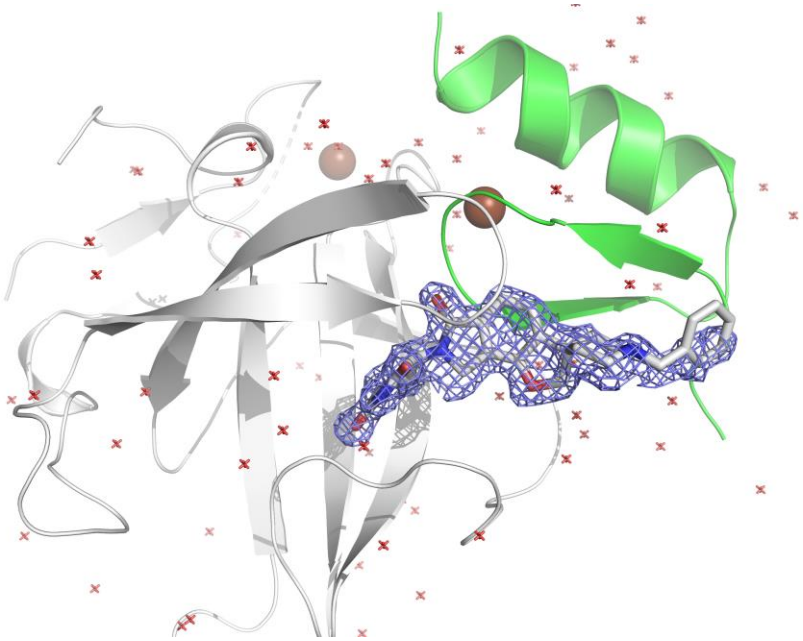
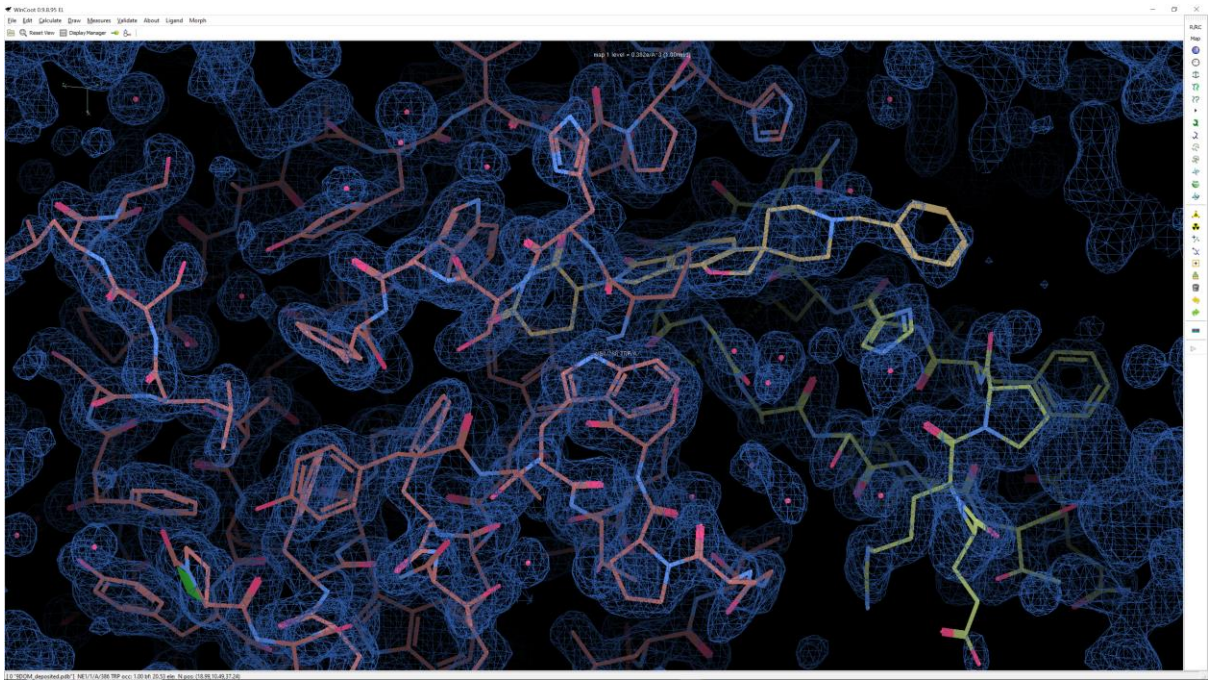


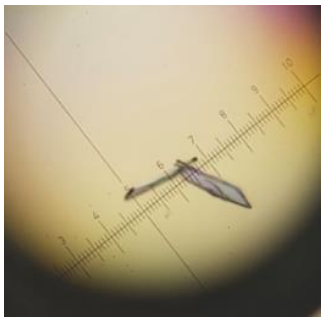
Supplementary Figure 1. The 2Fo-Fc difference electron density map. The 2Fo-Fc difference electron density map (blue grid contoured at 1 sigma) was derived from refinement of the molecular replacement solution of the structure prior to the addition of compound **5**. Compound **5** is shown with carbons in blue and the Cα atoms of proteins are represented as a grey cartoon for CRBN and green for IZKF2. The electron density map is shown for one of the 4 molecules in the asymmetric unit and the density map is of similar quality for each of the 4 copies. Figure generated with Pymol.



Supplementary Figure 2. 2Fo-Fc electron density around chains A (pink), B (yellow), and J (orange) contoured at 1 sigma. Figure generated with Coot version 0.9.8.95.

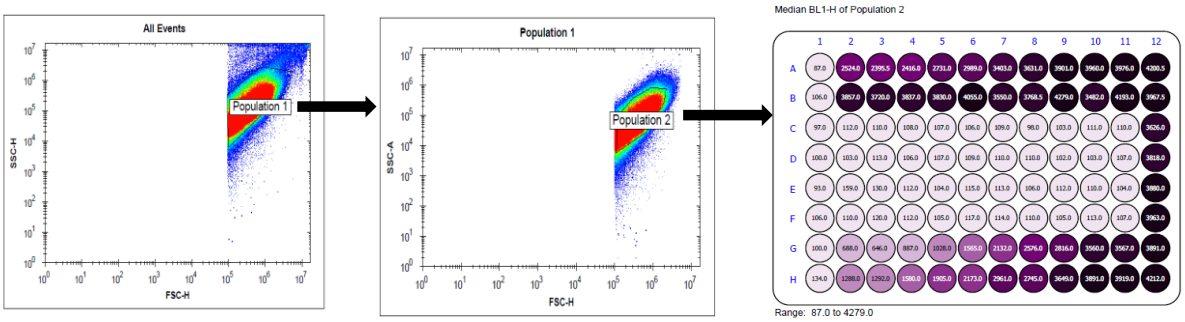
PBD ID	9DOM
Compound number	**5**
Data Collection Statistics	
Wavelength (A)	0.976
Beamline	NSLS2
Space Group	P21
a,b,c (A)	45.39, 69.24, 79.86
α, β, γ (deg)	90.00, 90.01, 90.0
Resolution (A) (Outer shell)	79.9 - 1.69 (1.73 - 1.69)
Completeness% (Outer shell)	84.6 (68.5)
Rmerge (Outer shell)	0.077 (0.653)
I/Sigma (Outer shell)	13.3 (2.1)
CC1/2 (Outer shell)	0.998 (0.826)
Refinement Statistics	
Number reflections	44747
Rwork (%)	21.1
Rfree (%)	23.4
Number of atoms	4637
Mean B-factor	22.8
R.m.s.d bonds (A)	0.009
R.m.s.d. angles (o)	1.9
Ramachandran	
Favoured (%)	98.8
Allowed (%)	1
Outlier (%)	0.2

0.4 M Magnesium chloride
0.1 M TRIS pH 7.5
27% w/v PEG 4000
1 ul + 1 ul hanging-drop
4℃

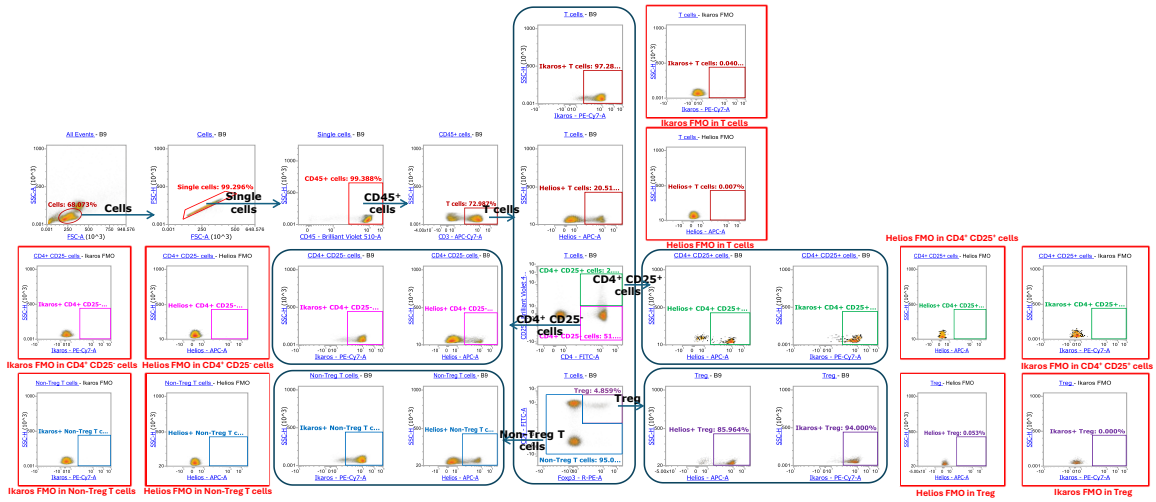


Supplementary Figure 3. Crystallization, X-ray data collection, and refinement statistics for compound 5.

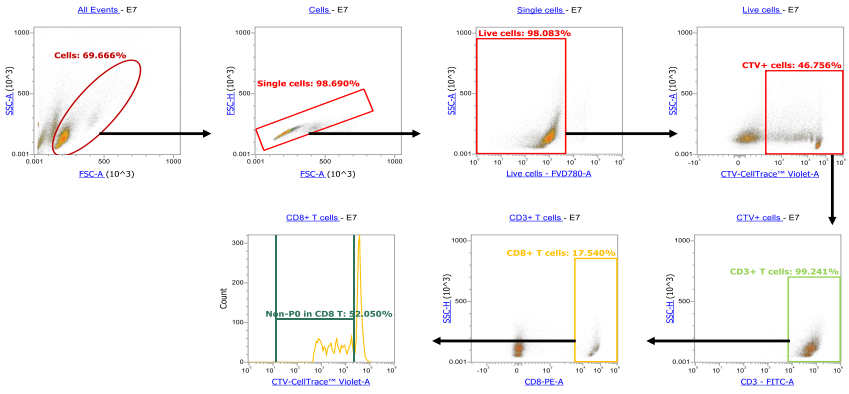
4a



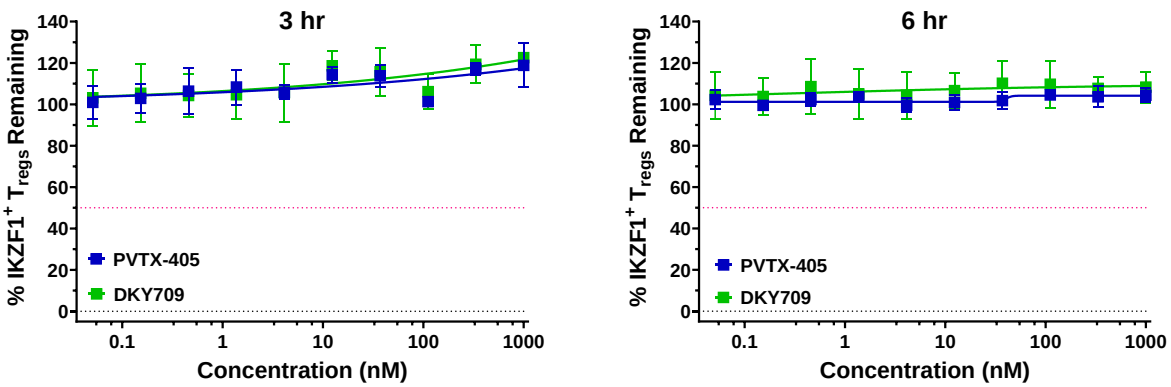
4b



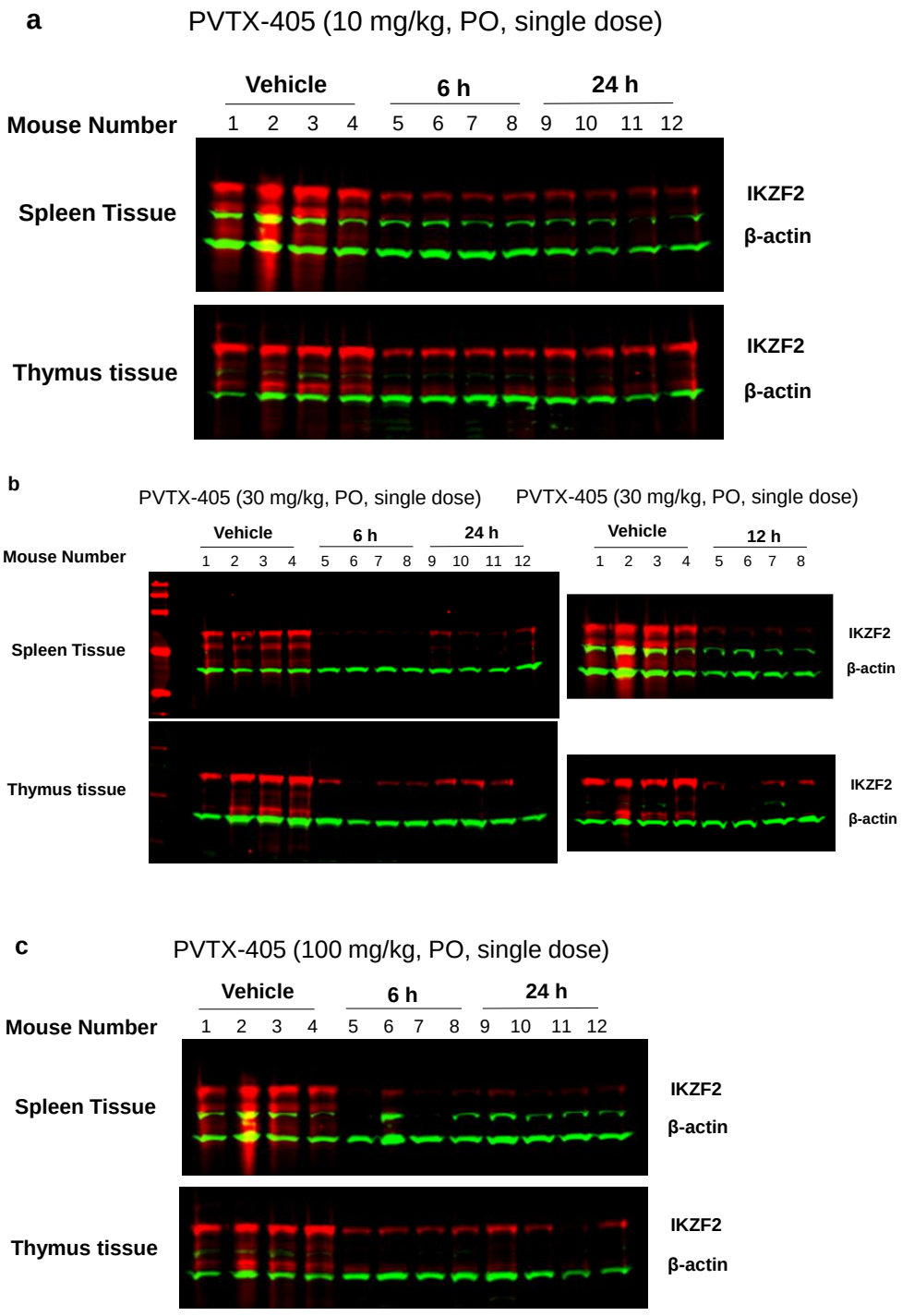
4c



Supplementary Figure 4. FACS Gating strategy. a Gating strategy for evaluating IKZF2 degradation in Jurkat cells as presented in Figure 4a of the manuscript. **b** Gating strategy for evaluating IKZF2 and IKZF1 degradation in human Tregs (CD4+/CD25+/FoxP3+) as presented in the Figure 4b (IKZF2) and supplementary Figure 4 (IKZF1) of the manuscript. **c** Gating strategy for evaluating proliferating effector T-cells (CD8+) as presented in the Figure 4c on the manuscript.



Supplementary Figure 5. Effect of PVTX-405 and DKY709 on IKZF1 in primary human Tregs *ex vivo*. Data presented as mean ± SD, with n = 2 biological replicates for each concentration. Source data are provided as a Source Data file.

