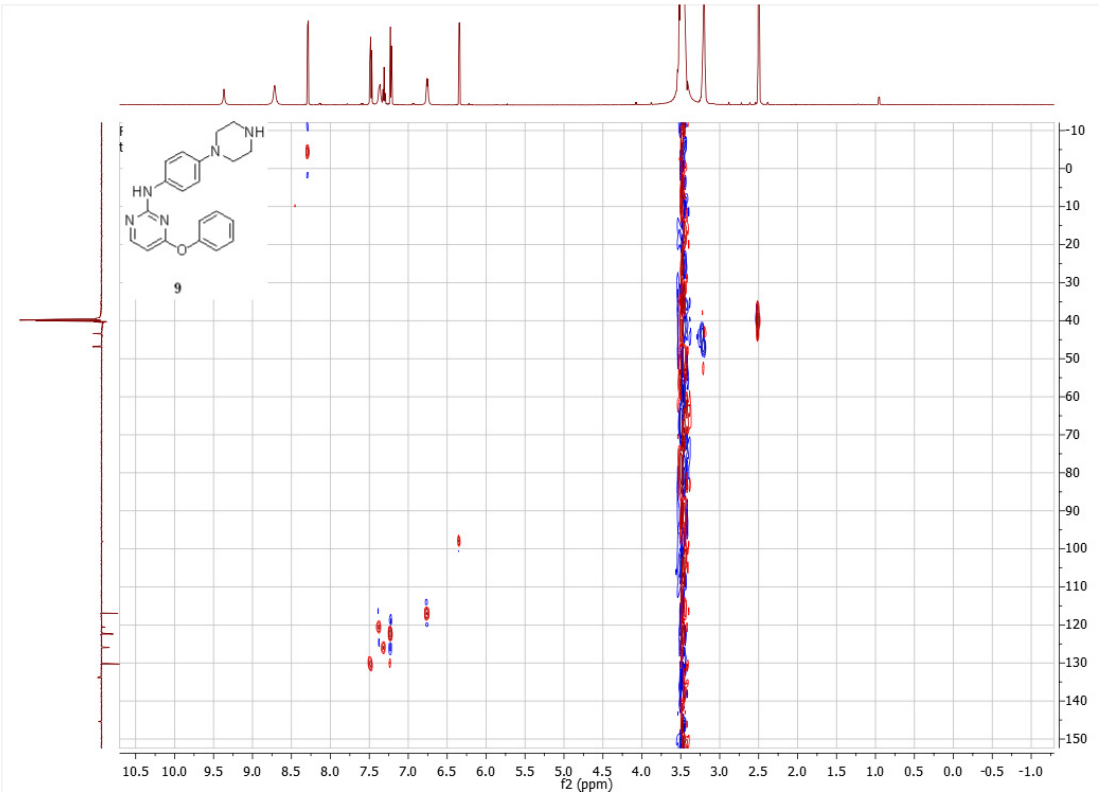
2-(2,6-dioxopiperidin-3-yl)-4-((2-oxo-2-(4-(4-((4-phenoxypyrimidin-2-yl)amino)phenyl)piperidin-1-yl)ethyl)amino)isoindoline-1,3-dione) (PS-32). The product (5.2 mg, 78%) was purified with prepHPLC with a reverse phase C18 column as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.11 (s, 1H), 9.50 (s, 1H), 8.33 (d, *J* = 5.6 Hz, 1H), 7.61 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.52 – 7.46 (m, 2H), 7.44 – 7.38 (m, 2H), 7.33 (t, *J* = 7.4 Hz, 1H), 7.26 – 7.22 (m, 2H), 7.17 – 7.12 (m, 2H), 7.08 (d, *J* = 7.0 Hz, 1H), 6.97 (d, *J* = 8.3 Hz, 2H), 6.40 (d, *J* = 5.6 Hz, 1H), 5.08 (dd, *J* = 12.8, 5.5 Hz, 1H), 4.54 (d, *J* = 12.9 Hz, 1H), 4.30 – 4.13 (m, 2H), 4.00 (d, *J* = 13.4 Hz, 1H), 3.15 – 3.08 (m, 1H), 2.89 (ddd, *J* = 16.8, 13.7, 5.5 Hz, 1H), 2.77 – 2.66 (m, 2H), 2.63 – 2.51 (m, 3H), 2.10 – 2.00 (m, 1H), 1.80 – 1.71 (m, 2H), 1.59 (tt, *J* = 12.5, 6.6 Hz, 1H), 1.44 (qd, *J* = 12.6, 4.3 Hz, 1H), 1.34 – 1.25 (m, 3H), 0.85 (t, *J* = 7.0 Hz, 1H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 172.09, 170.44, 169.07, 167.91, 165.88, 159.91, 159.43, 152.92, 145.77, 138.84, 138.08, 136.26, 132.93, 129.84, 127.08, 125.78, 122.33, 119.44, 117.15, 112.07, 111.29, 98.74, 49.15, 45.35, 44.25, 43.30, 42.17, 34.06, 33.64, 32.92, 32.14, 31.69, 29.92, 23.38, 23.00, 22.91, 18.06, 14.34. MS (ESI): m/z (%) 660.3 ($M+H^+$).

NMR spectra

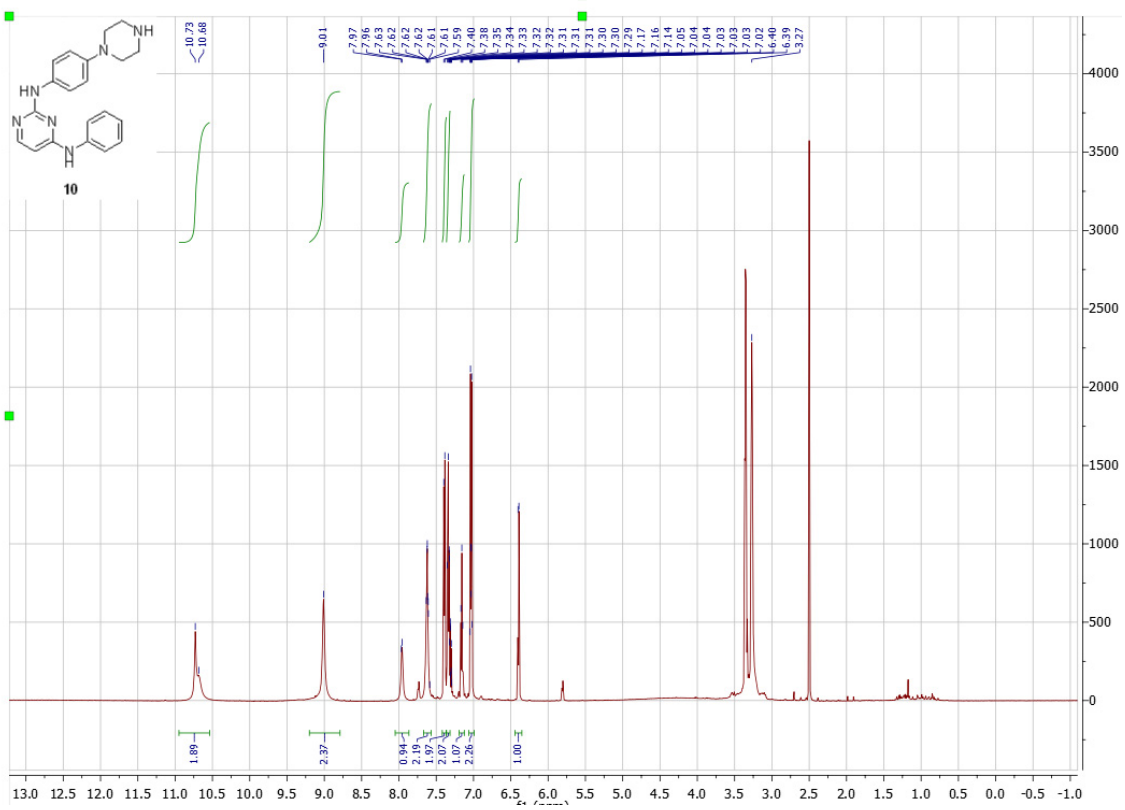
4-phenoxy-N-(4-(piperazin-1-yl)phenyl)pyrimidin-2-amine (9)