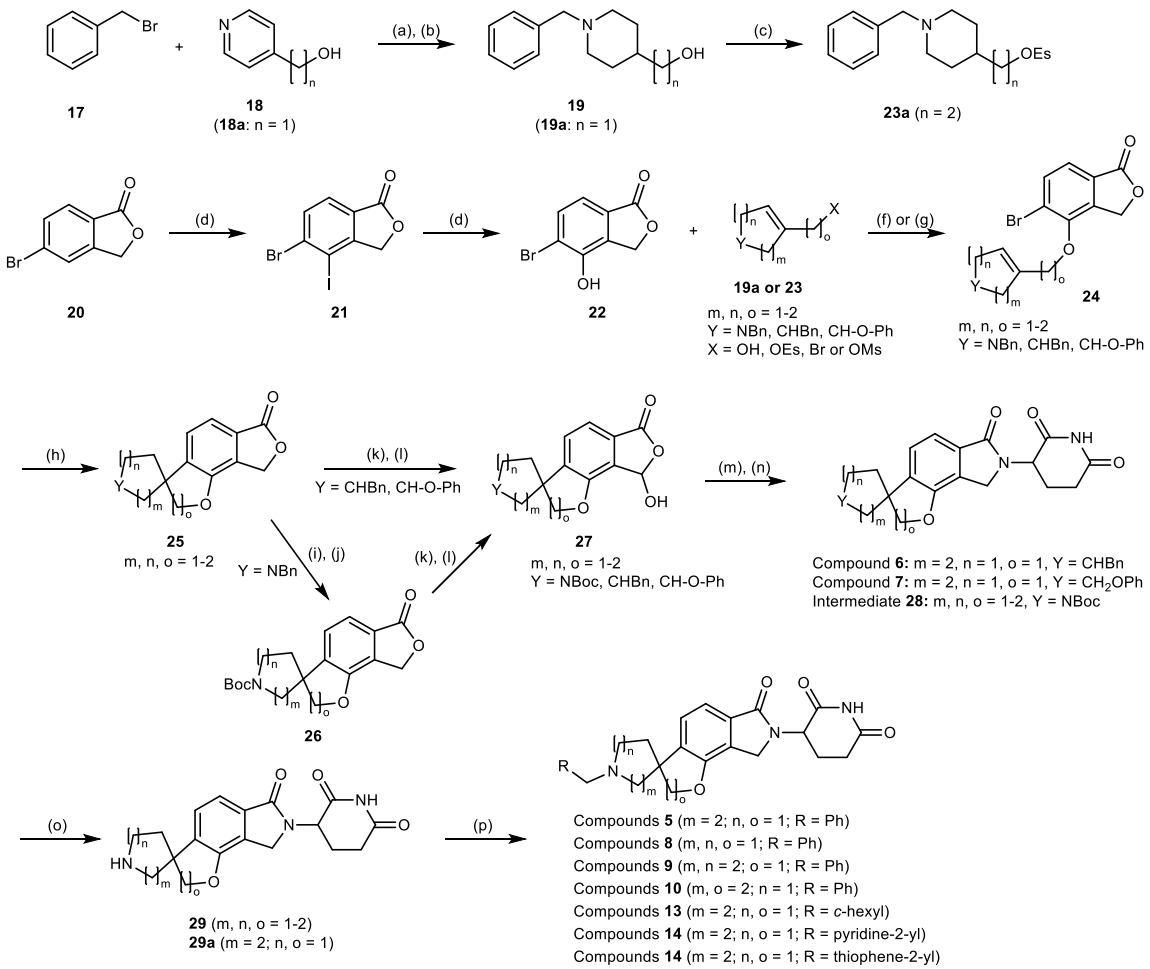


Supplementary Figure 6. IKZF2 degradation induced by PVTX-405 in the spleen and thymus tissues of *Crbn*^{I391V} Mice. a Single oral administration of PVTX-405 at 10 mg/kg and 6, 24 h time-points; **b** Single oral administration of PVTX-405 at 30 mg/kg and 6, 12, 24 h time-points; **c** Single oral administration of PVTX-405 at 100 mg/kg and 6, 24 h time-points. Representative blots from two independent experiments. The samples derive from the same experiment and that gels/blots were processed in parallel. Source data are provided in the Source Data file.

Supplementary Methods

General chemistry. Unless otherwise noted, all commercial materials were used as received. NMR spectra were recorded on a Bruker Ascend^{TM} 400 MHz spectrometer and calibrated using residual solvent peaks as internal references. In reported spectral data, the format (δ) chemical shift (multiplicity, *J* values in Hz, integration) was used with the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, hept = heptet, dd = doublet of doublets, and m = multiplet. Low resolution mass spectrometric (MS) analysis was carried out with a Waters UPLC ACQUITY QDa mass spectrometer. High resolution mass experiments were operated on an AB SCIEX 6600 QTOF LC/MS instrument with ESI ionization. Flash column chromatography was performed by Teledyne CombiFlash RF+ using RediSep Rf silica gel flash column. The final compounds were all purified by a C18 reverse phase preparative HPLC column (SunFire^{TM} Prep C18 OBD^{TM} 5 μm, 50×100 mm) with solvent A (0.1% TFA in H_2O) and solvent B (0.1% TFA in MeCN) as eluents at 60 mL/min flow rate. The purity of all the final compounds was measured and confirmed to be >95% by UPLC-MS or UPLC analysis (10−100% MeCN in H_2O containing 0.1% formic acid or TFA) with a C18 column (ACQUITY UPLC BEH C18 1.7 μm, 2.1 × 50 mm).

General synthetic procedures and characterization of compounds 5-10 and 13-15



Reagents and conditions: (a) DMF, 100 °C; (b) $NaBH_4$, EtOH, reflux; (c) DMAP, Et_3N, ethanesulfonyl chloride, DCM; (d) NIS, trifluoromethanesulfonic acid; (e) NaOH, DMA, Cu_2O; (f) DIAD, PPh_3, THF, rt; (g) K_2CO_3, DMF, 70 °C; (h) *n*-Bu_3SnH, AIBN, toluene, reflux; (i) α-chloroethyl chloroformate, DCE, 0 °C to reflux, then MeOH, reflux; (j) THF, Boc_2O, Et_3N, rt; (k) NaOH, rt; (l) MnO_2, DCM, rt; (m) 3-aminopiperidine-2,6-dione, $NaBH_3CN$, AcONa, AcOH, MeOH/DCM; (n) HATU, DIPEA, DMF; (o) TFA, DCM; (p) RCHO, $NaBH(Oac)_3$, DMF, rt.

Synthesis of 3-(1'-benzyl-6-oxo-6,8-dihydro-2H,7H-spiro[furo[2,3-e]isoindole-3,4'-piperidin]-7-yl)piperidine-2,6-dione (5). To a solution of pyridin-4-ylmethanol (**18a**, 100 g, 916 mmol, 1.0 eq.) in DMF (400 mL) was added BnBr (**17**, 172 g, 1.0 mol, 1.1 eq.). The mixture was stirred at 100 ℃ for 3 h. TLC showed that no starting material remained, and a new spot formed. The reaction mixture was dissolved in EtOH (1500 mL), then 45 g of $NaBH_4$ (1.19 mol, 1.3 eq.) was added into portion wise at 0 °C. The mixture was kept stirring at 0 °C for 1 h and then reflux for 2 h. The solvent was evaporated under reduced pressure, then water

was added, and the mixture was extracted with ethyl acetate. The combined organic phases were dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by silica gel column chromatograph (DCM:MeOH = 100:0-30:1) to afford (1-benzyl-1,2,3,6-tetrahydropyridin-4-yl)methanol as a viscous oil (**19a**, 107 g, 80% yield). LC/MS $[M + H]^+$ 204.14; ^1H NMR (400 MHz, $CDCl_3$): 7.25-7.41 (m, 5H), 5.59-5.66 (m, 1H), 4.01 (s, 2H), 3.60 (s, 2H), 2.95-3.02 (m, 2H), 2.61 (t, J = 5.8 Hz, 2H), 2.36 (s,br, 1H), 2.10-2.21 (m, 2H).

To a solution of 5-bromo-3H-isobenzofuran-1-one (**20**, 100 g, 1 eq.) in trifluoromethanesulfonic acid (1000 g, 10 eq) was added NIS (125 g, 1.2 eq.) in portions at 0 °C. After that, the mixture was warmed to room temperature and stirred overnight. TLC showed that no starting material remained, and two new spots formed. The reaction mixture was poured into ice-water and yellow solid was precipitated. The resulting mixture was filtered, and the filter cake was washed with ice cold water and dried to give a light yellow solid (100 g, yield 62%), which is a mixture of product 5-bromo-4-iodoisobenzofuran-1(3H)-one (**21**, major product, top spot on TLC) and by-product 5-bromo-6-iodoisobenzofuran-1(3H)-one (minor product, bottom spot on TLC). The crude product was directly used in the next step. Compound **21**: LC/MS $[M + H]^+$ 337.84; ^1H NMR (400 MHz, $CDCl_3$): 7.83 (d, J = 8.0 Hz, 1H), 7.77 (d, J = 8.0 Hz, 1H), 5.10 (s, 2H).

To a mixture of **21** (100 g, 1 eq.), sodium hydroxide (57.5 g, 5 eq.) in water (1000 mL, 1.5 M) and N,N-dimethylacetamide (600 mL) was added cuprous oxide (8.5 g, 0.2 eq.). The reaction mixture was heated at 80 °C and stirred for 12 h. TLC showed that compound **21** was completely consumed. Then the reaction mixture was poured into water (1000 mL), treated with solid K_2CO_3 until pH 8–9, and extracted with EtOAc. The aqueous layer was neutralized with 1 N hydrochloride solution and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography to give 5-bromo-4-

hydroxyisobenzofuran-1(3H)-one (**22**) as a yellow solid (42 g, 39% yield). LC/MS $[M + H]^+$ 228.94; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.89 (s, 1H), 7.72 (d, J = 8.0 Hz, 1H), 7.23 (d, J = 8.0 Hz, 1H), 5.34 (s, 2H).

To a solution of **22** (20 g, 1.0 eq.) in 200 mL of THF was added (1-benzyl-1,2,3,6-tetrahydropyridin-4-yl)methanol (**19a**, 23.1 g, 1.3 eq.) and PPh_3 (34.4 g, 1.55 eq.). The mixture was cooled to 0°C and DIAD (27.1 mL, 1.55 eq.) was added dropwise, after which the resulting mixture was stirred at rt overnight. Then the solvent was removed at reduced pressure and the crude product was purified by silica gel column chromatography using 0-100% EtOAc/hexane as the eluting solvent. The desired product 4-((1-benzyl-1,2,3,6-tetrahydropyridin-4-yl)methoxy)-5-bromoisobenzofuran-1(3H)-one (**24a**) was obtained as a yellow foam (17.7 g, yield 49%). LC/MS $[M + H]^+$ 414.0.

To a solution of **24a** (14.8 g, 35.7 mmol, 1.0 eq.) in toluene (150 mL) was added n-Bu_3SnH (41.6 g, 142.9 mmol, 4.0 eq.) and AIBN (0.6 g, 3.57 mmol, 0.1 eq.). The mixture was heated at reflux and kept stirring overnight. TLC (PE:EA = 1:1) showed that no starting material remained, and new spots formed. The reaction mixture was cooled to rt, poured into saturated aq. KF solution (100 mL), and then stirred overnight. The resulting mixture was extracted with EtOAc, and the combined organic layers were washed with brine and dried over Na_2SO_4. The crude product was purified by silica gel column chromatography (DCM:MeOH = 50:1) to give 1'-benzyl-2H-spiro[benzo[2,1-b:3,4-c']difuran-3,4'-piperidin]-6(8H)-one (**25a**) as a white solid (7.1 g, 60% yield). LC/MS $[M + H]^+$ 336.15.

To a solution of **25a** (10 g, 29.8 mmol, 1.0 eq.) in DCE (100 mL) was added α-chloroethyl chloroformate (1.0 eq.) at 0 °C. The mixture was heated at reflux for 1 h. Then the mixture was concentrated under reduced pressure. The residue was dissolved in MeOH and heated at reflux for few hours. The solvent was evaporated in vacuo to give a crude product 2H-spiro[benzo[2,1-b:3,4-c']difuran-3,4'-piperidin]-6(8H)-one. The resulting crude mixture

was dissolved in THF (100 mL), and then 4.5 g of triethylamine (44.7 mmol, 1.5 eq.) and Boc_2O (38.7 mmol, 1.3 eq.) was added. The mixture was stirred at rt for 3 h, after which the solvent was evaporated under reduced pressure. Then water was added, and the mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel flash chromatograph to give product *tert*-butyl 6-oxo-6,8-dihydro-2H-spiro[benzo[2,1-b:3,4-c']difuran-3,4'-piperidine]-1'-carboxylate (**26a**, 6.0 g, 60% yield). LC/MS $[M + H]^+$ 346.16.

To a solution of **26a** (15 g, 1 eq.) in THF (100 mL) and water (100 mL) was added NaOH (8.7 g, 5 eq). The mixture was stirred at 20 °C for 16 h. TLC (ethyl acetate: hexane = 1:1) showed that the reaction was complete. The mixture was adjusted to pH = 5-6 with aq. HCl (1 M) and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product 1'-(*tert*-butoxycarbonyl)-7-(hydroxymethyl)-2H-spiro[benzofuran-3,4'-piperidine]-6-carboxylic acid was directly used in the next step. LC/MS (ESI) m/z: 364.17. The crude product (15 g, crude, 1 eq.) was dissolved in DCM (300 mL) and then MnO_2 (20 eq.) was added. The mixture was stirred at 20 °C for 1 h. TLC showed that the reaction was complete. The reaction mixture was diluted with DCM and filtered through a pad of Celite. The filtrate was concentrated in vacuum and the crude product was purified by silica gel column chromatography (DCM: MeOH = 10:1) to give product *tert*-butyl 8-hydroxy-6-oxo-6,8-dihydro-2H-spiro[benzo[2,1-b:3,4-c']difuran-3,4'-piperidine]-1'-carboxylate (**27a**) as a yellow solid (8 g, 60% yield). LC/MS $[M + H]^+$ 362.15.

To a mixture of **27a** (3 g, 1.0 eq.) in MeOH (20 mL) and DCM (20 mL) was added 3-aminopiperidine-2,6-dione (4.0 g, 3 eq., TFA salt), AcONa (3.08 g, 6.0 eq.) and AcOH (5.1 mL, 10.0 eq.). The mixture was stirred at 25 °C for 2 h, then $NaBH_3CN$ (1.57 g, 3.0 eq.) was added, and the mixture was stirred for another 30 min. LC-MS showed that the reaction was complete.

Next, the reaction mixture was quenched with water and concentrated under reduced pressure. The residue was suspended in MeCN/H_2O (1:1, 30 mL) and the mixture was stirred at 0-5 ^oC overnight. The mixture was filtered, and the filter cake was washed with MeCN/H_2O (1:1) and dried to afford the crude intermediate 1'-(*tert*-butoxycarbonyl)-7-(((2,6-dioxopiperidin-3-yl)amino)methyl)-2H-spiro[benzofuran-3,4'-piperidine]-6-carboxylic acid as a solid (900 mg, 60% yield). LC/MS (ESI) m/z: 474.22. To a solution of the resulting crude intermediate (900 mg 1.0 equiv) in DMF (15 mL) was added HATU (795 mg, 1.1 equiv) and DIPEA (0.72 mL, 3.0 equiv). The reaction was stirred at rt for 30 min. LC-MS indicated a new main peak with desired MS formed. Then the reaction was quenched with water, and the mixture was extracted with EtOAc, washed with brine, and dried over Na_2SO_4. The product *tert*-butyl 7-(2,6-dioxopiperidin-3-yl)-6-oxo-7,8-dihydro-2H,6H-spiro[furo[2,3-e]isoindole-3,4'-piperidine]-1'-carboxylate (**28a**) was obtained as a brown solid (675 mg, 75% yield). LC/MS $[M + H]^+$ 456.21. ^1H NMR (400 MHz, Chloroform-*d*) δ 8.00 (s, 1H), 7.50 (d, J = 7.7 Hz, 1H), 7.28 (s, 1H), 5.23 (dd, J = 13.3, 5.1 Hz, 1H), 4.55 (d, J = 1.4 Hz, 2H), 4.46 (d, J = 16.0 Hz, 1H), 4.32 (d, J = 16.0 Hz, 1H), 4.15 (s, 2H), 3.01 – 2.77 (m, 4H), 2.38 (dd, J = 13.1, 5.0 Hz, 1H), 2.29 – 2.17 (m, 1H), 1.92 (t, J = 12.5 Hz, 2H), 1.83 – 1.72 (m, 2H), 1.52 (s, 9H).

28a was treated with TFA in DCM at room temperature to de-protect the N-Boc group to provide product 3-(6-oxo-6,8-dihydro-2H,7H-spiro[furo[2,3-e]isoindole-3,4'-piperidin]-7-yl)piperidine-2,6-dione (**29a**) as a white solid. UPLC-MS $[M + H]^+$ 356.15; ^1H NMR (400 MHz, DMSO-d_6) δ 10.97 (s, 1H), 9.27 (s, 2H), 7.38 – 7.22 (m, 2H), 5.09 (dd, J = 13.3, 5.1 Hz, 1H), 4.71 – 4.59 (m, 2H), 4.39 (d, J = 17.2 Hz, 1H), 4.23 (d, J = 17.2 Hz, 1H), 3.33 – 3.25 (m, 2H), 3.07 – 2.84 (m, 3H), 2.64 – 2.54 (m, 1H), 2.48 – 2.35 (m, 1H), 2.26 – 2.13 (m, 2H), 2.03 – 1.93 (m, 1H), 1.92 – 1.79 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 172.86, 170.94, 167.66, 153.45, 137.81, 133.46, 122.87, 122.58, 115.94, 80.68, 51.70, 44.48, 43.15, 40.59, 31.83, 31.20, 22.34; HRMS (ESI) *m/z* calcd. For $C_{19}H_{22}N_3O_4$ $[M + H]^+$ 356.1610, found 356.1622.

To a solution of **29a** (37 mg, 1.0 eq.) and benzaldehyde (10.4 μL, 1.3 eq.) in DMF (3 mL) was added $NaBH(OAc)_3$ (68 mg, 4.0 eq.) in two potions. The reaction was stirred overnight and then quenched with water (2 mL). The mixture was directly purified by pre-HPLC ($MeCN/H_2O$ = 20-100% in 80 min, 60 mL/min) to give the final compound 3-(1'-benzyl-6-oxo-6,8-dihydro-2H,7H-spiro[furo[2,3-e]isoindole-3,4'-piperidin]-7-yl)piperidine-2,6-dione (**5**) as a white solid (40 mg, 91% yield). UPLC-MS $[M + H]^+$ 446.30; ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.62 – 7.49 (m, 5H), 7.41 (d, J = 7.7 Hz, 1H), 7.31 (d, J = 7.7 Hz, 1H), 5.13 (dd, J = 13.3, 5.1 Hz, 1H), 4.76 – 4.62 (m, 2H), 4.51 – 4.34 (m, 4H), 3.64 – 3.47 (m, 2H), 3.25 – 3.13 (m, 2H), 2.96 – 2.83 (m, 1H), 2.82 – 2.73 (m, 1H), 2.57 – 2.44 (m, 1H), 2.31 – 2.11 (m, 3H), 2.11 – 2.01 (m, 2H); ^{13}C NMR (101 MHz, MeOD) δ 174.60, 172.21, 170.96, 155.31, 138.48, 134.84, 132.35, 131.32, 130.41, 130.31, 124.48, 124.10, 117.50, 81.73, 61.85, 53.80, 50.87, 46.26, 44.67, 34.14, 32.35, 23.95; HRMS (ESI) *m/z* calcd. For $C_{26}H_{28}N_3O_4$ $[M + H]^+$ 446.2080, found 446.2069.