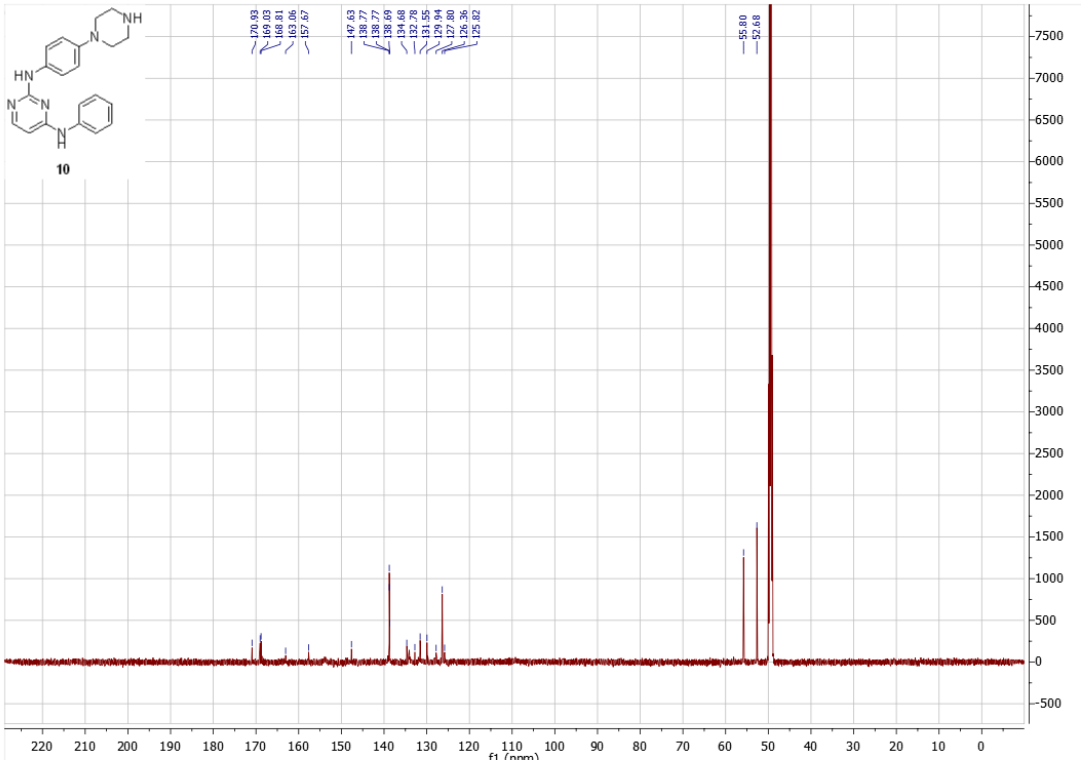




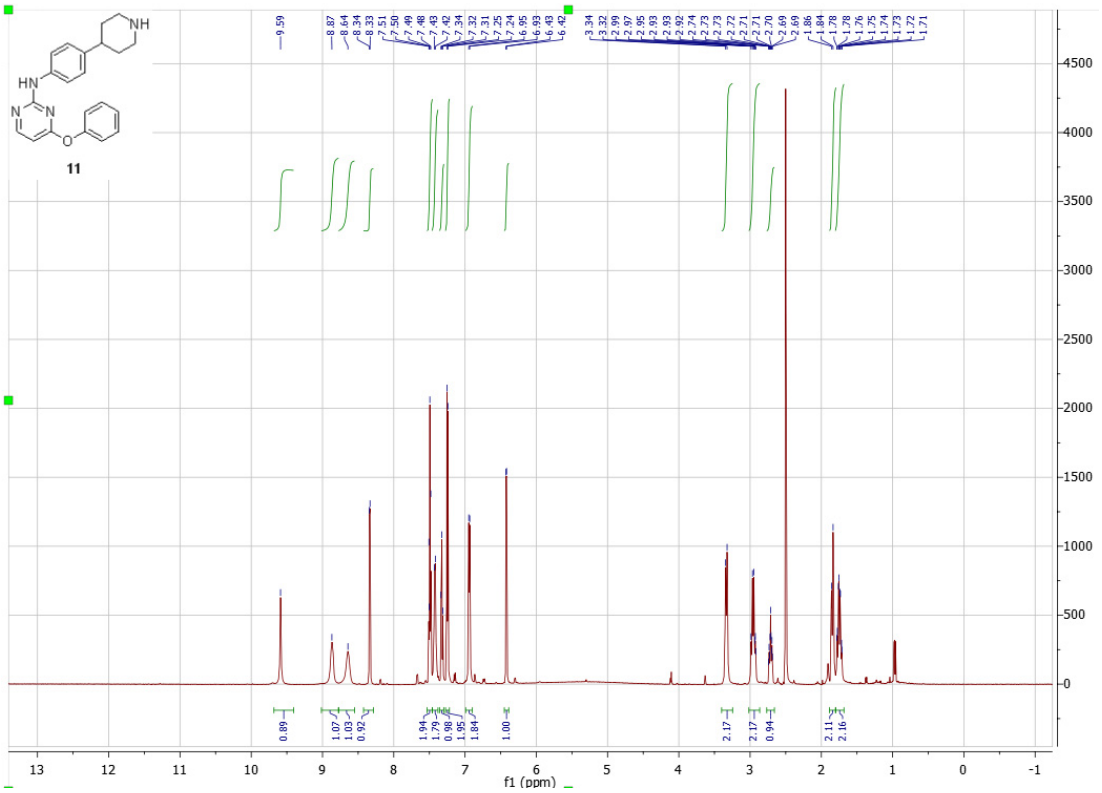

NH
HN
N
N
O
9
f2 (ppm)

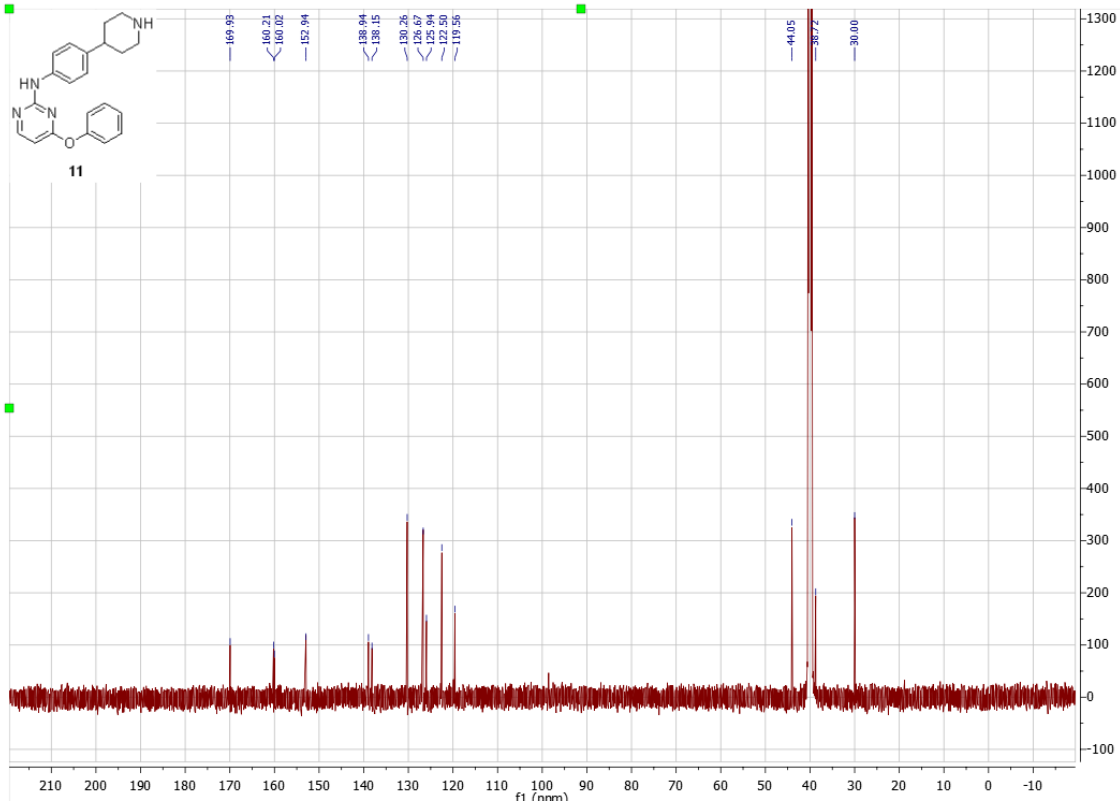
N4-phenyl-N2-(4-(piperazin-1-yl)phenyl)pyrimidine-2,4-diamine (10)