Synthesis of 3-(4-benzyl-6'-oxo-6',8'-dihydro-2'H,7'H-spiro[cyclohexane-1,3'-furo[2,3-e]isoindol]-7'-yl)piperidine-2,6-dione (6). Compound **6** was prepared using a similar procedure for synthesizing compound **5** from intermediate **22** and (4-benzylcyclohex-1-en-1-yl)methanol. LC-MS (ESI): mass calced for: $C_{27}H_{28}N_2O_4$ 444.53; m/z found, 445.1 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.96 (s, 1H), 7.37 (ddd, J = 65.6, 21.5, 7.5 Hz, 4H), 7.22 – 7.07 (m, 3H), 5.21 – 5.00 (m, 1H), 4.63 – 3.84 (m, 4H), 3.17 (s, 1H), 2.90 (t, J = 13.1 Hz, 1H), 2.82 – 2.72 (m, 1H), 2.58 (d, J = 20.3 Hz, 2H), 2.42 (d, J = 18.5 Hz, 1H), 2.28 (s, 1H), 1.99 (s, 1H), 1.91 – 1.75 (m, 1H), 1.74 – 1.38 (m, 5H), 1.31 (s, 1H), 1.09 (d, J = 10.7 Hz, 1H).

Synthesis of 3-(6'-oxo-4-phenoxy-6',8'-dihydro-2'H,7'H-spiro[cyclohexane-1,3'-furo[2,3-e]isoindol]-7'-yl)piperidine-2,6-dione (7). Compound **7** was prepared using a similar procedure for synthesizing compound **5** from intermediate **22** and (4-phenoxycyclohex-

1-en-1-yl)methanol. LC-MS (ESI): mass calced for: $C_{26}H_{26}N_2O_5$ 446.50; m/z found, 447.2 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.97 (s, 1H), 7.68 – 7.16 (m, 4H), 7.13 – 6.81 (m, 3H), 5.79 (s, 1H), 5.33 (s, 1H), 5.08 (d, J = 13.6 Hz, 1H), 4.89 (s, 1H), 4.61 (t, J = 18.1 Hz, 1H), 4.53 – 4.11 (m, 3H), 3.94 (dd, J = 25.1, 14.8 Hz, 1H), 3.37 (s, 1H), 2.98 – 2.88 (m, 1H), 2.74 (d, J = 12.9 Hz, 1H), 2.67 (s, 1H), 2.61 (s, 1H), 2.43 (d, J = 14.0 Hz, 1H), 2.08 (s, 1H), 2.00 – 1.89 (m, 2H), 1.77 (d, J = 15.1 Hz, 1H), 1.65 – 1.38 (m, 2H), 1.18 – 0.83 (m, 1H).

Synthesis of 3-(1'-benzyl-6-oxo-6,8-dihydro-2H,7H-spiro[furo[2,3-e]isoindole-3,3'-pyrrolidin]-7-yl)piperidine-2,6-dione (8). Compound **8** was prepared using a similar procedure for synthesizing compound **5** from intermediate **22** and (1-benzyl-2,5-dihydro-1H-pyrrol-3-yl)methanol. LC-MS (ESI): mass calcd. for $C_{25}H_{25}N_3O_4$, 431.18; m/z found, 432.2 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.79 (s, 1H), 8.13 (s, 1H), 7.46 - 7.32 (m, 7H), 5.11 - 5.06 (m, 1H), 4.60 - 4.58 (m, 2H), 4.38 (dd, J = 17.2, 4.6 Hz, 1H), 4.23 (d, J = 17.2 Hz, 1H), 3.65 (s, 2H), 3.03 - 2.65 (m, 3H), 2.64 - 2.52 (m, 2H), 2.49 - 2.35 (m, 2H), 2.17 (s, 2H), 2.02 - 1.93 (m, 1H).

Synthesis of 3-(1-benzyl-6'-oxo-6',8'-dihydro-2'H,7'H-spiro[azepane-4,3'-furo[2,3-e]isoindol]-7'-yl)piperidine-2,6-dione (9). Compound **9** was prepared using a similar procedure for synthesizing compound **5** from intermediate **22** and (1-benzyl-2,3,6,7-tetrahydro-1H-azepin-4-yl)methanol. LC-MS (ESI): mass calcd. for $C_{27}H_{29}N_3O_4$, 459.21; m/z found, 460.4 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.99 (d, J = 21.7 Hz, 1H), 8.14 (s, 1H), 7.62 (d, J = 7.2 Hz, 1H), 7.58 – 7.12 (m, 6H), 5.09 (t, J = 13.4 Hz, 1H), 4.69 (s,1H), 4.47 – 4.22 (m, 2H), 4.15 (t, J = 12.4 Hz, 1H), 3.96 (s, 1H), 3.75 (d, J = 49.5 Hz, 2H), 3.47 (d, J = 31.5 Hz, 1H), 3.31 – 3.18 (m, 2H), 2.91 (dd, J = 21.0, 9.2 Hz, 1H), 2.63 (dd, J = 39.5, 22.9 Hz, 2H), 2.33 (d, J = 9.2 Hz, 2H), 1.98 (dt, J = 112.6, 46.6 Hz, 5H).

Synthesis of 3-(1-benzyl-7'-oxo-2',3',7',9'-tetrahydro-8'H-spiro[piperidine-4,4'-pyrano[2,3-e]isoindol]-8'-yl)piperidine-2,6-dione (10). Compound **10** was prepared using a

similar procedure for synthesizing compound **5** from intermediate **22** and 2-(1-benzyl-1,2,3,6-tetrahydropyridin-4-yl)ethan-1-ol. UPLC-MS $[M + H]^+$ 460.32; ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.62 – 7.50 (m, 5H), 7.48 (d, J = 8.0 Hz, 1H), 7.35 (d, J = 8.1 Hz, 1H), 5.11 (dd, J = 13.3, 5.1 Hz, 1H), 4.48 – 4.33 (m, 4H), 4.33 – 4.24 (m, 2H), 3.53 – 3.41 (m, 2H), 3.39 – 3.32 (m, 2H), 2.95 – 2.83 (m, 1H), 2.82 – 2.73 (m, 1H), 2.56 – 2.31 (m, 3H), 2.27 – 2.11 (m, 3H), 2.00 – 1.89 (m, 2H); ^{13}C NMR (101 MHz, MeOD) δ 174.63, 172.22, 171.26, 151.37, 133.46, 132.89, 132.35, 131.67, 131.37, 130.46, 130.41, 128.33, 116.37, 63.86, 61.84, 53.77, 49.50, 46.89, 35.83, 33.06, 32.36, 30.33, 24.05; HRMS (ESI) *m/z* calcd. For $C_{27}H_{30}N_3O_4$ $[M + H]^+$ 460.2236, found 460.2257.

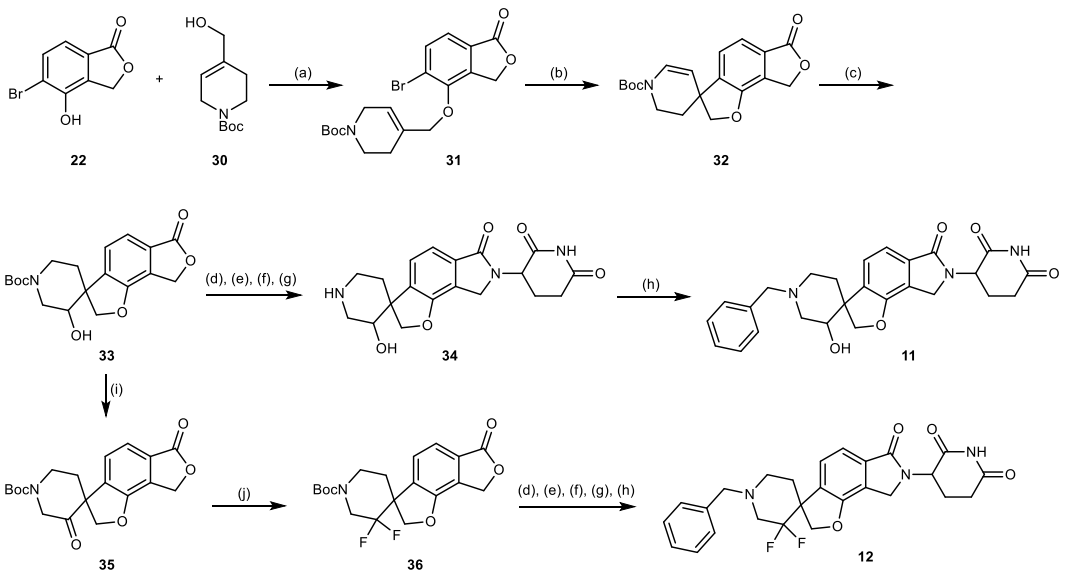
Synthesis of 3-(1'-(cyclohexylmethyl)-6-oxo-6,8-dihydro-2H,7H-spiro[furo[2,3-e]isoindole-3,4'-piperidin]-7-yl)piperidine-2,6-dione (13). Compound **13** was prepared using a similar procedure for synthesizing compound **5**. UPLC-MS $[M + H]^+$ 452.36; ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.43 (d, J = 7.7 Hz, 1H), 7.34 (d, J = 7.7 Hz, 1H), 5.14 (dd, J = 13.3, 5.2 Hz, 1H), 4.73 – 4.65 (m, 2H), 4.51 – 4.36 (m, 2H), 3.70 – 3.62 (m, 2H), 3.17 – 3.06 (m, 2H), 3.03 (d, J = 6.8 Hz, 2H), 2.96 – 2.84 (m, 1H), 2.82 – 2.73 (m, 1H), 2.57 – 2.43 (m, 1H), 2.38 – 2.26 (m, 2H), 2.21 – 2.13 (m, 1H), 2.11 – 2.01 (m, 2H), 1.90 – 1.69 (m, 6H), 1.41 – 1.23 (m, 3H), 1.17 – 1.01 (m, 2H); ^{13}C NMR (101 MHz, MeOD) δ 174.59, 172.21, 170.94, 155.30, 138.59, 134.81, 124.46, 124.13, 117.50, 81.82, 64.55, 53.80, 51.70, 46.27, 44.61, 34.05, 34.01, 32.35, 31.72, 26.89, 26.46, 23.95; HRMS (ESI) *m/z* calcd. For $C_{26}H_{34}N_3O_4$ $[M + H]^+$ 452.2549, found 452.2542.

Synthesis of 3-(6-oxo-1'-(pyridin-2-ylmethyl)-6,8-dihydro-2H,7H-spiro[furo[2,3-e]isoindole-3,4'-piperidin]-7-yl)piperidine-2,6-dione (14). Compound **14** was prepared using a similar procedure for synthesizing compound **5**. UPLC-MS $[M + H]^+$ 447.30; ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.72 (d, J = 4.3 Hz, 1H), 7.93 (td, J = 7.7, 1.7 Hz, 1H), 7.53 (d, J = 7.8 Hz, 1H), 7.51 – 7.45 (m, 1H), 7.45 – 7.40 (m, 2H), 5.14 (dd, J = 13.3, 5.2 Hz, 1H), 4.73 –

4.67 (m, 2H), 4.63 – 4.51 (m, 2H), 4.51 – 4.37 (m, 2H), 3.71 – 3.62 (m, 2H), 3.38 – 3.32 (m, 2H), 2.96 – 2.85 (m, 1H), 2.82 – 2.73 (m, 1H), 2.57 – 2.44 (m, 1H), 2.41 – 2.31 (m, 2H), 2.21 – 2.13 (m, 1H), 2.13 – 2.04 (m, 2H); ^{13}C NMR (101 MHz, MeOD) δ 174.63, 172.24, 171.00, 155.36, 151.03, 150.98, 139.23, 138.47, 134.83, 125.68, 125.57, 124.50, 124.30, 117.51, 82.03, 61.19, 53.83, 51.50, 46.30, 44.52, 33.81, 32.35, 23.96; HRMS (ESI) *m/z* calcd. For $C_{25}H_{27}N_4O_4$ $[M + H]^+$ 447.2032, found 447.2058.

Synthesis of 3-(6-oxo-1'-(thiophen-3-ylmethyl)-6,8-dihydro-2H,7H-spiro[furo[2,3-e]isoindole-3,4'-piperidin]-7-yl)piperidine-2,6-dione (15). Compound **15** was prepared using a similar procedure for synthesizing compound **5**. UPLC-MS $[M + H]^+$ 452.03; ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.78 – 7.69 (m, 1H), 7.66 – 7.59 (m, 1H), 7.42 (d, J = 7.7 Hz, 1H), 7.37 – 7.21 (m, 2H), 5.14 (dd, J = 13.3, 5.2 Hz, 1H), 4.75 – 4.64 (m, 2H), 4.51 – 4.35 (m, 4H), 3.65 – 3.53 (m, 2H), 3.23 – 3.09 (m, 2H), 2.96 – 2.84 (m, 1H), 2.83 – 2.74 (m, 1H), 2.57 – 2.42 (m, 1H), 2.30 – 2.03 (m, 5H); HRMS (ESI) *m/z* calcd. For $C_{24}H_{26}N_3O_4S$ $[M + H]^+$ 452.1644, found 452.1664.

Synthetic procedures and characterization of compounds 11 and 12



Reagents and conditions: (a) DIAD, PPh_3, THF, rt; (b) $Pd(OAc)_2$, NaOAc, TEA in DMF at 70^{o}C; (c) BH_3.THF, -78^{o}C to 0^{o}C; (d) NaOH, THF/H_2O; (e) DMP, $NaHCO_3$, DCM; (f) 3-aminopiperidine-2,6-dione, $NaBH_3CN$ (5 eq), NaOAc, AcOH (50 eq), MeOH, 40 ^{o}C, 3 h; (g) TFA, DCM; (h) benzaldehyde, $NaBH(OAc)_3$, DMF, rt; (i) DMP, DCM, rt; (j) triethylammonium fluoride, N,N-Diethyl-S,S-difluoro-sulfiliminium tetrafluoroborate, TEA, rt

Synthesis of 3-(1'-benzyl-3'-hydroxy-6-oxo-6,8-dihydro-2H,7H-spiro[furo[2,3-e]isoindole-3,4'-piperidin]-7-yl)piperidine-2,6-dione (11). To a solution of 5-bromo-4-hydroxyisobenzofuran-1(3H)-one (**22**, 10.0 g, 43.60 mmol, 1.0 eq) in THF (300 mL) were added tert-butyl- 4-(hydroxymethyl)-3,6-dihydropyridine-1(2H)-carboxylate (**30**, 11.18 g, 52.4 mmol, 1.2 eq) and triphenylphosphine (17.18 g, 65.4 mmol, 14.62 mL, 1.5 eq). The mixture was stirred under N_2 at 0 ºC for 20 min. Then DIAD (13.24 g, 65.4 mmol, 12.74 mL, 1.5 eq) was dropwise added to above mixture and the mixture was stirred at room temperature overnight. After evaporation, the crude product was purified by flash column chromatography on silica gel (ethyl acetate in petroleum ether, from 0% to 20%) to afford tert-butyl 4-(((5-bromo-1-oxo-1,3-dihydroisobenzofuran-4-yl)oxy)methyl)-3,6-dihydropyridine-1(2H)-carboxylate (**31**, 18.0 g, yield 97%) as a yellow solid. LC-MS (ESI): mass calcd. for $C_{19}H_{22}BrNO_5$, 424.29; m/z found, 369.9 $[M+H-56]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ 7.83 (d, J = 8.0 Hz, 1H), 7.45 (d, J = 8.0 Hz, 1H), 5.89 (s, 1H), 5.66 (s, 2H), 4.66 (s, 2H), 3.88 (s, 2H), 3.48 (dd, J = 14.6, 9.0 Hz, 2H), 2.22 (s, 2H), 1.41 (s, 9H).

To a solution of tert-butyl 4-(((5-bromo-1-oxo-1,3-dihydroisobenzofuran-4-yl)oxy)methyl)-3,6-dihydropyridine-1(2H)-carboxylate (**31**, 15.00 g, 23.70 mmol, 1.0 eq) in DMF (90.0 mL) were added sodium formate (1.77 g, 26.07 mmol, 1.1 eq), palladium diacetate (531 mg, 2.37 mmol, 0.1 eq), sodium acetate (4.86 g, 59.1 mmol, 2.5 eq), and TEA (4.32g, 26.07 mmol, 3.99 mL, 1.1 eq). The mixture was stirred under N_2 at 70 ºC for 16 h. After cooled to room temperature, the mixture was filtered and the cake was washed with EA (300 mL). The filtrate was washed with brine (60 mL x 3), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified with flash column chromatography on silica gel (ethyl acetate in petroleum ether, from 0% to 30%) to afford tert-butyl 6-oxo-2',3',6,8-tetrahydro-1'H,2H-spiro[benzo[2,1-b:3,4-c']difuran-3,4'-pyridine]-1'-carboxylate (**32**, 5.10 g, yield 62.7%) as a colorless oil. LC-MS (ESI): mass calcd. for

$C_{19}H_{21}NO_5$, 343.38; m/z found, 344.1 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.41 (d, J = 7.6 Hz, 1H), 7.34 (d, J = 7.6 Hz, 1H), 7.03 - 6.98 (m, 1H), 5.38 (s, 2H), 4.88 - 4.82 (m, 1H), 4.60 (d, J = 9.2 Hz, 1H), 4.39 (d, J = 9.2 Hz, 1H), 3.81 - 3.75 (m, 1H), 3.47 - 3.41 (m, 1H), 2.02 - 1.98 (m, 1H), 1.84 - 1.78 (m, 1H), 1.47 (s, 9H).