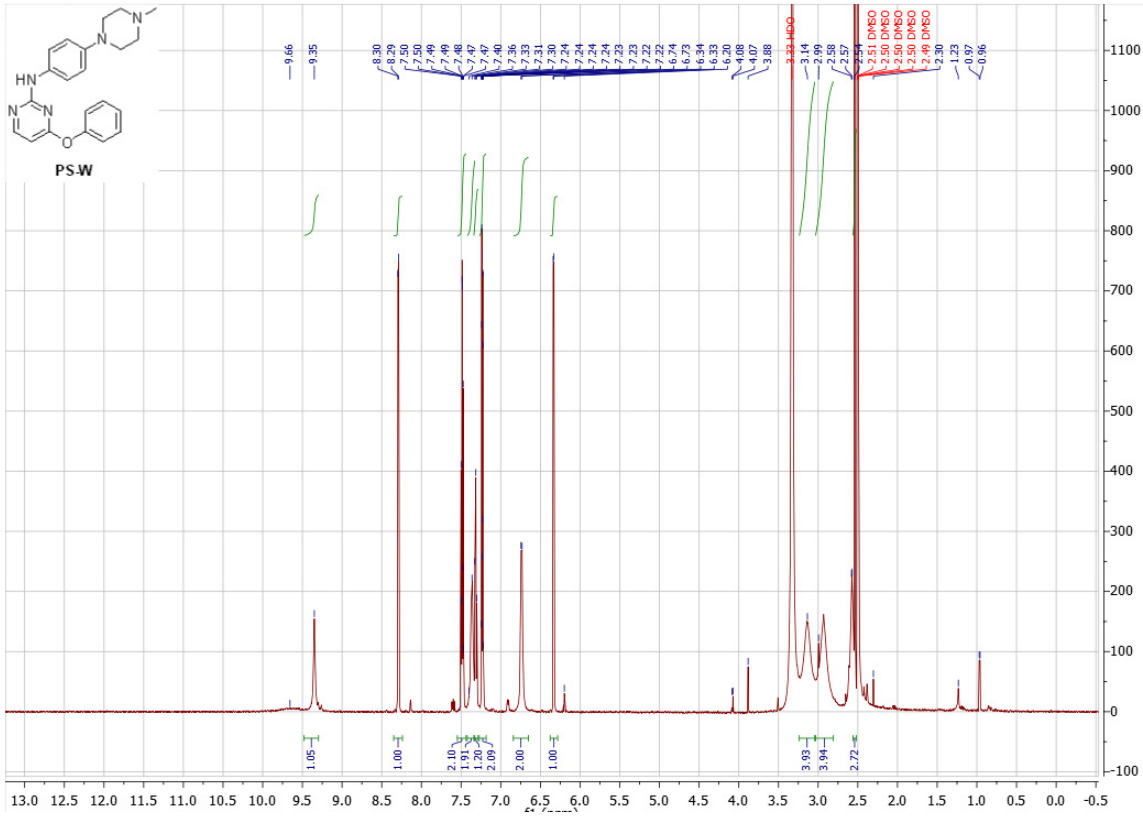


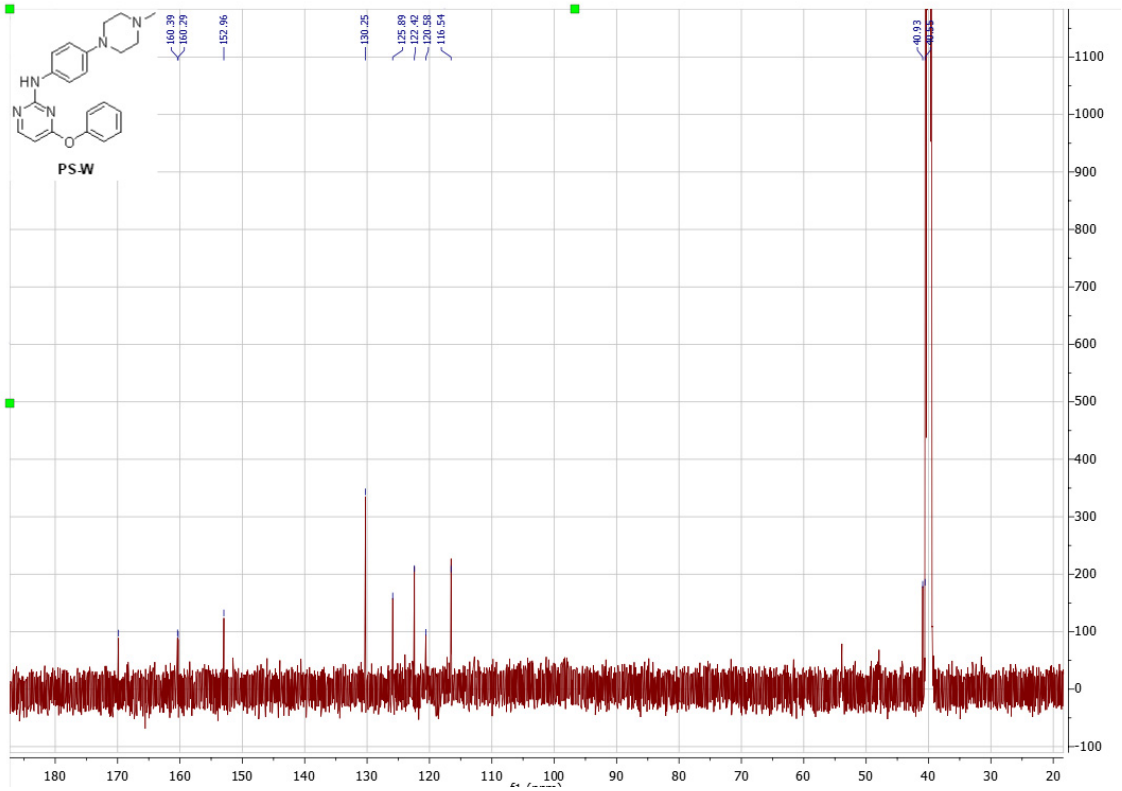
***4-phenoxy-N-(4-(piperidin-4-yl)phenyl)pyrimidin-2-amine* (11)**



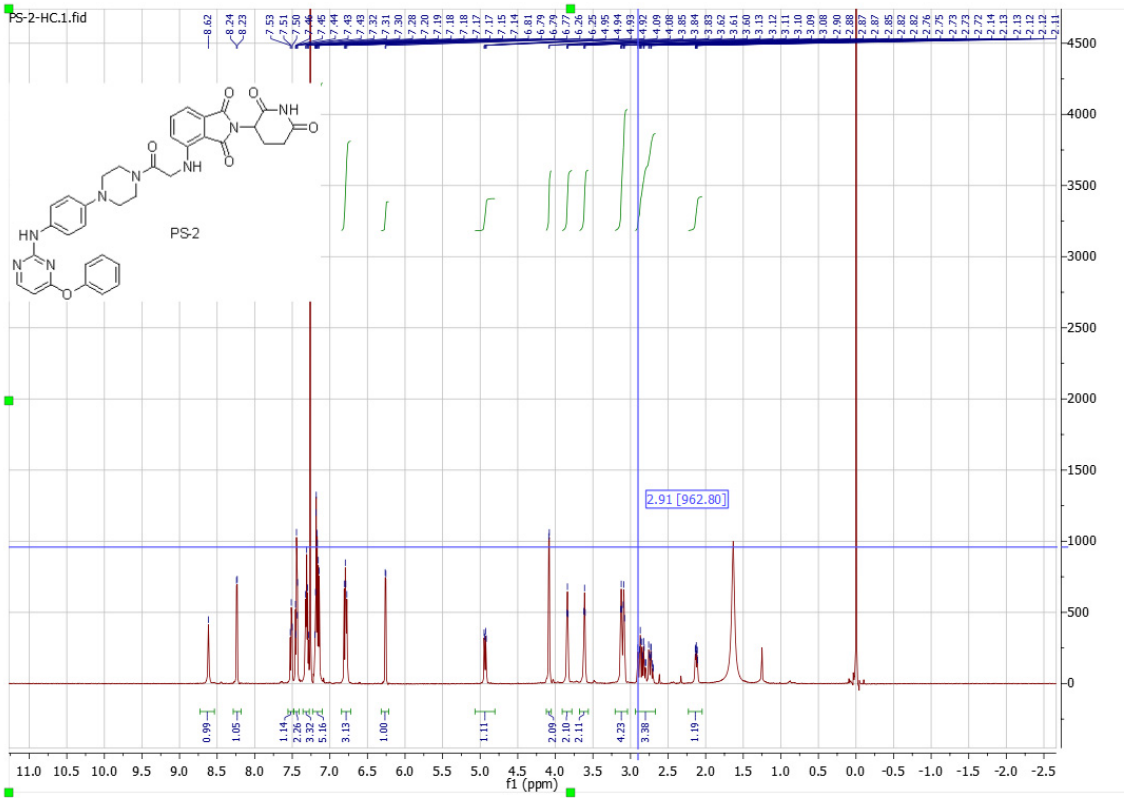


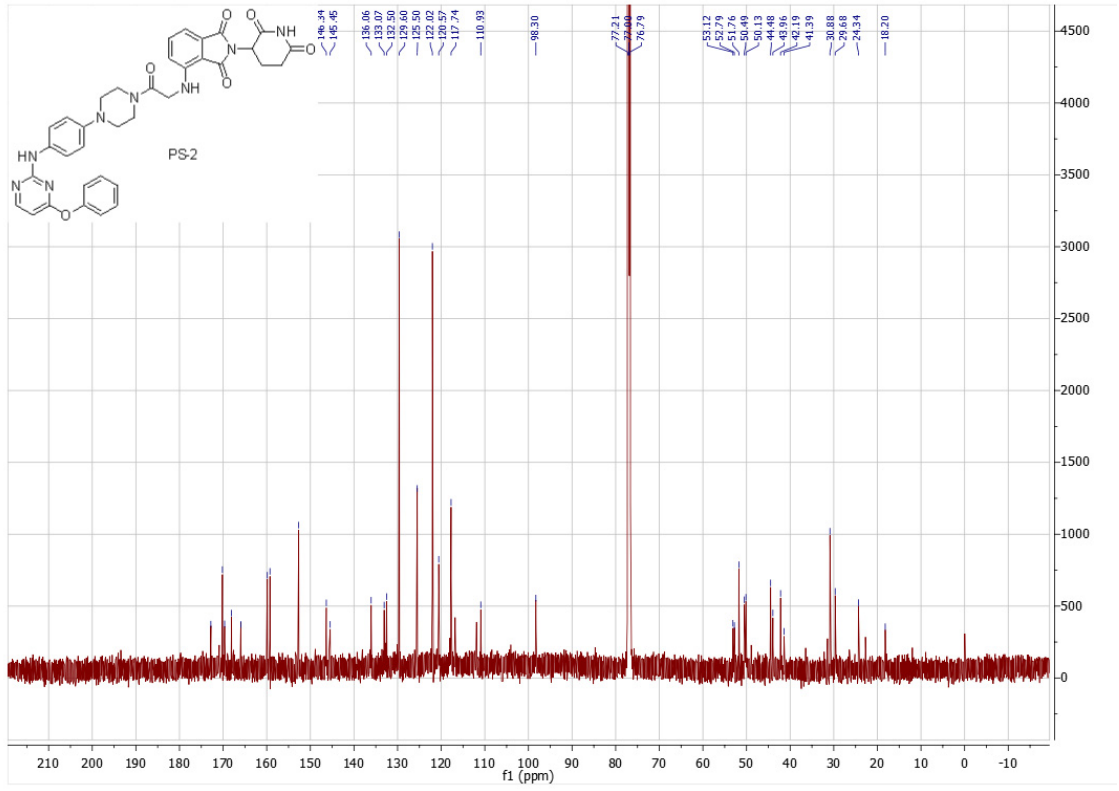
N-(4-(4-methylpiperazin-1-yl)phenyl)-4-phenoxypyrimidin-2-amine (PS-W)



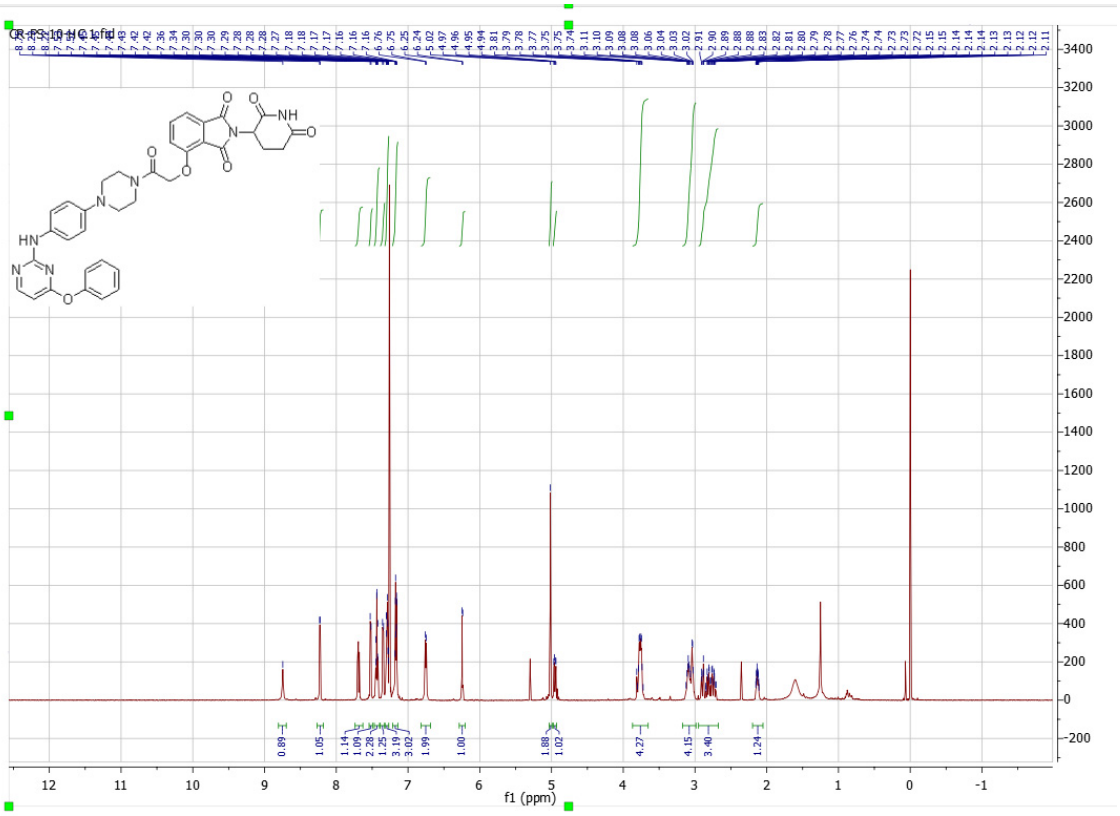


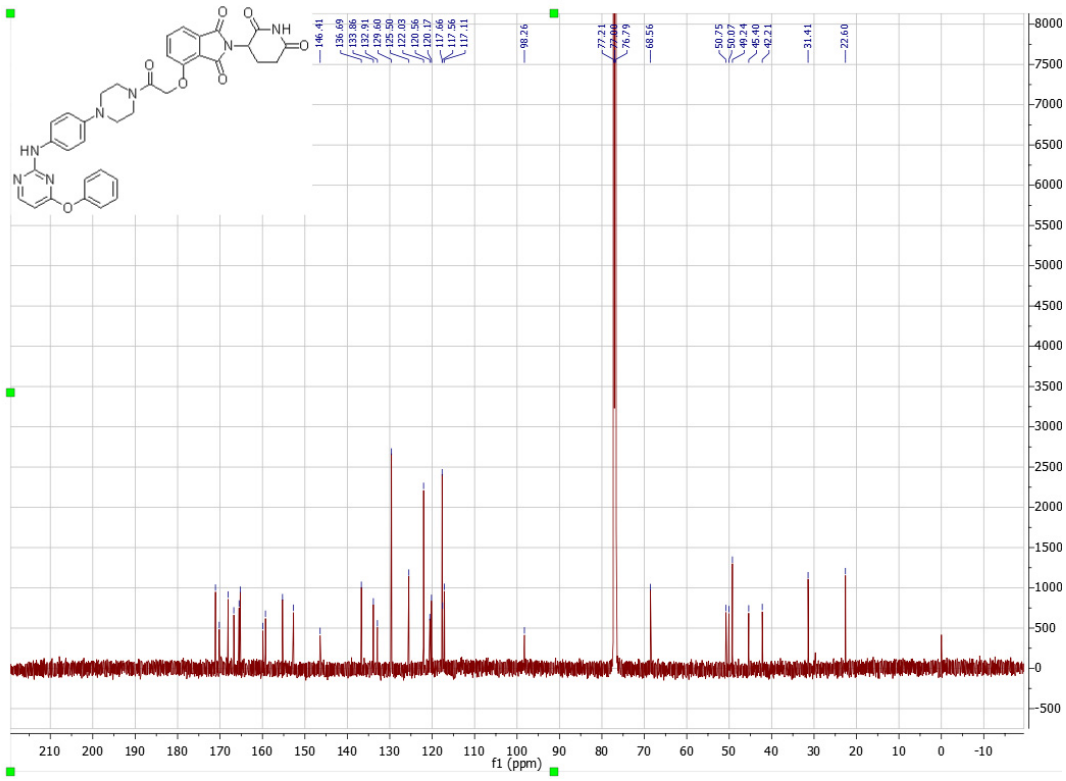
2-(2,6-dioxopiperidin-3-yl)-4-((2-oxo-2-(4-(4-((4-phenoxypyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethyl)amino)isoindoline-1,3-dione (PS2).