To a solution of tert-butyl 6-oxo-2',3',6,8-tetrahydro-1'H,2H-spiro[benzo[2,1-b:3,4-c']difuran-3,4'-pyridine]-1'-carboxylate (**32**, 5.10 g, 14.87 mmol, 1.0 eq) in THF (60.0 mL) was added BH_3-THF (1 N) (37.1 mL, 37.1 mmol, 2.5 eq) under N_2 at -78 °C. The reaction was allowed to slowly warm to 0 °C and stirred at 0 °C for 5 hrs. Water (10 mL) was added to above mixture, followed by sodium perborate (6.07 g, 74.2 mmol, 5.0 eq). The resulting mixture was stirred overnight. The mixture was diluted with DCM (100 mL), washed with brine (50 mL x 2), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified with flash column chromatography on silica gel (ethyl acetate in petroleum ether, from 0% to 40%) to afforded tert-butyl 3'-hydroxy-6-oxo-6,8-dihydro-2H-spiro[benzo[2,1-b:3,4-c']difuran-3,4'-piperidine]-1'-carboxylate (**33**, 2.93 g, yield 54%) as a white powder. LC-MS (ESI): mass calcd. for $C_{19}H_{23}NO_6$, 361.39; m/z found, 306.1 $[M+H-56]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.51 (d, J = 7.6 Hz, 1H), 7.37 (d, J = 7.6 Hz, 1H), 5.35 (d, J = 2.6 Hz, 2H), 5.31 (d, J = 4.6 Hz, 1H), 4.83 (d, J = 9.0 Hz, 1H), 4.53 (d, J = 9.0 Hz, 1H), 4.01 (s, 1H), 3.87 (s, 1H), 3.76 - 3.73 (m, 1H), 2.83 - 2.56 (m, 2H), 1.88 - 1.74 (m, 2H), 1.43 (s, 9H).

To a mixture of tert-butyl 3'-hydroxy-6-oxo-6,8-dihydro-2H-spiro[benzo[2,1-b:3,4-c']difuran-3,4'-piperidine]-1'-carboxylate (**33**, 400 mg, 1.11 mmol, 1.0 eq) in THF (9.00 mL), MeOH (9.00 mL), and H_2O (3.00 mL) was added NaOH (89 mg, 2.21 mmol, 2.0 eq) and the reaction mixture was stirred at 40 °C for 1 h. After cooled to room temperature, the reaction mixture was diluted with EA (20 mL), adjusted to pH = 4-5 with aqueous HCl solution (3 N), and extracted with EA (40 mL x 4). The organic layer was washed with brine (20 mL x 2), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to

provide 1'-(tert-butoxycarbonyl)-3'-hydroxy-7-(hydroxymethyl)-2H-spiro[benzofuran-3,4'-piperidine]-6-carboxylic acid (400 mg, yield 95%) as a colorless oil. The crude product was directly used in next step without purification. LC-MS (ESI): mass calcd. for $C_{19}H_{25}NO_7$, 379.41; m/z found, 378.3 $[M-H]^-$.

A mixture of 1'-(tert-butoxycarbonyl)-3'-hydroxy-7-(hydroxymethyl)-2H-spiro[benzofuran-3,4'-piperidine]-6-carboxylic acid (420 mg, 1.11 mmol, 1.0 eq) and active manganese dioxide (1.92 g, 22.1 mmol, 20.0 eq) in DCM (40.0 mL) was stirred at room temperature for 16 hrs. After filtration via a short column, the filtrate was collected and concentrated under reduced pressure to 1'-(tert-butoxycarbonyl)-7-formyl-3'-hydroxy-2H-spiro[benzofuran-3,4'-piperidine]-6-carboxylic acid (260 mg, yield 62%) as a colorless oil. The crude product was directly used in next step without purification. LC-MS (ESI): mass calcd. for $C_{19}H_{21}F_2NO_6$, 377.39; m/z found, 378.1 $[M+H]^+$.

To a solution of 1'-(tert-butoxycarbonyl)-7-formyl-3'-hydroxy-2H-spiro[benzofuran-3,4'-piperidine]-6-carboxylic acid (260 mg, 689 μmol, 1.0 eq) and 3-aminopiperidine-2,6-dione hydrochloride (227 mg, 1.38 mmol, 2.0 eq) in DMF (10.0 mL) was added with acetic acid (1.0 mL) and the reaction mixture was stirred at 40 °C for 2 hrs. Sodium triacetoxyborohydride (438 mg, 2.07 mmol, 3.0 eq) was added to above mixture and the resulting mixture was stirred at 40 °C for 16 hrs. After cooled to room temperature, the mixture was dissolved in EA (40 mL), washed with brine (30 mL x 3), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (MeOH in DCM, 10%) to afford tert-butyl 7-(2,6-dioxopiperidin-3-yl)-3'-hydroxy-6-oxo-7,8-dihydro-2H,6H-spiro[furo[2,3-e]isoindole-3,4'-piperidine]-1'-carboxylate (260 mg, yield 80%) as a grey solid. LC-MS (ESI): mass calcd. for $C_{24}H_{29}N_3O_7$, 471.51; m/z found, 472.2 $[M+H]^+$.

To a solution of tert-butyl 7-(2,6-dioxopiperidin-3-yl)-3'-hydroxy-6-oxo-7,8-dihydro-2H,6H-spiro[furo[2,3-e]isoindole-3,4'-piperidine]-1'-carboxylate (260 mg, 551 μmol, 1.0 eq) in DCM (5.0 mL) was added TFA (1.7 mL, 22.1 mmol, 40.0 eq) and the reaction mixture was stirred at room temperature for 6 hrs. The mixture was cautiously concentrated under reduced pressure to afford 3-(3'-hydroxy-6-oxo-6,8-dihydro-2H,7H-spiro[furo[2,3-e]isoindole-3,4'-piperidin]-7-yl)piperidine-2,6-dione trifluoroacetate (**34**, 100 mg, yield 48%) as a yellow oil. The crude product was directly used in next step without further purification. LC-MS (ESI): mass calcd. for $C_{19}H_{21}N_3O_5$, 371.39; m/z found, 372.2 $[M+H]^+$.

To a solution of 3-(3'-hydroxy-6-oxo-6,8-dihydro-2H,7H-spiro[furo[2,3-e]isoindole-3,4'-piperidin]-7-yl)piperidine-2,6-dione trifluoroacetate (**34**, 80.0 mg, 1 eq, 215 μmol) in DMF (10.0 mL) was added benzaldehyde (45.7 mg, 44.2 μL, 2 Eq, 431 μmol), and the reaction was stirred at 40 °C for 2 hrs. Then it was added with sodium triacetoxyborohydride (137 mg, 95.8 μL, 3 eq, 646 μmol) and the mixture was stirred at 40 °C for 16 hrs. After it was cooled to room temperature, the mixture was dissolved with EA (40 mL), washed with brine (30 mL x 3), dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by silica gel chromatography on silica gel (MeOH in DCM, from 0% to 20%) to afford 3-(1'-benzyl-3'-hydroxy-6-oxo-6,8-dihydro-2H,7H-spiro[furo[2,3-e]isoindole-3,4'-piperidin]-7-yl)piperidine-2,6-dione (**11**, 40.0 mg, 40.2 %) as a white solid. LC-MS (ESI): mass calcd. for $C_{26}H_{27}N_3O_5$, 461.52; m/z found, 462.1 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.95 (s, 1H), 7.38 - 7.23 (m, 7H), 5.08 - 5.04 (m, 1H), 4.95 (s, 1H), 4.77 - 4.75 (m, 1H), 4.45 - 4.31 (m, 2H), 4.19 (d, J = 17.0 Hz, 1H), 3.81 (dd, J = 10.4, 4.4 Hz, 1H), 3.56 (d, J = 13.2 Hz, 1H), 3.44 (d, J = 13.2 Hz, 1H), 2.95 - 2.71 (m, 3H), 2.63 - 2.54 (m, 1H), 2.43 - 2.39 (m, 1H), 2.02 - 1.88 (m, 3H), 1.77 - 1.72 (m, 2H).

Synthesis of 3-(1'-benzyl-3',3'-difluoro-6-oxo-6,8-dihydro-2H,7H-spiro[furo[2,3-e]isoindole-3,4'-piperidin]-7-yl)piperidine-2,6-dione (**12**). To a solution of tert-butyl 3'-

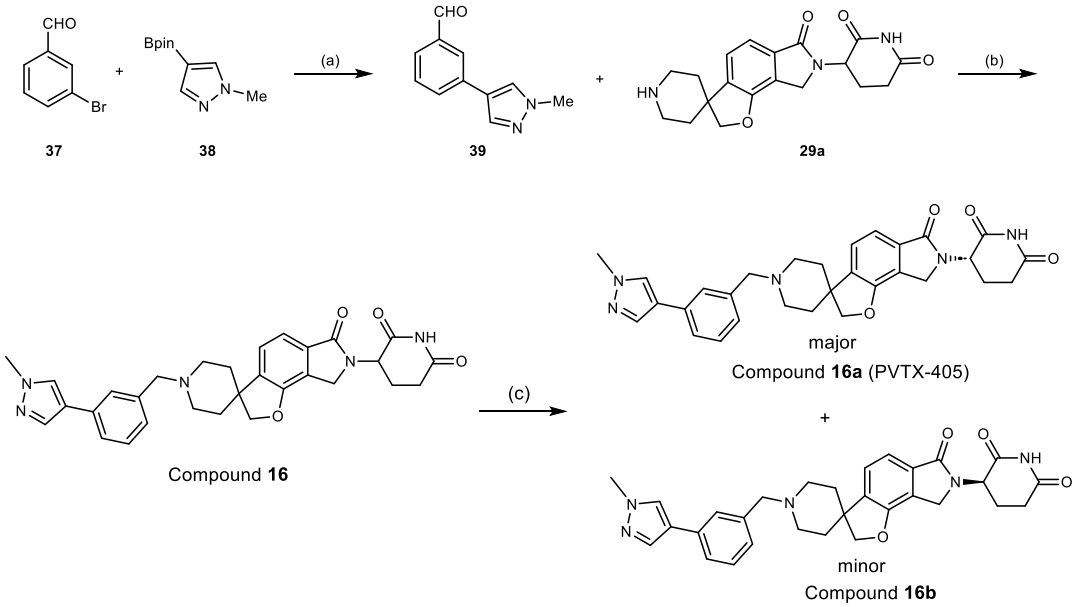
hydroxy-6-oxo-6,8-dihydro-2H-spiro[benzo[2,1-b:3,4-c']difuran-3,4'-piperidine]-1'-carboxylate (**33**, 2.90 g, 8.10 mmol, 1.0 eq) in DCM (60.0 mL) was added Dess-Martin periodinane (8.60 g, 20.35 mmol, 2.5 eq). The mixture was stirred at room temperature for 4 h. The reaction mixture was quenched with aqueous sodium thiosulfate solution (60 mL) and extracted with DCM (90 mL x 3). The organic layer was washed with saturated aqueous NaHCO3 solution (60 mL x 2), brine (40 mL x 2), dried over anhydrous sodium sulfate, and filtered. The filtrate was concentrated under reduced pressure to afford tert-butyl 3',6-dioxo-6,8-dihydro-2H-spiro[benzo[2,1-b:3,4-c']difuran-3,4'-piperidine]-1'-carboxylate (**35**, 2.12 g, yield 72.6%) as a colorless oil. The crude product was directly used in next step without further purification. LC-MS (ESI): mass calcd. for $C_{19}H_{21}NO_6$, 359.38; m/z found, 360.1 $[M+H]^+$.

To a solution of tert-butyl 3',6-dioxo-6,8-dihydro-2H-spiro[benzo[2,1-b:3,4-c']difuran-3,4'-piperidine]-1'-carboxylate (**35**, 2.0 g, 5.57 mmol, 1.0 eq), triethylammonium fluoride (5.38 g, 5.44 mL, 33.4 mmol, 6.0 eq), and N,N-Diethyl-S,S-difluoro-sulfiliminium tetrafluoroborate (XtalFluor-E) (5.73 g, 25.0 mmol, 4.5 eq) in DCM (100.0 mL) was added TEA (1.41 g, 1.94 mL, 13.9 mmol, 2.5 eq) at 0 °C and the mixture was stirred at 25 °C for 1 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution (60 mL) and extracted with DCM (100 mL x 3). The separated organic phase was washed with brine (100 mL x 3), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography on silica gel (ethyl acetate in petroleum ether, from 0% to 30%) to afford tert-butyl 3',3'-difluoro-6-oxo-6,8-dihydro-2H-spiro[benzo[2,1-b:3,4-c']difuran-3,4'-piperidine]-1'-carboxylate (**36**, 1.0 g, yield 47%) as a yellow oil. LC-MS (ESI): mass calcd. for $C_{19}H_{21}F_2NO_5$, 381.38; m/z found, 382.1 $[M+H]^+$.

Analogous synthesis following the procedure of **11** afforded 3-(1'-benzyl-3',3'-difluoro-6-oxo-6,8-dihydro-2H,7H-spiro[furo[2,3-e]isoindole-3,4'-piperidin]-7-yl)piperidine-2,6-dione (**12**) as a yellow solid. LC-MS (ESI): mass calcd. for $C_{26}H_{25}F_2N_3O_4$, 481.50; m/z found, 482.6

[M+H]$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.99 (d, J = 3.6 Hz, 1H), 8.17 (s, 1H), 7.50 (d, J = 7.4 Hz, 1H), 7.41 - 7.23 (m, 6H), 5.12 - 5.08 (m, 1H), 4.87 - 4.85 (m, 1H), 4.55 (t, J = 8.0 Hz, 1H), 4.41 (t, J = 16.4 Hz, 1H), 4.24 (t, J = 16.4 Hz, 1H), 3.65 (s, 2H), 3.09 (s, 1H), 2.95 - 2.81 (m, 2H), 2.69 - 2.54 (m, 2H), 2.46 - 2.36 (m, 1H), 2.28 (t, J = 11.2 Hz, 1H), 2.14 (t, J = 11.6 Hz, 1H), 2.04 - 1.88 (m, 2H).

Synthetic procedures and characterization of compounds 16, 16a and 16b



Reagents and conditions: (a) $Pd(PPh_{3)4}$, Na_2CO_3, dioxane/H_2O, 90 ^oC; (b) $NaBH(OAc)_3$, DMF, rt; (c) Chiral SFC condition (see experimental section)

Synthesis of 3-(1'-(3-(1-methyl-1H-pyrazol-4-yl)benzyl)-6-oxo-6,8-dihydro-2H,7H-spiro[furo[2,3-e]isoindole-3,4'-piperidin]-7-yl)piperidine-2,6-dione (16). A mixture of 3-bromobenzaldehyde (**37**, 500 mg, 1.0 eq), 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (**38**, 843 mg, 1.5 eq), $Pd(PPh_3)_4$ (937 mg, 0.3 eq) and Na_2CO_3 (1.0 g, 3.5 eq) in dioxane/H_2O (15 mL: 1.5 mL) was degassed and purged 3 times with N_2, and then the mixture was stirred at 90 ^oC for 3 h. UPLC-MS indicated that a main peak with the desired MS formed. The mixture was cooled, diluted with water and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (*n*-

Hexane:EtOAc = 100:0 to 60:40) to give compound 3-(1-methyl-1H-pyrazol-4-yl)benzaldehyde (**39**, 455 mg, 91% yield). UPLC-MS $[M + H]^+$ 186.08; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.03 (s, 1H), 7.96 (t, J = 1.6 Hz, 1H), 7.81 (s, 1H), 7.75 – 7.70 (m, 2H), 7.69 (s, 1H), 7.52 (t, J = 7.7 Hz, 1H), 3.96 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 192.42, 137.03, 136.88, 133.81, 131.33, 129.62, 128.06, 127.37, 126.00, 122.00, 39.27.

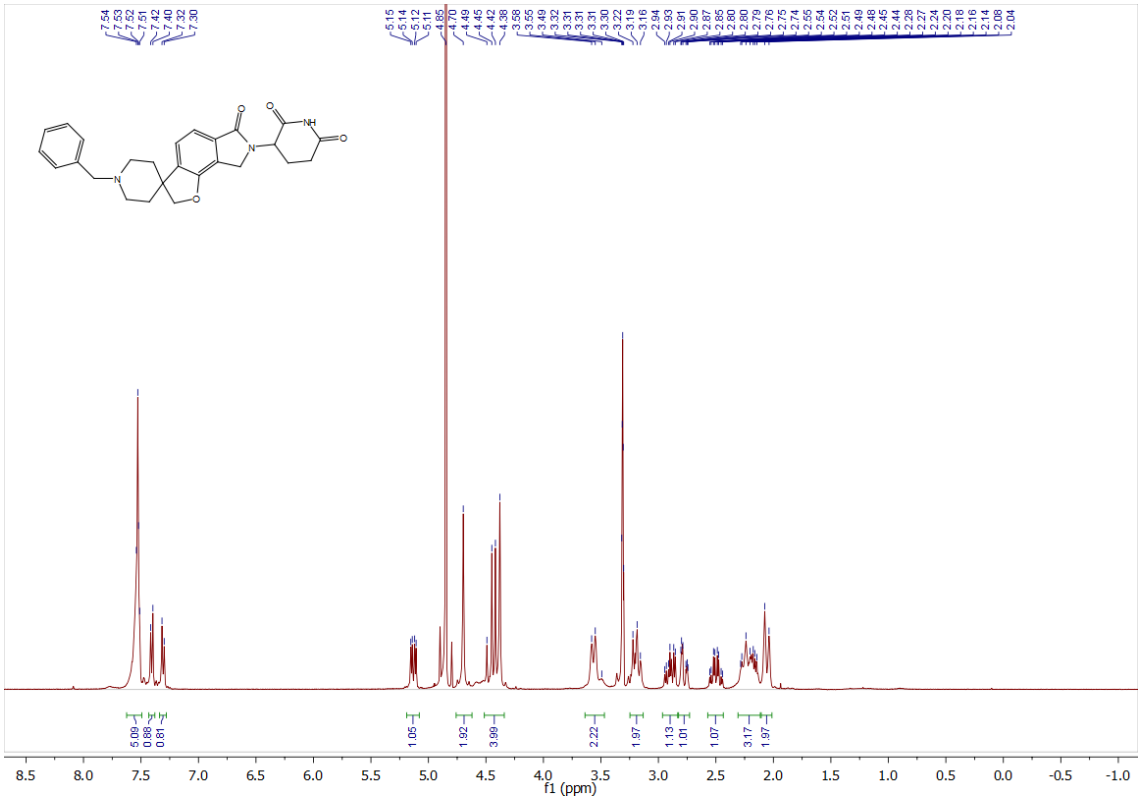
To a solution of **29a** (37 mg, 1.0 eq.) and 3-(1-methyl-1H-pyrazol-4-yl)benzaldehyde (**39**, 22 mg, 1.5 eq.) in DMF (3 mL) was added $NaBH(OAc)_3$ (68 mg, 4.0 eq.) in two potions. The reaction was stirred overnight and then quenched with water (2 mL). The mixture was directly purified by pre-HPLC ($MeCN/H_2O$ = 20-100% in 80 min, 60 mL/min) to give the final compound 3-(1'-(3-(1-methyl-1H-pyrazol-4-yl)benzyl)-6-oxo-6,8-dihydro-2H,7H-spiro[furo[2,3-e]isoindole-3,4'-piperidin]-7-yl)piperidine-2,6-dione (**16**) as a white solid (21 mg, 50% yield). UPLC-MS $[M + H]^+$ 526.12; ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.03 (s, 1H), 7.88 (s, 1H), 7.75 (s, 1H), 7.70 (d, J = 7.8 Hz, 1H), 7.51 (t, J = 7.7 Hz, 1H), 7.46 – 7.30 (m, 3H), 5.12 (dd, J = 13.3, 5.1 Hz, 1H), 4.71 (s, 2H), 4.53 – 4.34 (m, 4H), 3.95 (s, 3H), 3.66 – 3.53 (m, 2H), 3.28 – 3.15 (m, 2H), 2.96 – 2.84 (m, 1H), 2.84 – 2.74 (m, 1H), 2.57 – 2.43 (m, 1H), 2.36 – 2.21 (m, 2H), 2.21 – 2.13 (m, 1H), 2.13 – 2.00 (m, 2H); ^{13}C NMR (101 MHz, MeOD) δ 174.82, 172.23, 171.08, 155.28, 138.45, 137.52, 135.21, 134.80, 131.05, 130.93, 130.01, 129.55, 128.95, 128.12, 124.49, 124.13, 123.42, 117.57, 81.74, 61.91, 53.82, 51.02, 46.30, 44.71, 39.10, 34.17, 32.32, 23.95; HRMS (ESI) *m/z* calcd. For $C_{30}H_{32}N_5O_4$ $[M + H]^+$ 526.2454, found 526.2452.

Synthesis of (S)-3-(1'-(3-(1-methyl-1H-pyrazol-4-yl)benzyl)-6-oxo-6,8-dihydro-2H,7H-spiro[furo[2,3-e]isoindole-3,4'-piperidin]-7-yl)piperidine-2,6-dione (16a, PVTX-405) and (R)-3-(1'-(3-(1-methyl-1H-pyrazol-4-yl)benzyl)-6-oxo-6,8-dihydro-2H,7H-spiro[furo[2,3-e]isoindole-3,4'-piperidin]-7-yl)piperidine-2,6-dione (16b). 3-(1'-(3-(1-methyl-1H-pyrazol-4-yl)benzyl)-6-oxo-6,8-dihydro-2H,7H-spiro[furo[2,3-e]isoindole-3,4'-

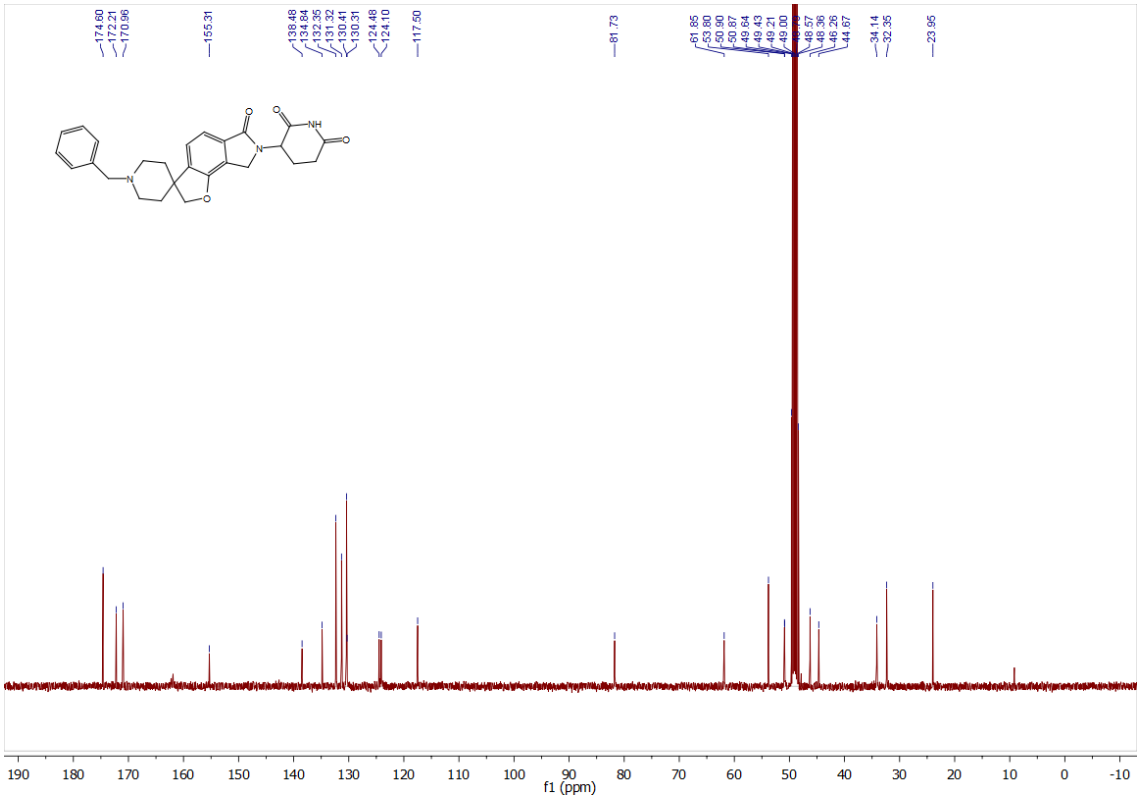
piperidin]-7-yl)piperidine-2,6-dione (**16**, ~100 mg, R/S mixture) was separated by SFC Prep-HPLC with IMADZU PREP SOLUTION SFC with ChiralPak IH (ChiralPak IH, 250 x 21.2 mm I.D., 5 μm), and mobile phase of A for CO_2 and B for EtOH + 0.1% $NH_3.H_2O$ 5-99% over 5 h, at a flow rate of 40 mL/min to give (*S*)-3-(1'-(3-(1-methyl-1H-pyrazol-4-yl)benzyl)-6-oxo-6,8-dihydro-2H,7H-spiro[furo[2,3-e]isoindole-3,4'-piperidin]-7-yl)piperidine-2,6-dione (**16a,** PVTX-405, 30.0 mg, yield 20.0%, 100% e.e) as a white solid and (*R*)-3-(1'-(3-(1-methyl-1H-pyrazol-4-yl)benzyl)-6-oxo-6,8-dihydro-2H,7H-spiro[furo[2,3-e]isoindole-3,4'-piperidin]-7-yl)piperidine-2,6-dione (**16b**, 12.7 mg, yield 8.5%, 96.5% e.e.) as a white solid. Compound **16a** (PVTX-405): UPLC-MS $[M + H]^+$ 526.17; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.96 (s, 1H), 8.13 (s, 1H), 7.88 – 7.81 (m, 1H), 7.53 – 7.47 (m, 1H), 7.47 – 7.38 (m, 2H), 7.35 – 7.23 (m, 2H), 7.16 (d, J = 7.6 Hz, 1H), 5.08 (dd, J = 13.3, 5.1 Hz, 1H), 4.59 – 4.47 (m, 2H), 4.37 (d, J = 17.1 Hz, 1H), 4.21 (d, J = 17.1 Hz, 1H), 3.87 (s, 3H), 3.51 (s, 2H), 2.98 – 2.76 (m, 3H), 2.63 – 2.54 (m, 1H), 2.48 – 2.35 (m, 1H), 2.13 – 2.00 (m, 2H), 2.00 – 1.86 (m, 3H), 1.76 – 1.60 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 172.84, 170.96, 167.80, 153.51, 139.19, 138.87, 135.98, 132.96, 132.50, 128.66, 127.79, 126.57, 125.43, 123.62, 123.24, 122.26, 121.87, 115.56, 80.83, 62.37, 51.65, 50.18, 44.40, 44.19, 38.62, 36.02, 31.19, 22.36; HRMS (ESI) *m/z* calcd. For $C_{30}H_{32}N_5O_4$ $[M + H]^+$ 526.2454, found 526.2491. Compound **16b**: UPLC-MS $[M + H]^+$ 526.3; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.96 (s, 1H), 8.13 (s, 1H), 7.84 (s, 1H), 7.49 (s, 1H), 7.47 – 7.39 (m, 2H), 7.34 – 7.24 (m, 2H), 7.19 – 7.11 (m, 1H), 5.08 (dd, J = 13.3, 5.1 Hz, 1H), 4.53 (s, 2H), 4.45 – 4.32 (m, 1H), 4.27 – 4.14 (m, 1H), 3.86 (s, 3H), 3.51 (s, 2H), 2.98 – 2.77 (m, 3H), 2.63 – 2.53 (m, 1H), 2.47 – 2.34 (m, 1H), 2.13 – 2.01 (m, 2H), 2.01 – 1.86 (m, 3H), 1.75 – 1.62 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 172.84, 170.95, 167.79, 153.51, 139.19, 138.87, 135.97, 132.96, 132.49, 128.66, 127.79, 126.56, 125.42, 123.61, 123.26, 122.25, 121.85, 115.55, 80.82, 62.37, 51.64, 50.18, 44.39, 44.19, 38.62, 36.02, 31.18, 22.34; HRMS (ESI) *m/z* calcd. For $C_{30}H_{32}N_5O_4$ $[M + H]^+$ 526.2454, found 526.2467.

NMR spectra for the final compounds and key intermediates

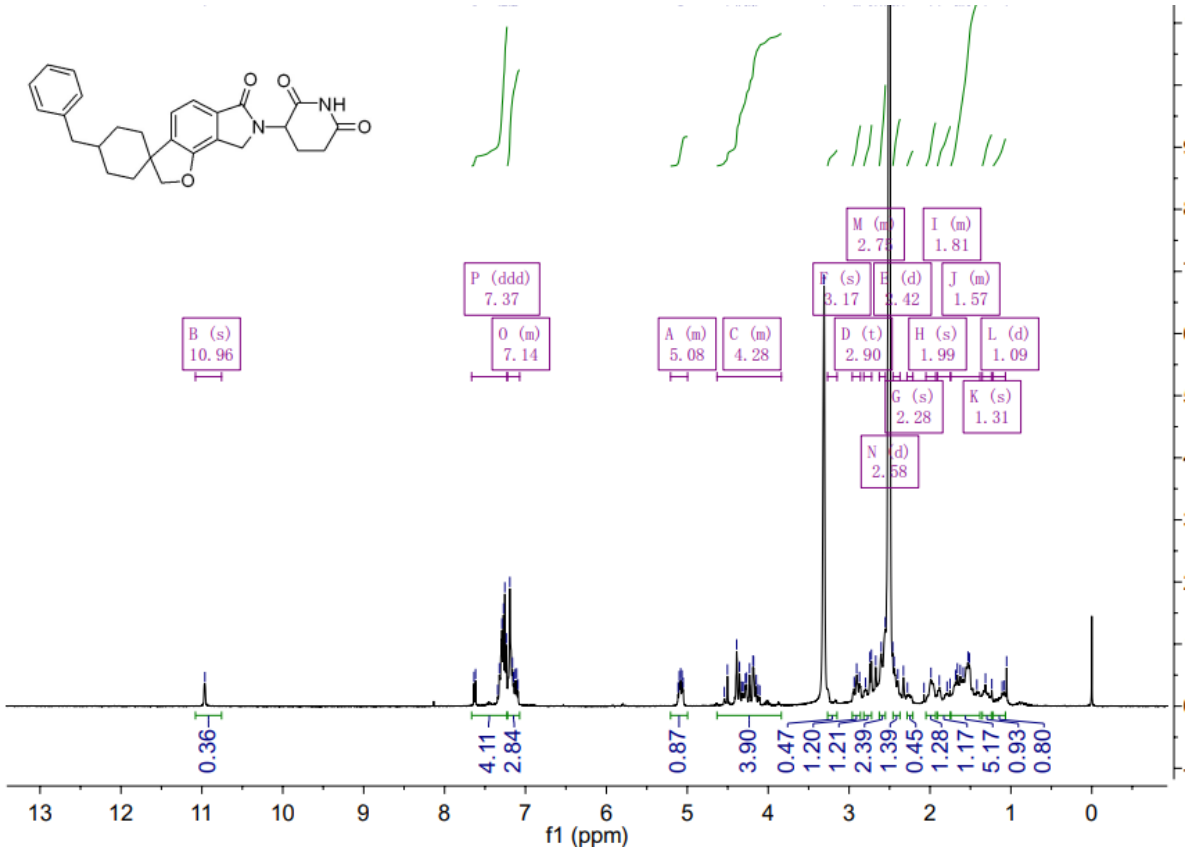
^{1}H-NMR Spectrum for the Final Compound **5**



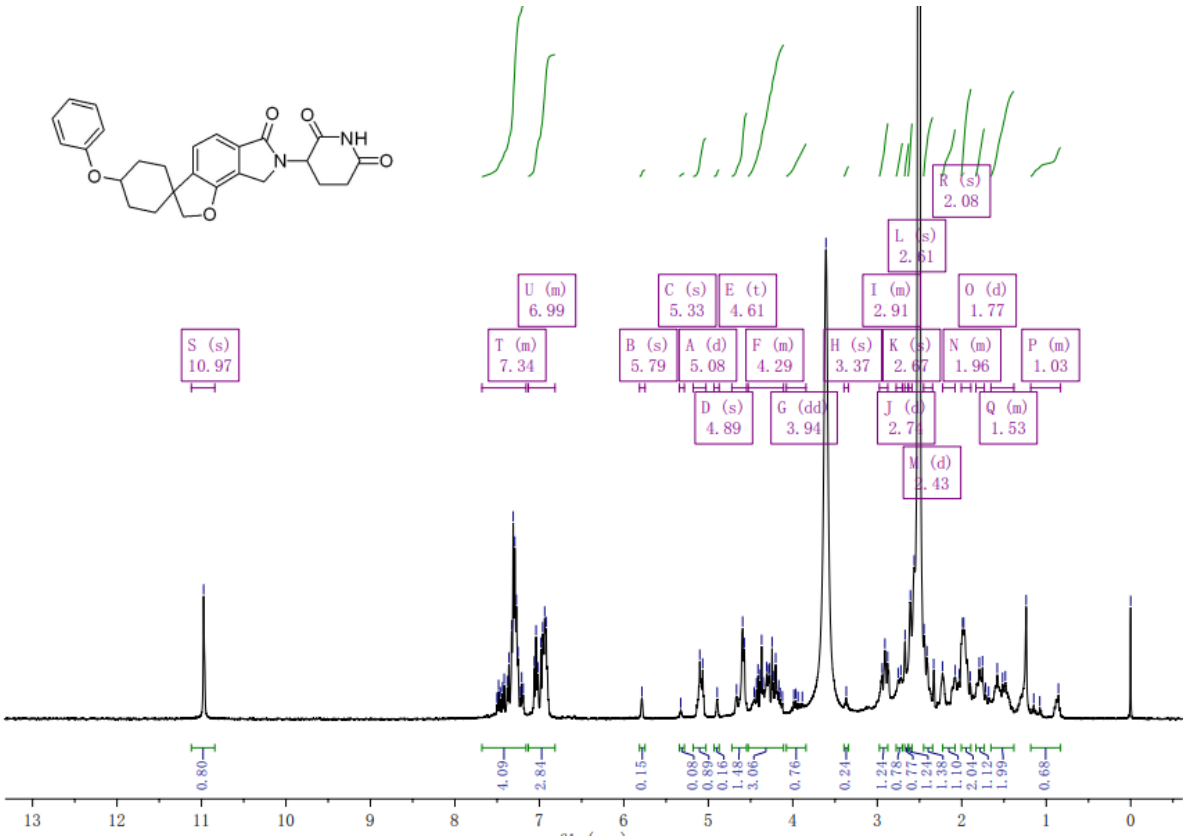
^{13}C-NMR spectrum for the final compound **5**



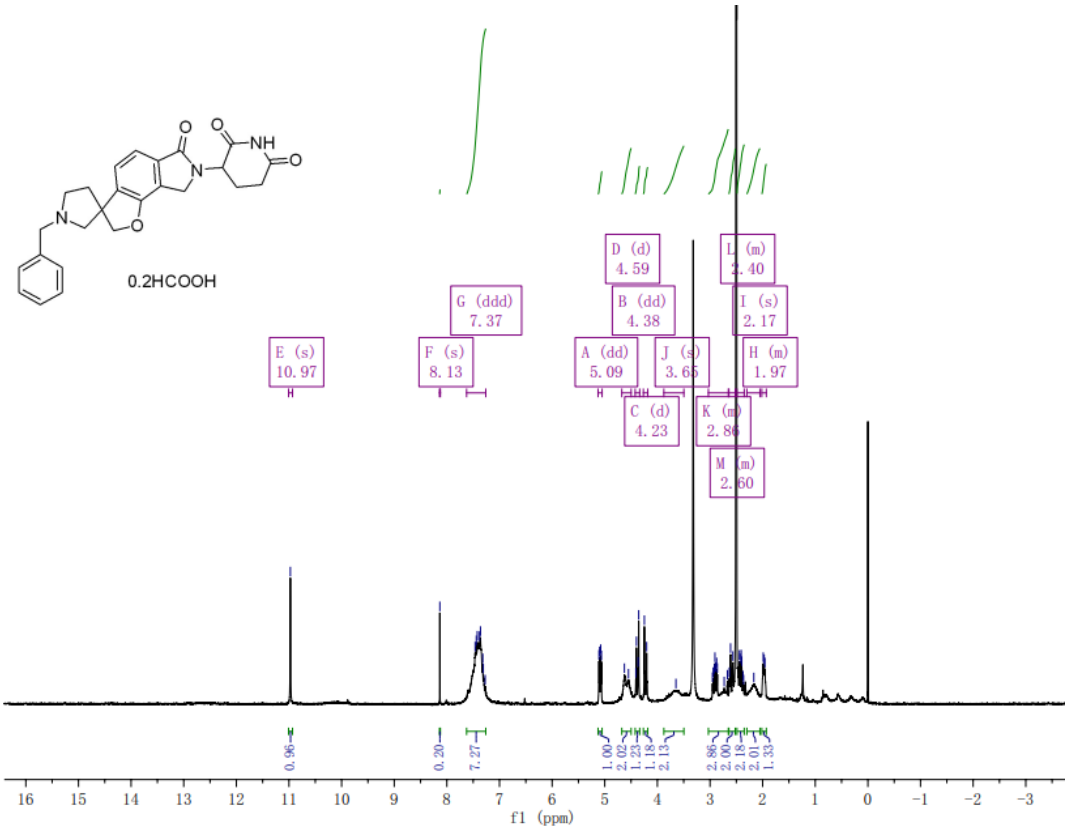
[1]H-NMR spectrum for the final compound **6**