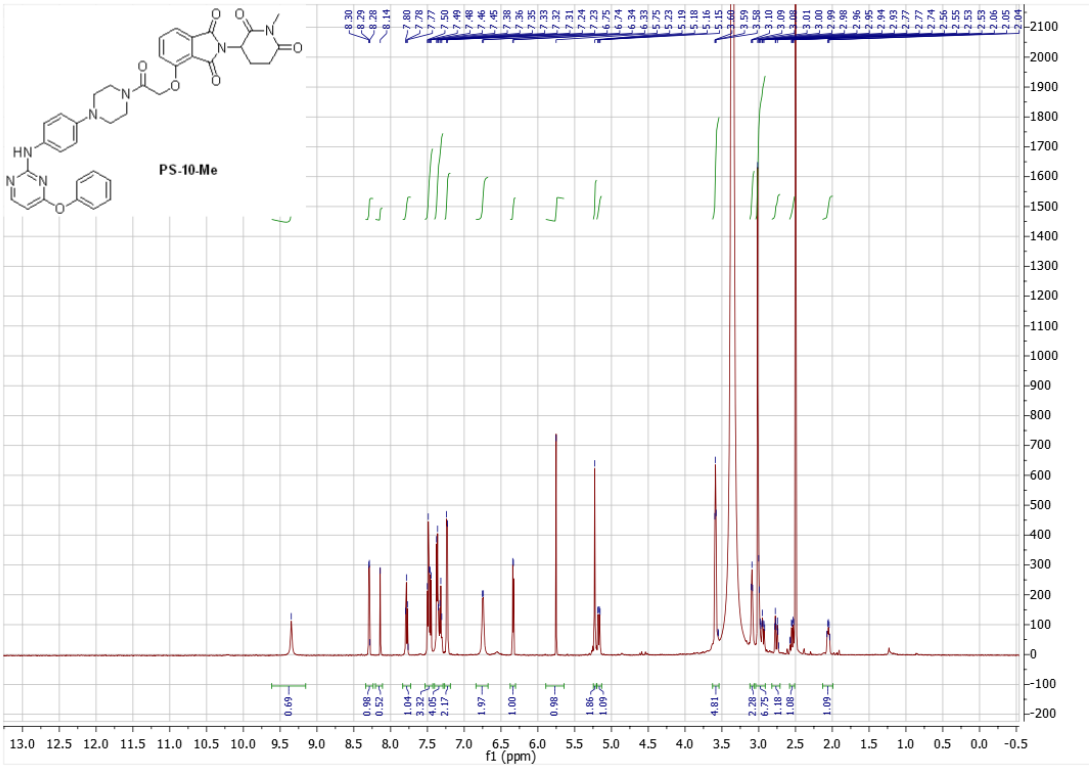


2-(2,6-dioxopiperidin-3-yl)-4-(2-oxo-2-(4-(4-((4-phenoxypyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethoxy)isoindoline-1,3-dione (PS-10).

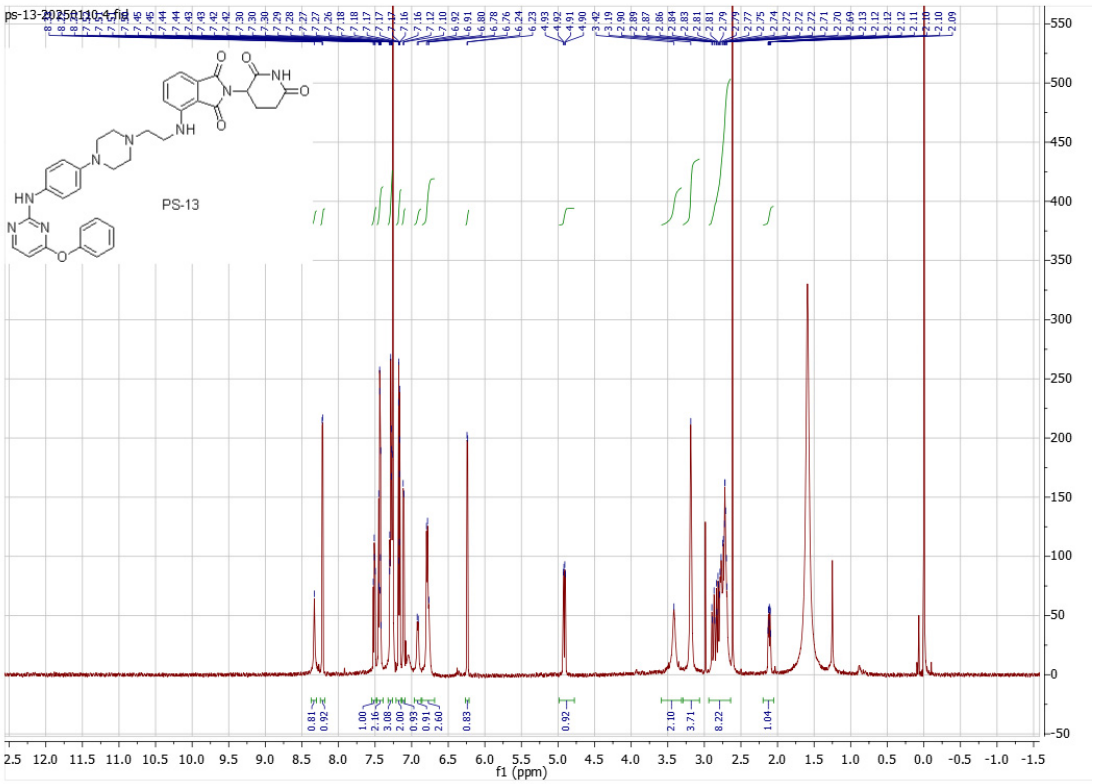


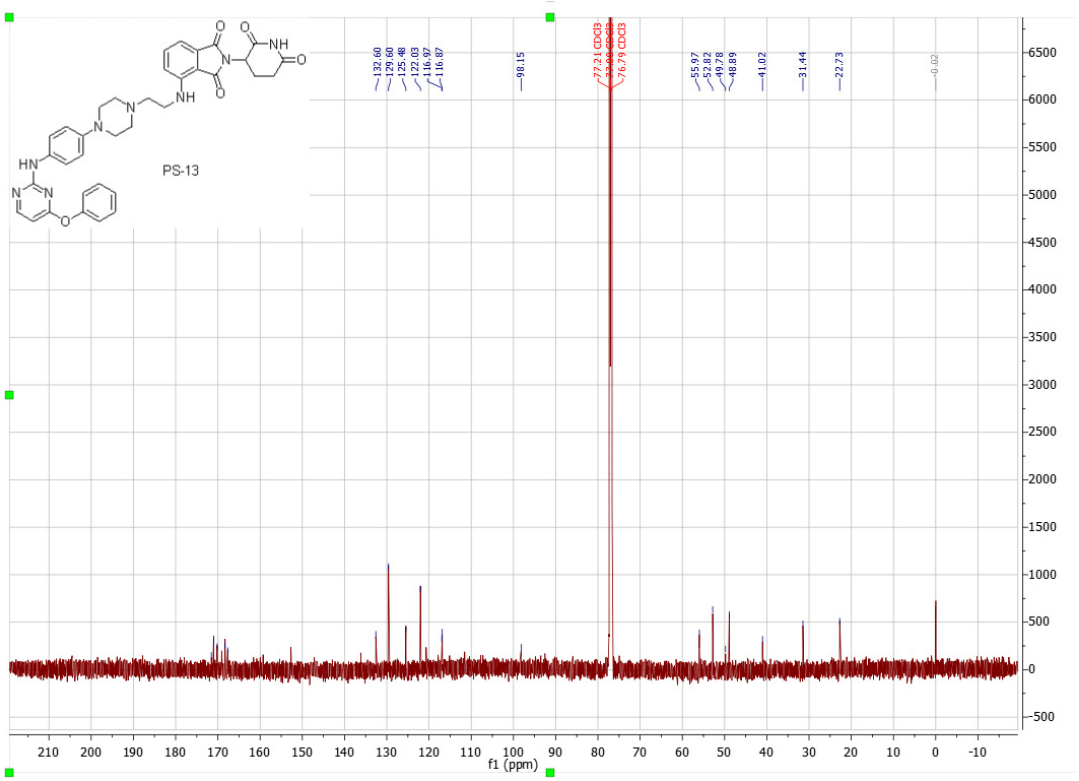


2-(1-methyl-2,6-dioxopiperidin-3-yl)-4-(2-oxo-2-(4-(4-((4-phenoxypyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethoxy)isoindoline-1,3-dione (PS-10-Me).

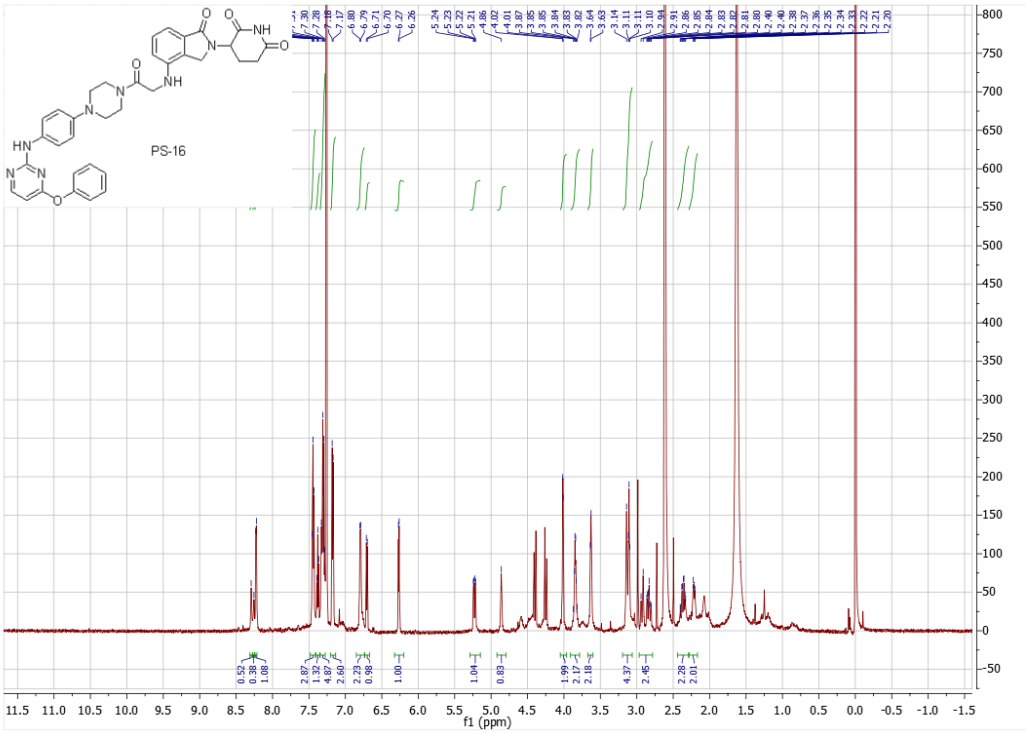


2-(2,6-dioxopiperidin-3-yl)-4-((2-(4-(4-((4-phenoxypyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethyl)amino)isoindoline-1,3-dione (PS-13)