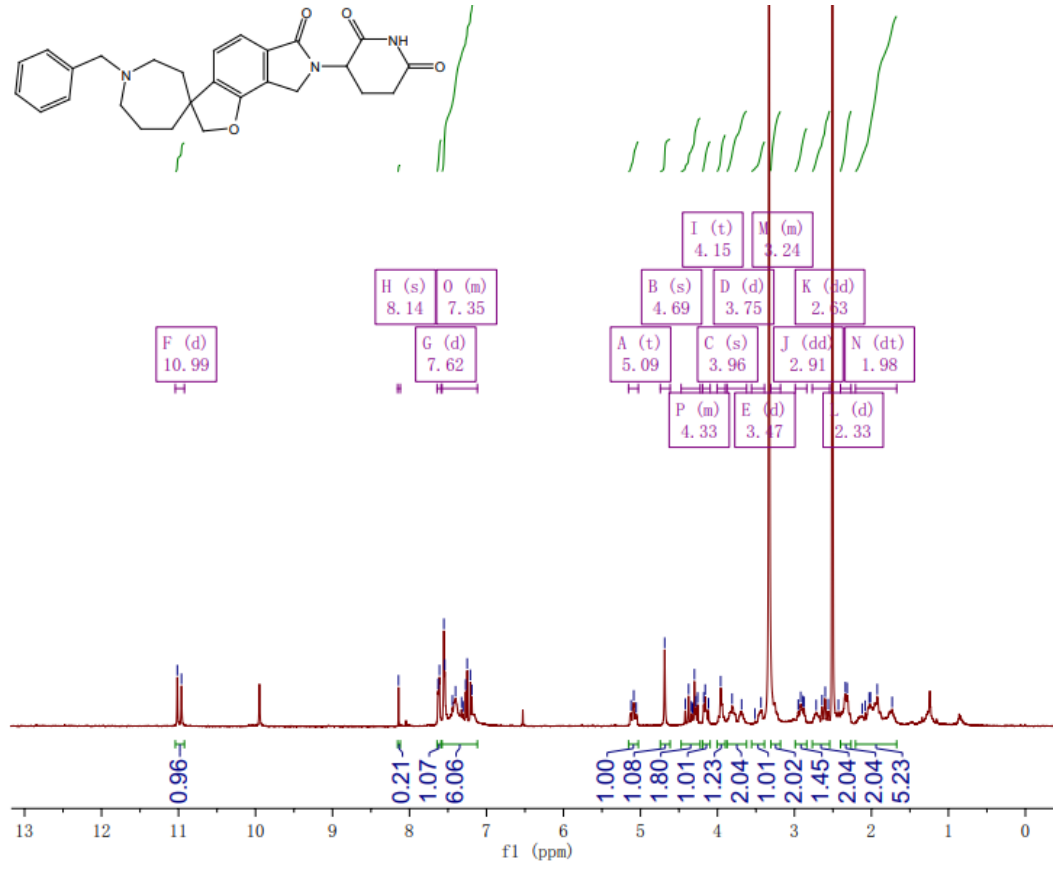
[1]H-NMR spectrum for the final compound **7**



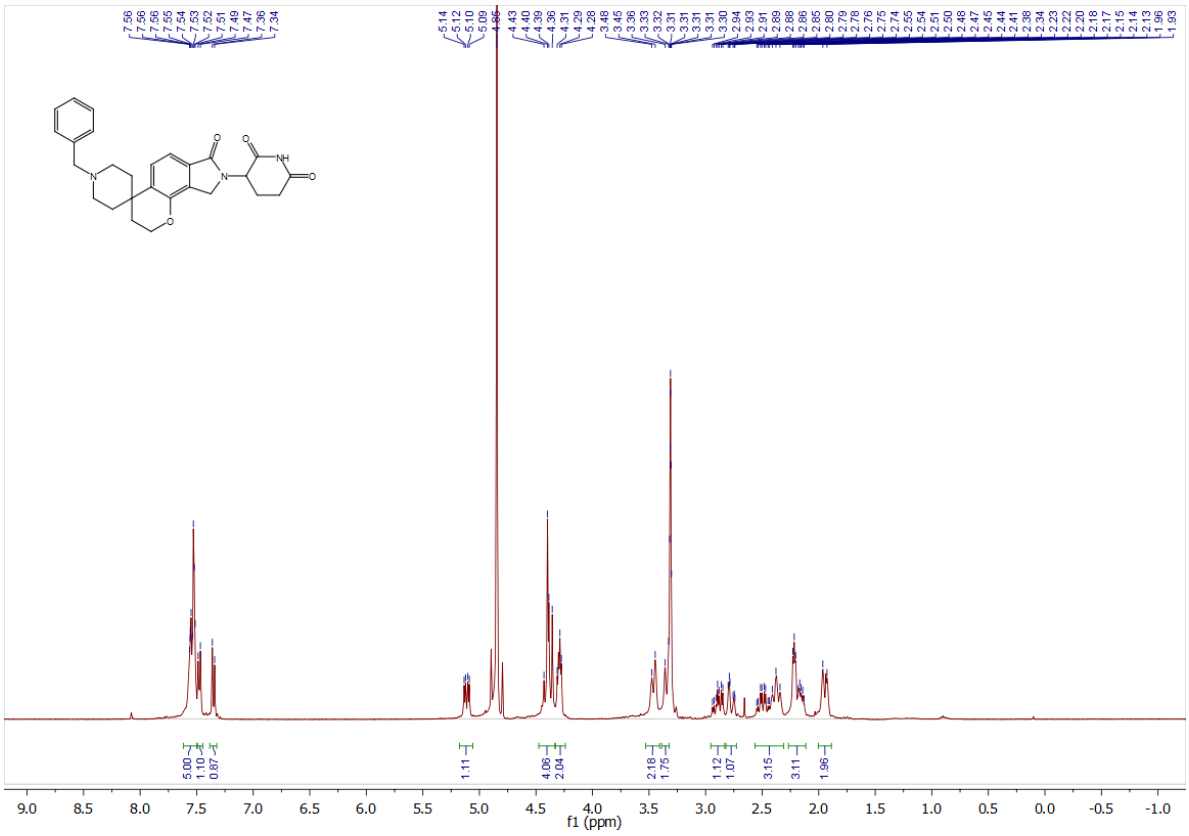
[1]H-NMR spectrum for the final compound **8**



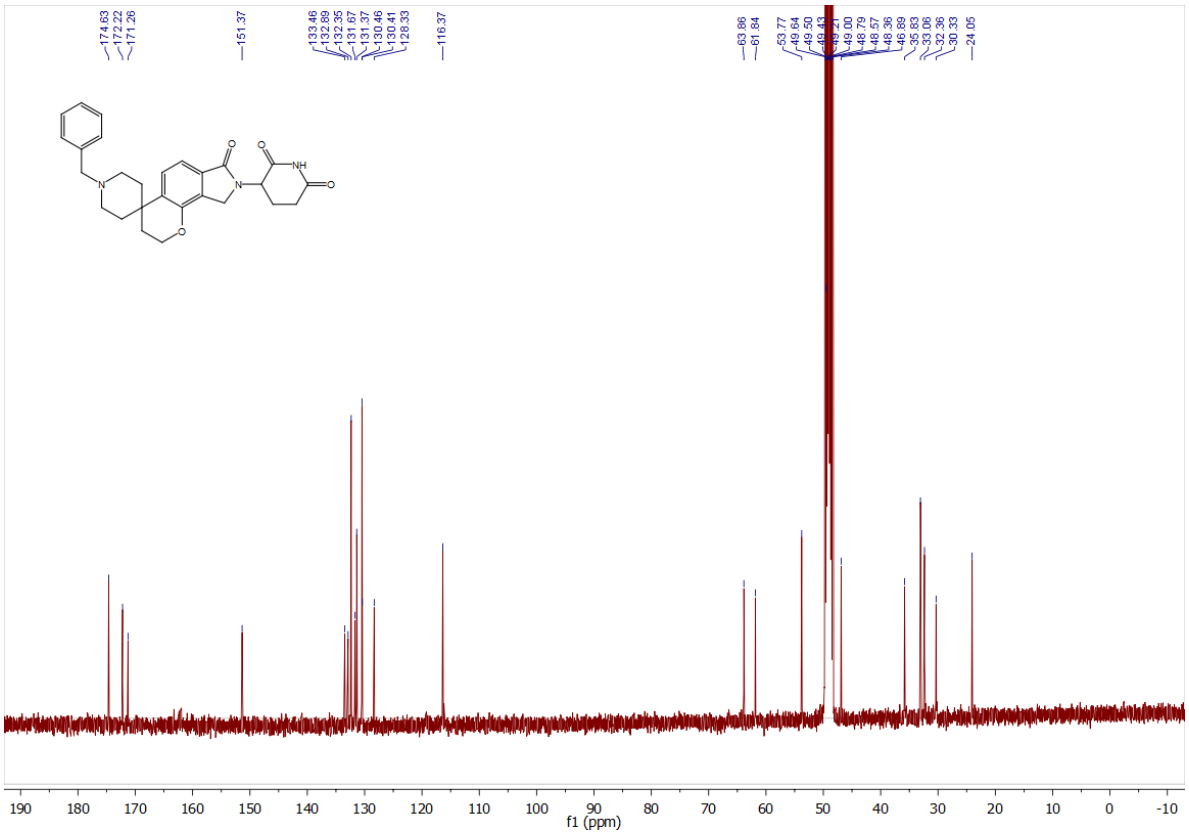
[1]H-NMR spectrum for the final compound **9**



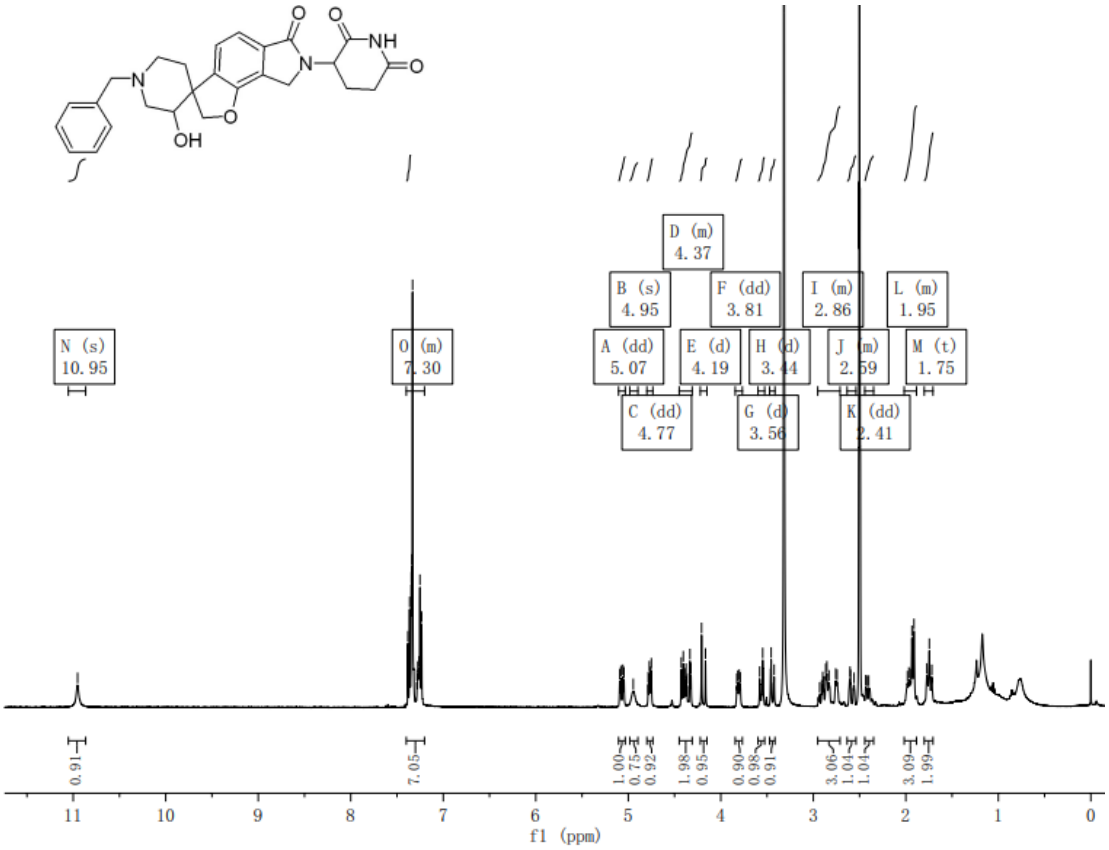
^{1}H-NMR spectrum for the final compound **10**



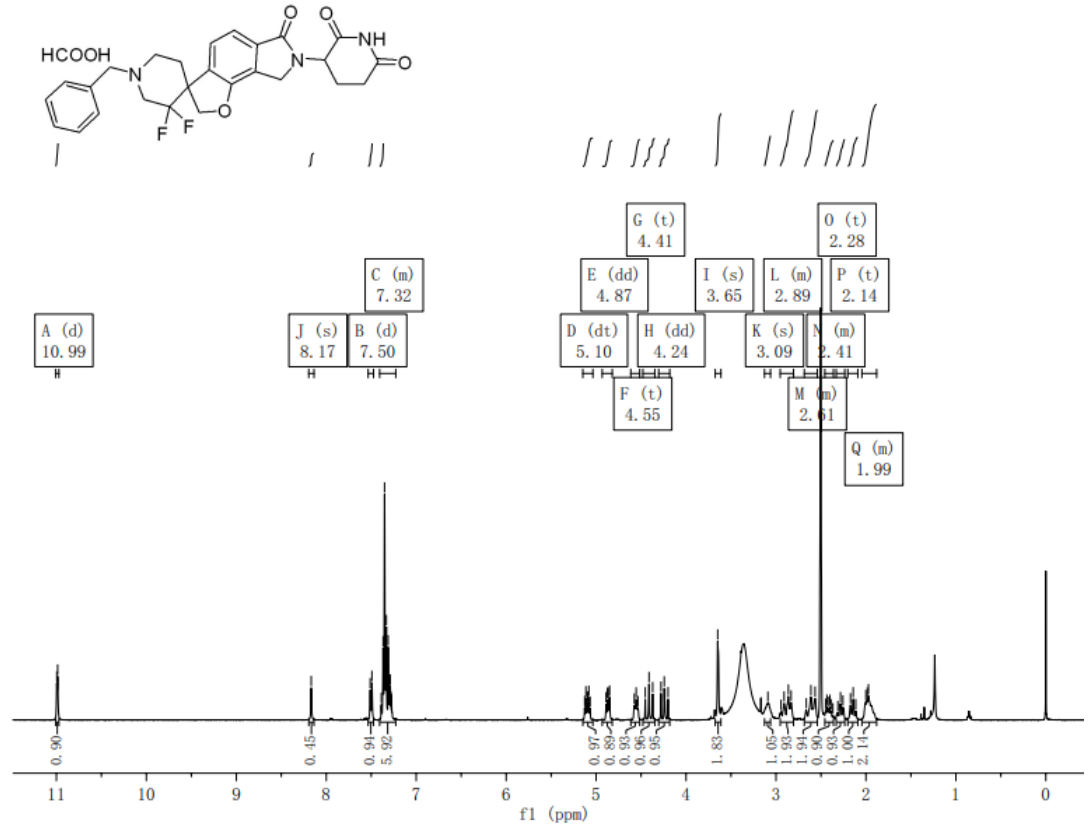
^{13}C-NMR spectrum for the final compound **10**



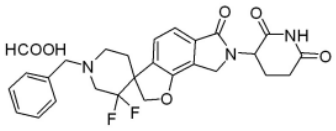
[1]H-NMR spectrum for the final compound **11**