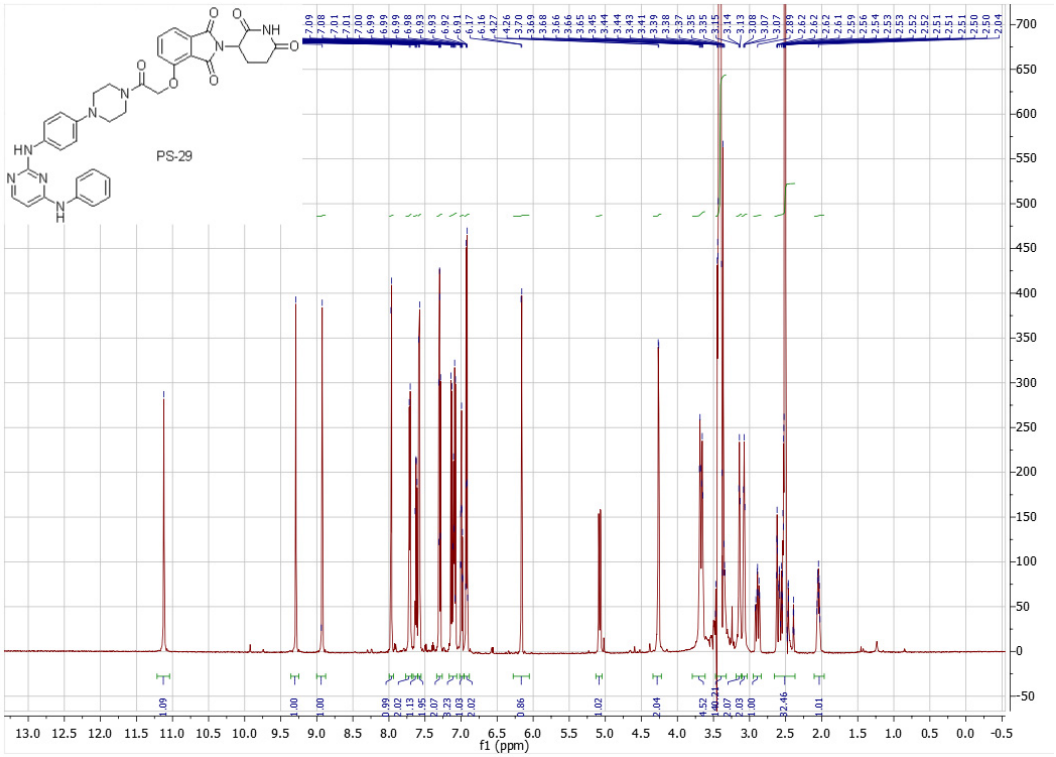


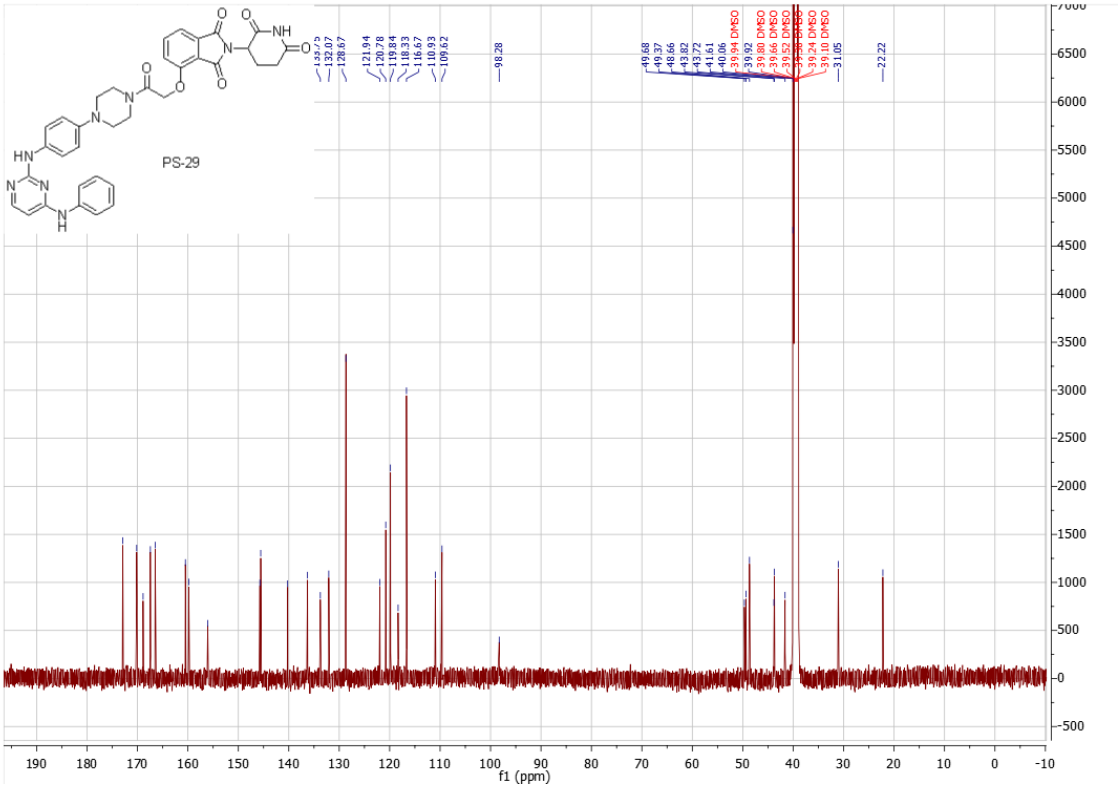


3-(1-oxo-4-((2-oxo-2-(4-(4-((4-phenoxypyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethyl)amino)isoindolin-2-yl)piperidine-2,6-dione (PS-16).

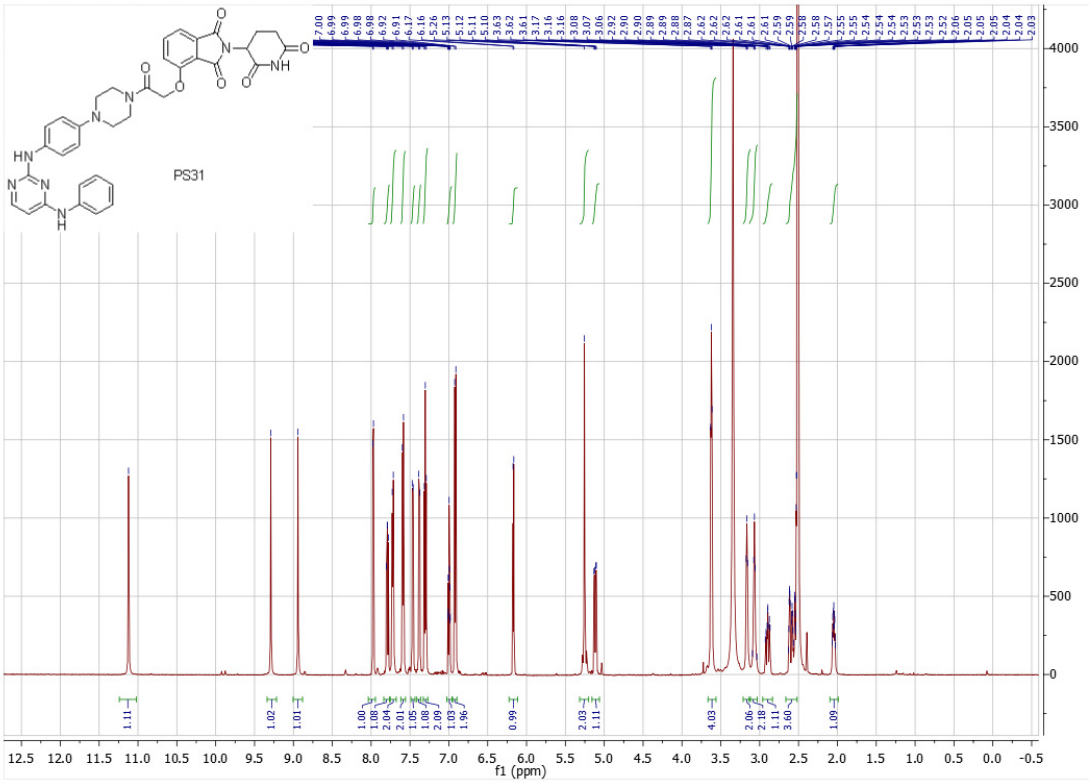


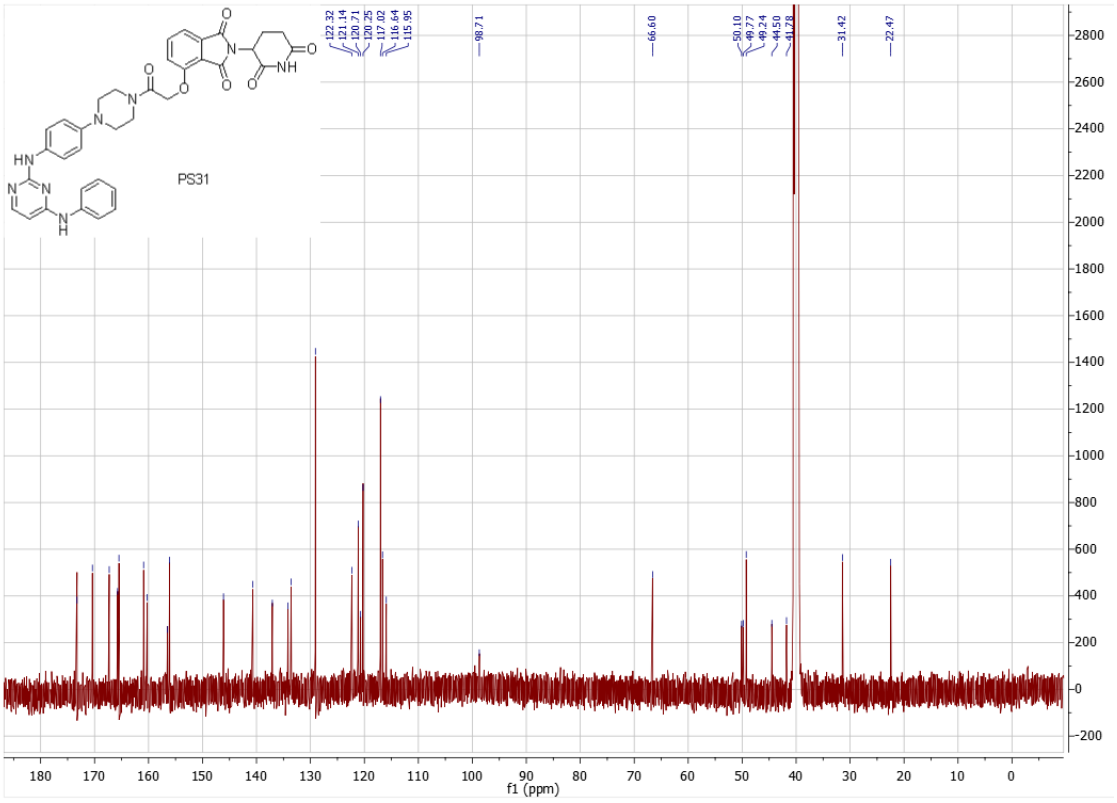
2-(2,6-dioxopiperidin-3-yl)-4-(2-oxo-2-(4-(4-((4-(phenylamino)pyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethoxy)isoindoline-1,3-dione (PS-29).

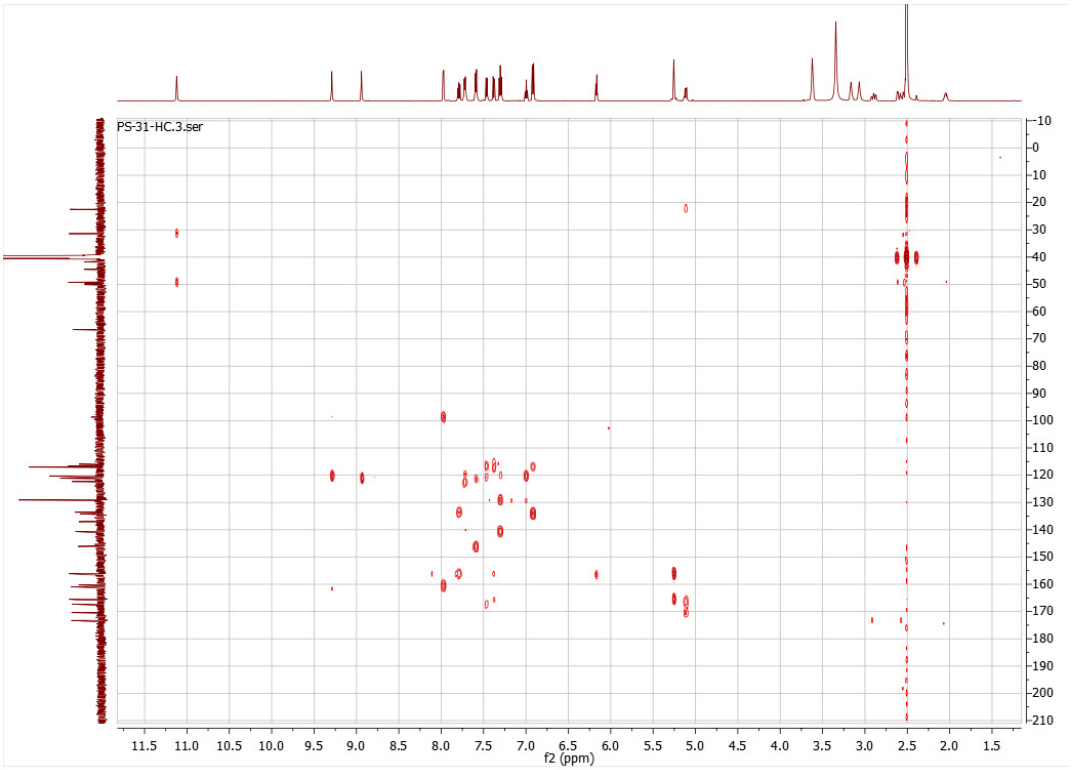




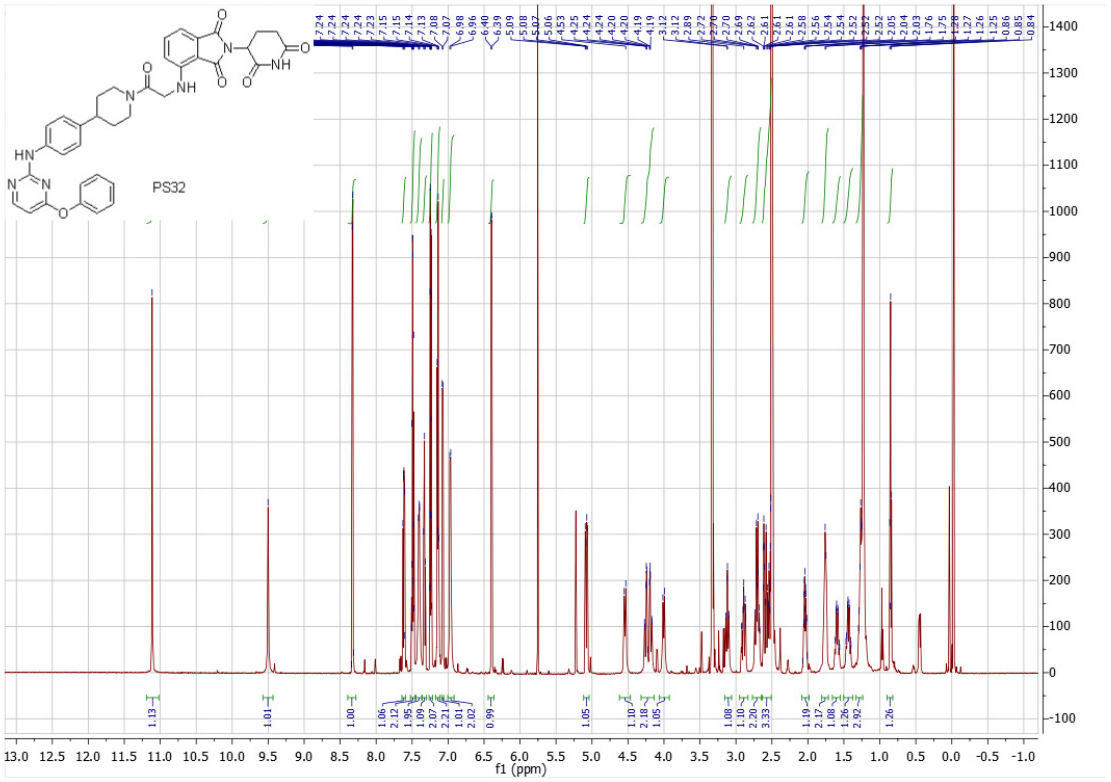
2-(2,6-dioxopiperidin-3-yl)-4-(2-oxo-2-(4-(4-((4-(phenylamino)pyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethoxy)isoindoline-1,3-dione (PS-31).

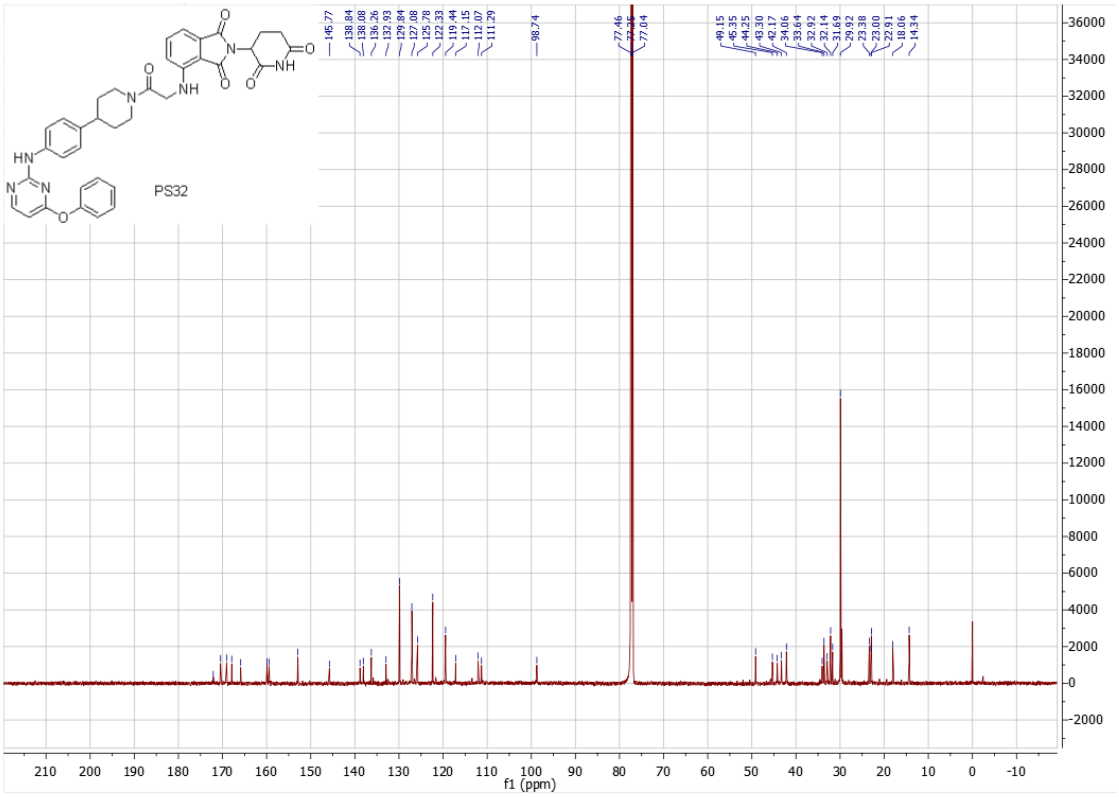





PS-31-HC.3.ser
f2 (ppm)

2-(2,6-dioxopiperidin-3-yl)-4-((2-oxo-2-(4-(4-((4-phenoxypyrimidin-2-yl)amino)phenyl)piperidin-1-yl)ethyl)amino)isoindoline-1,3-dione) (PS-32).





Reference

1. Wu, X. *et al*. Discovery of KDM5A inhibitors: Homology modeling, virtual screening and structure–activity relationship analysis. *Bioorg. Med. Chem. Lett.* **26**, 2284–2288 (2016).
2. Xu, Y. *et al*. Discovery of novel 2,4-disubstituted pyrimidines as Aurora kinase inhibitors. *Bioorg. Med. Chem. Lett.* **30**, 126885 (2020).