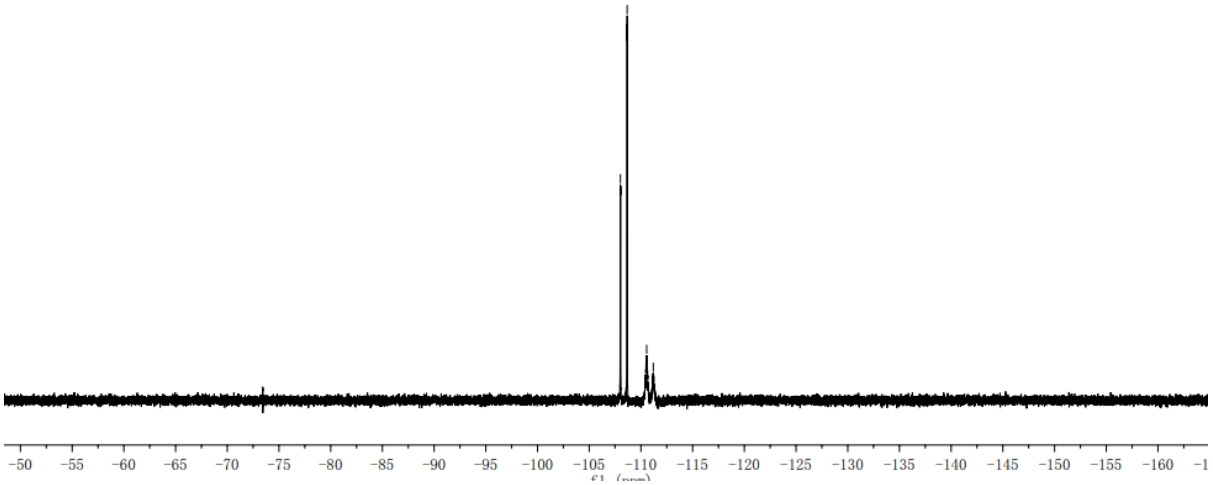


[1]H-NMR spectrum for the final compound **12**

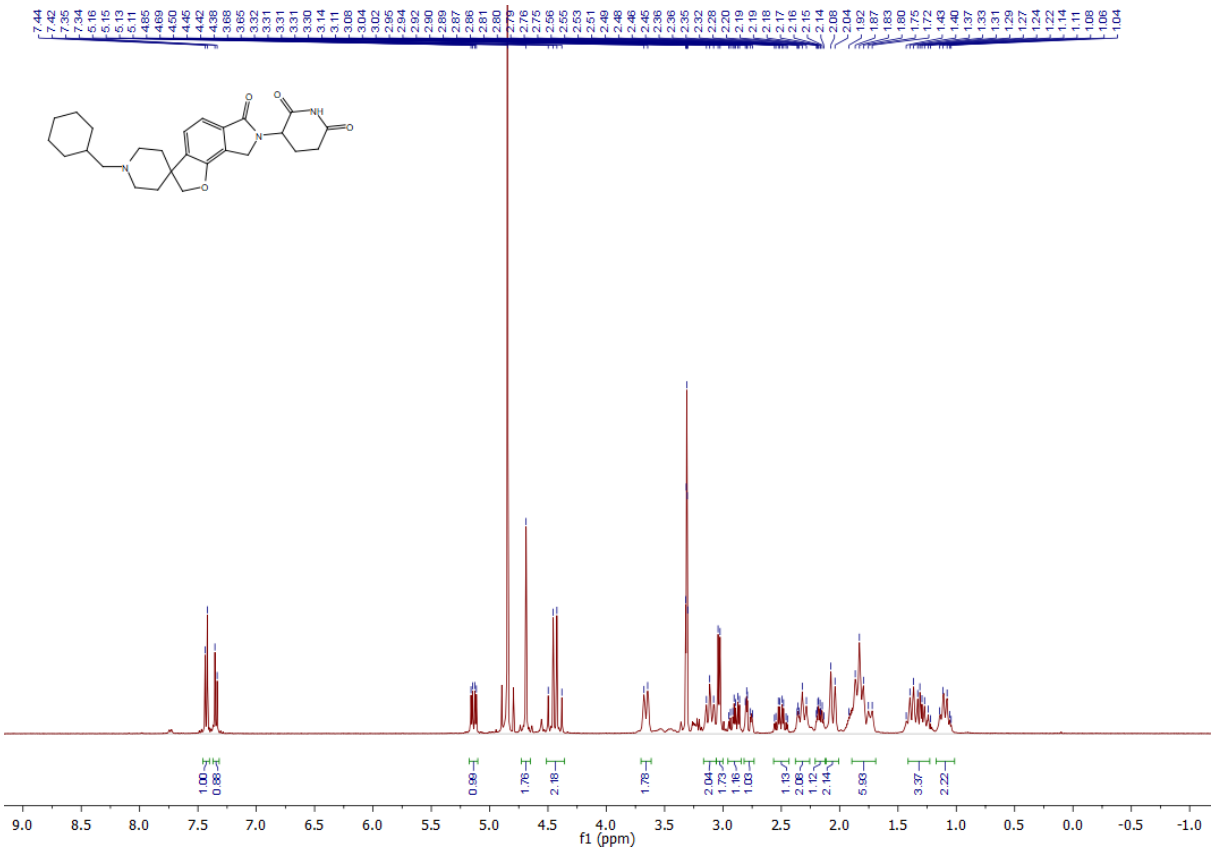


^{19}F-NMR spectrum for the final compound **12**

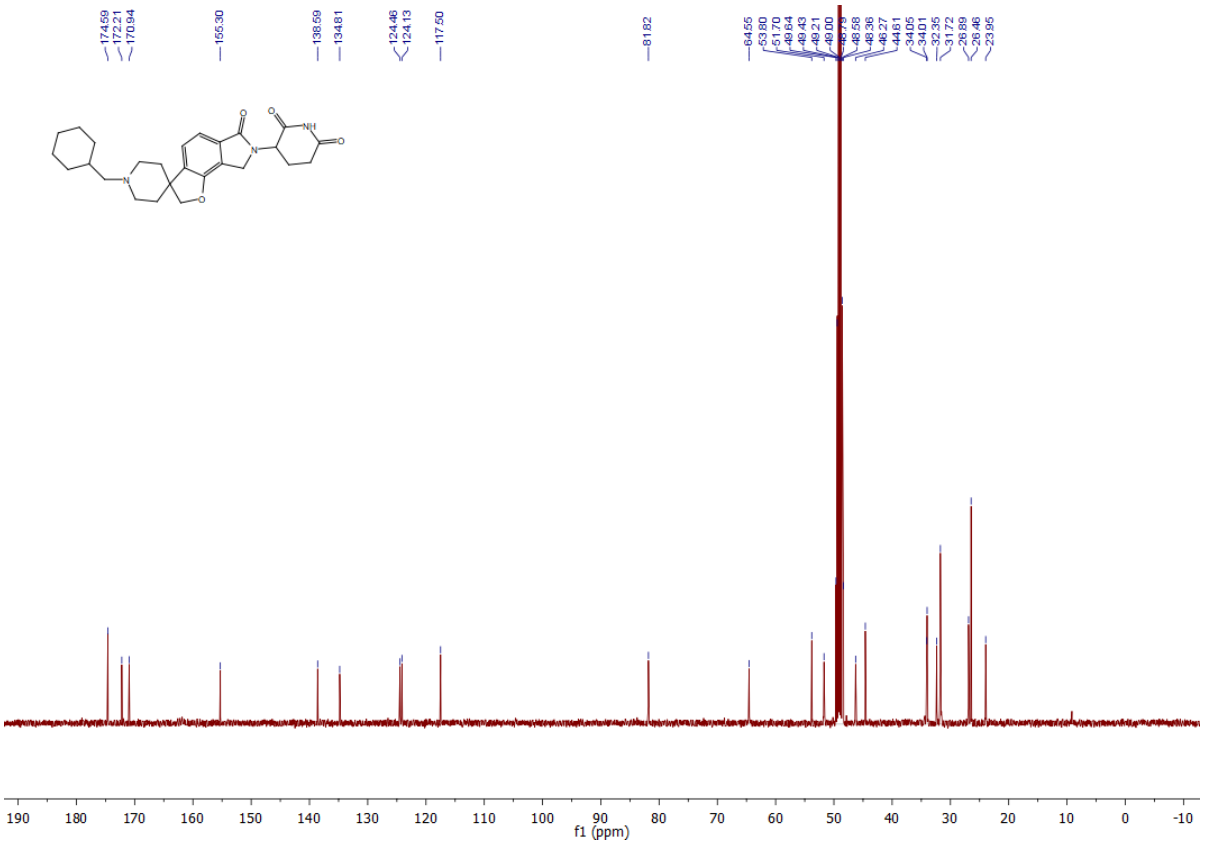




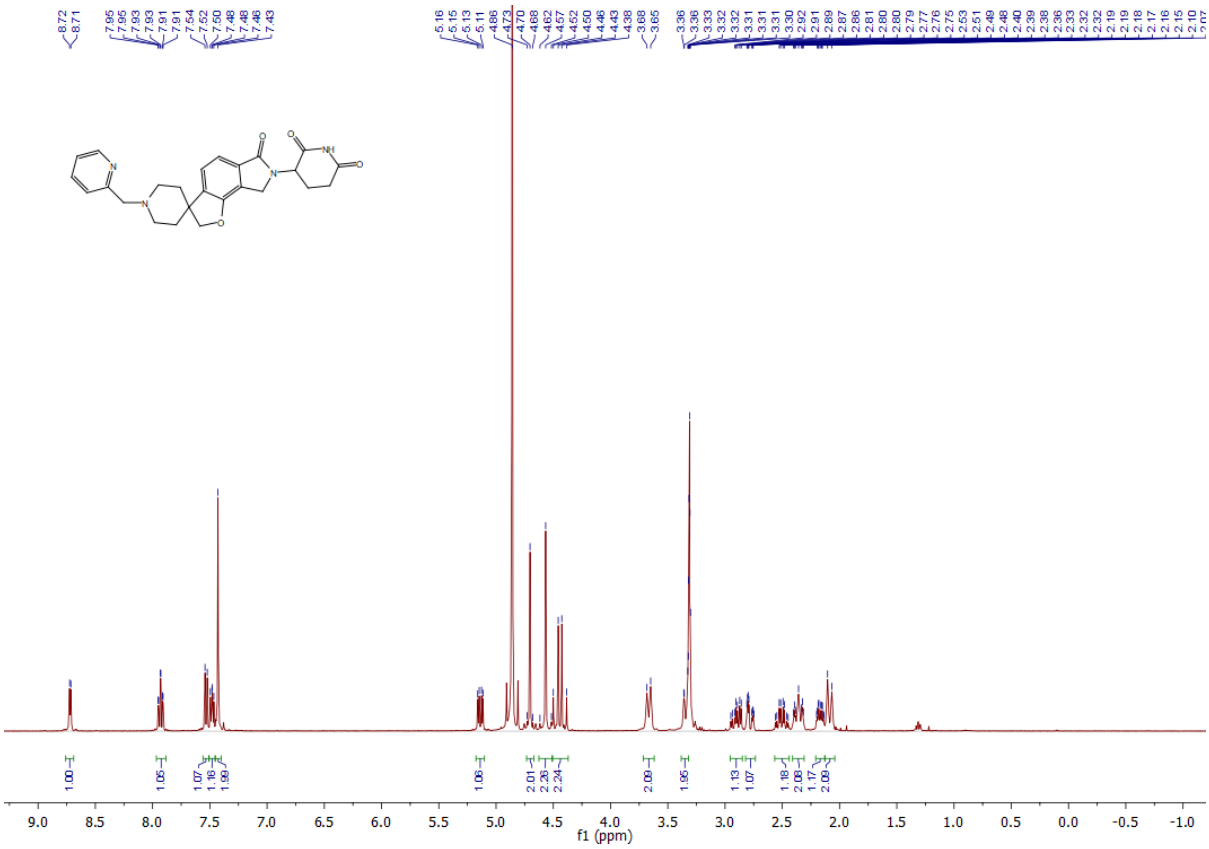
^{1}H-NMR spectrum for the final compound **13**



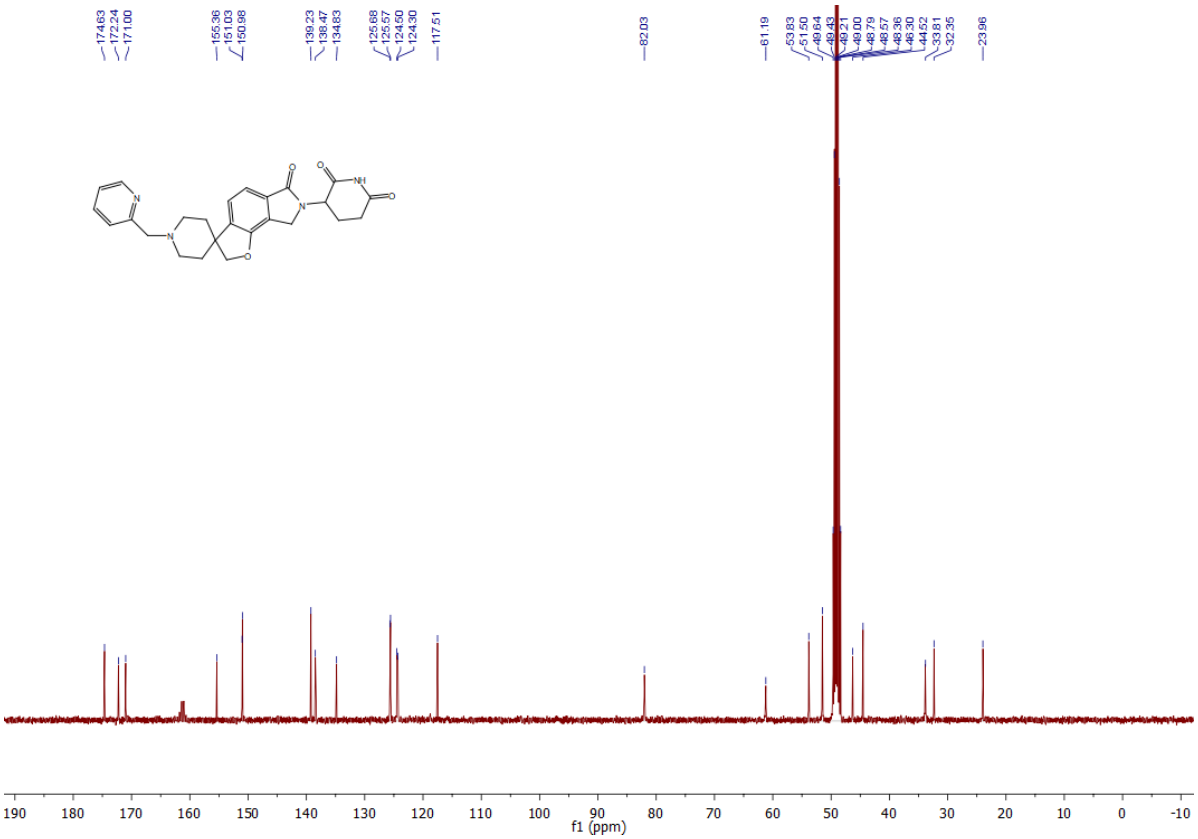
^{13}C-NMR spectrum for the final compound **13**



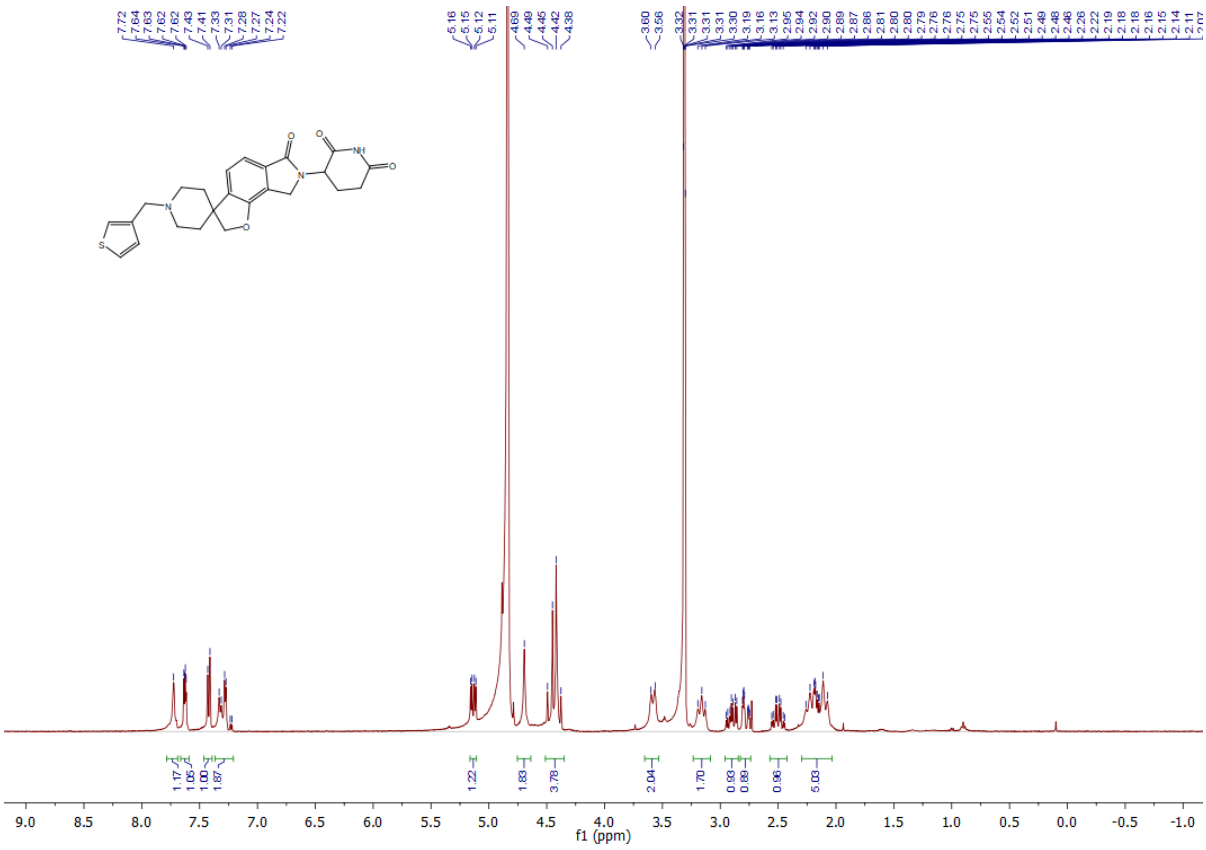
^{1}H-NMR spectrum for the final compound **14**



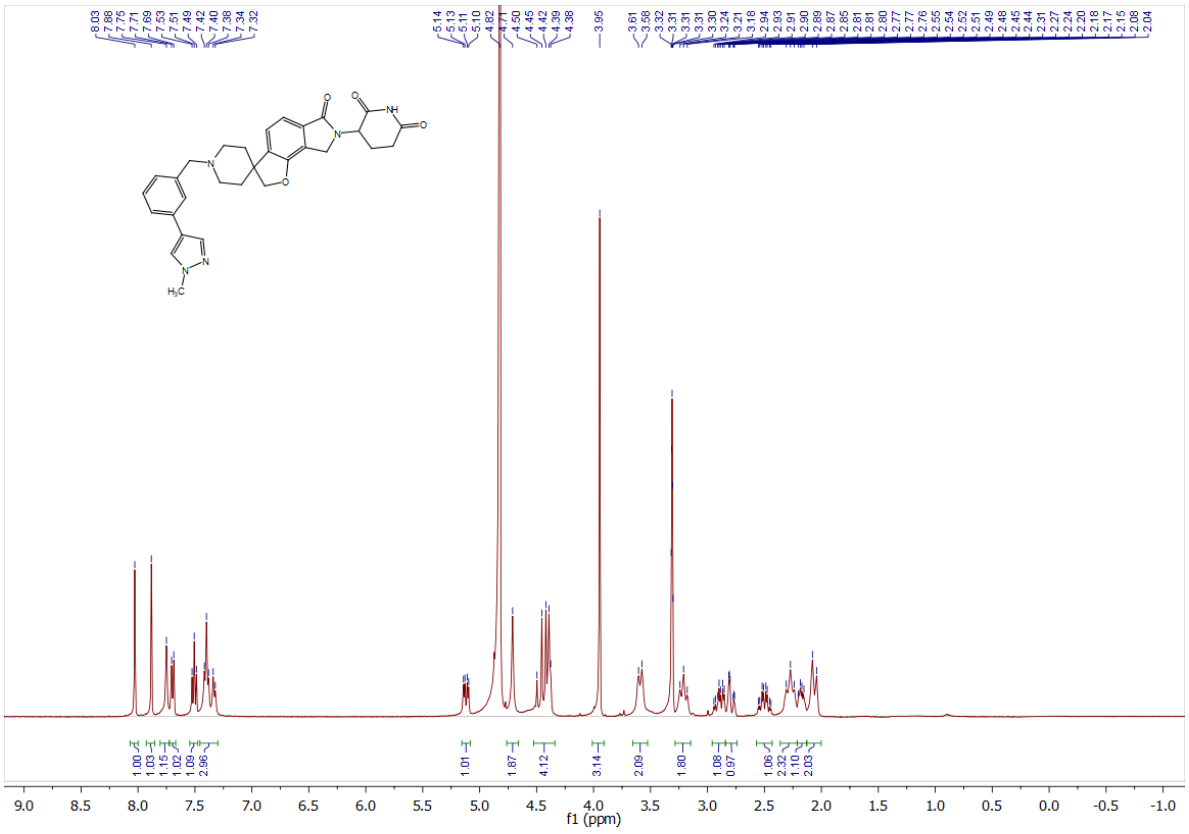
^{13}C-NMR spectrum for the final compound **14**



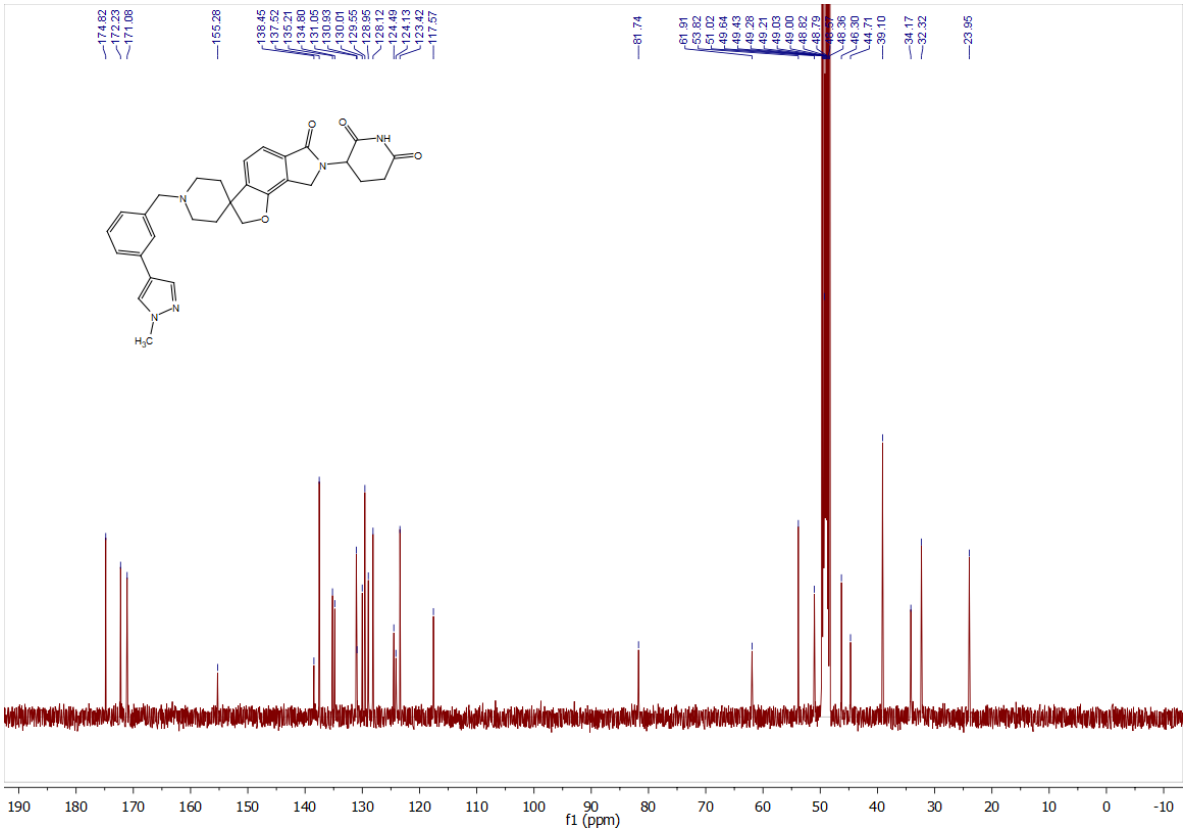
^{1}H-NMR spectrum for the final compound **15**



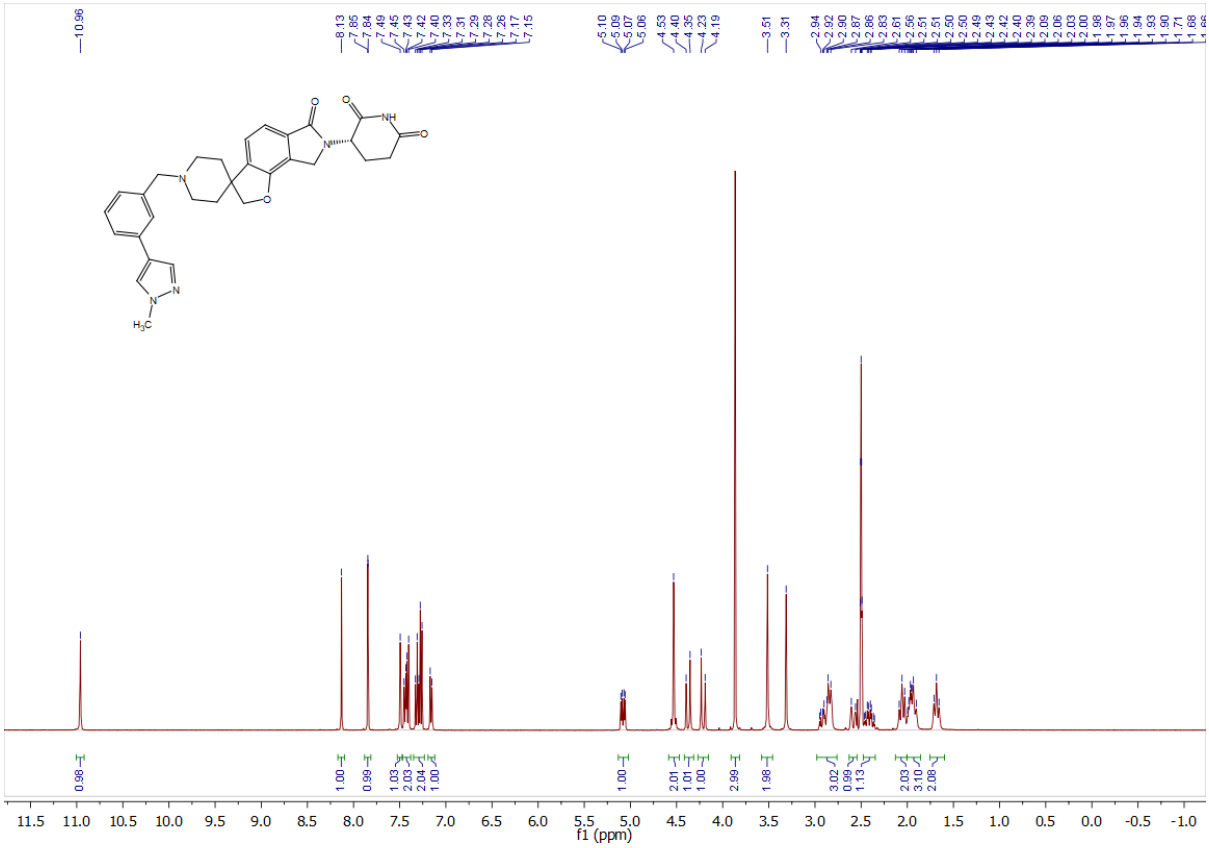
^{1}H-NMR spectrum for the final compound **16**



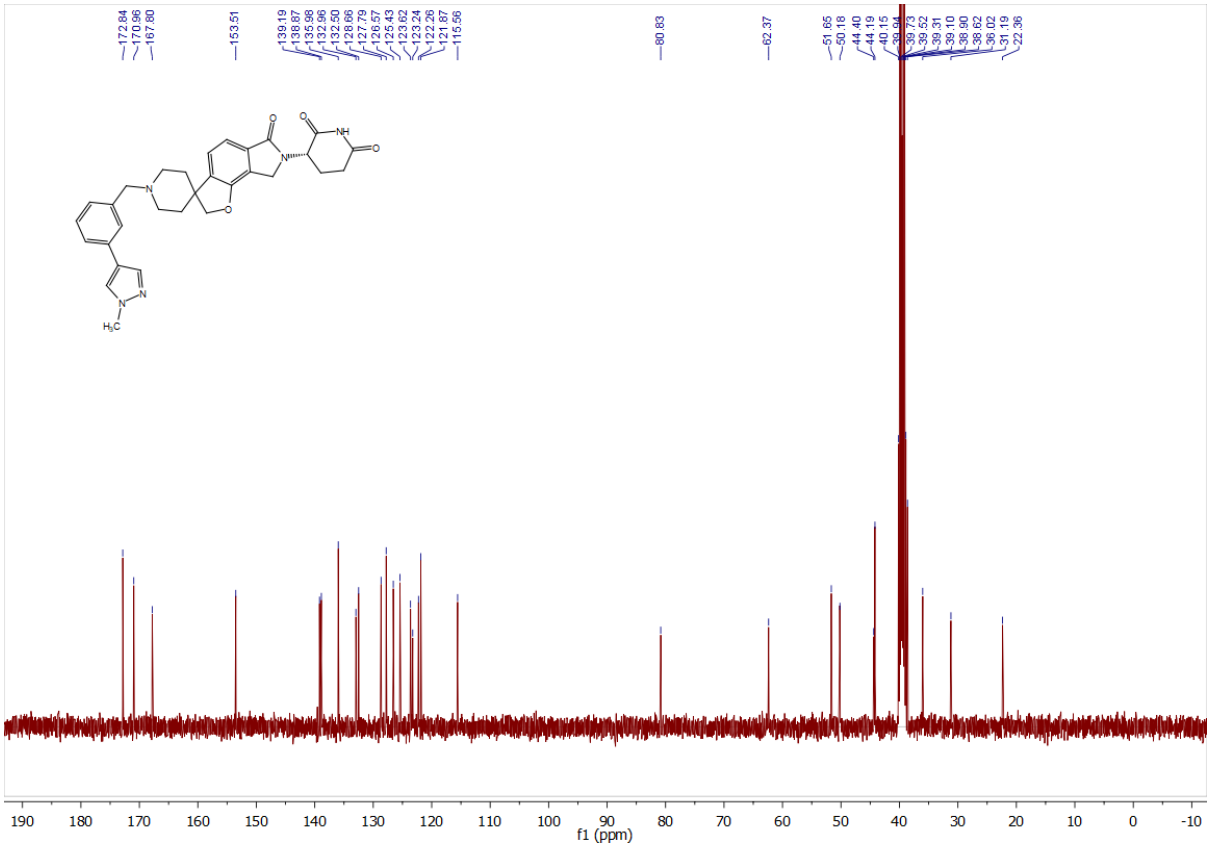
^{13}C-NMR spectrum for the final compound **16**



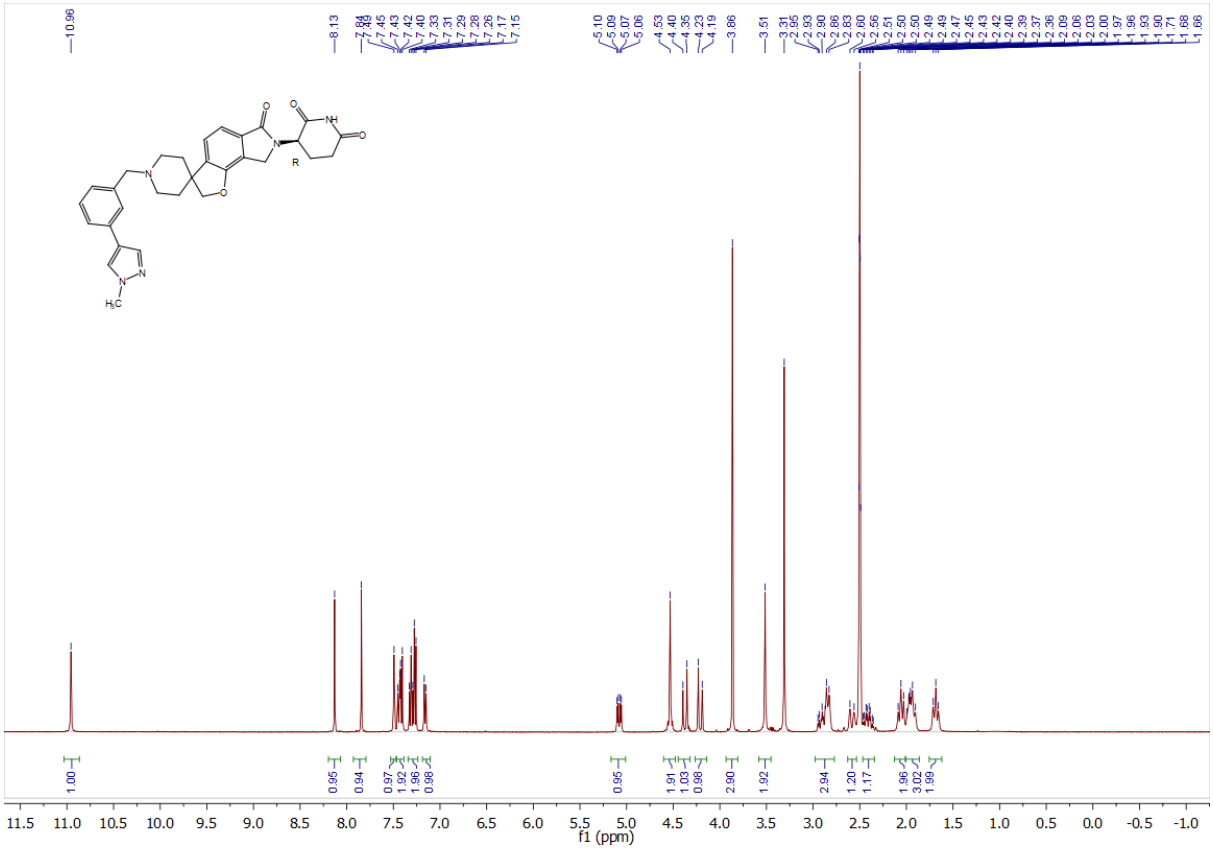
[1]H-NMR spectrum for the final compound **16a** (PVTX-405)



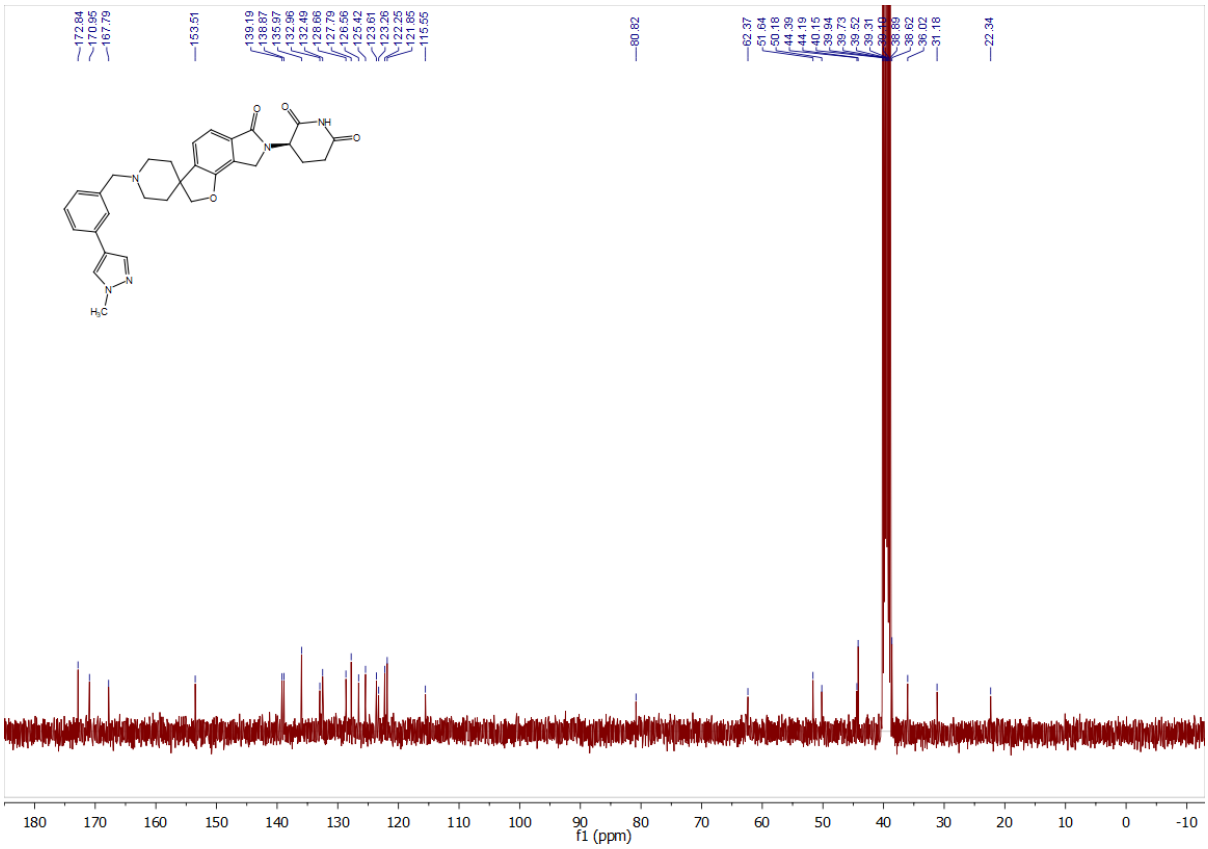
[13]C-NMR spectrum for the final compound **16a** (PVTX-405)



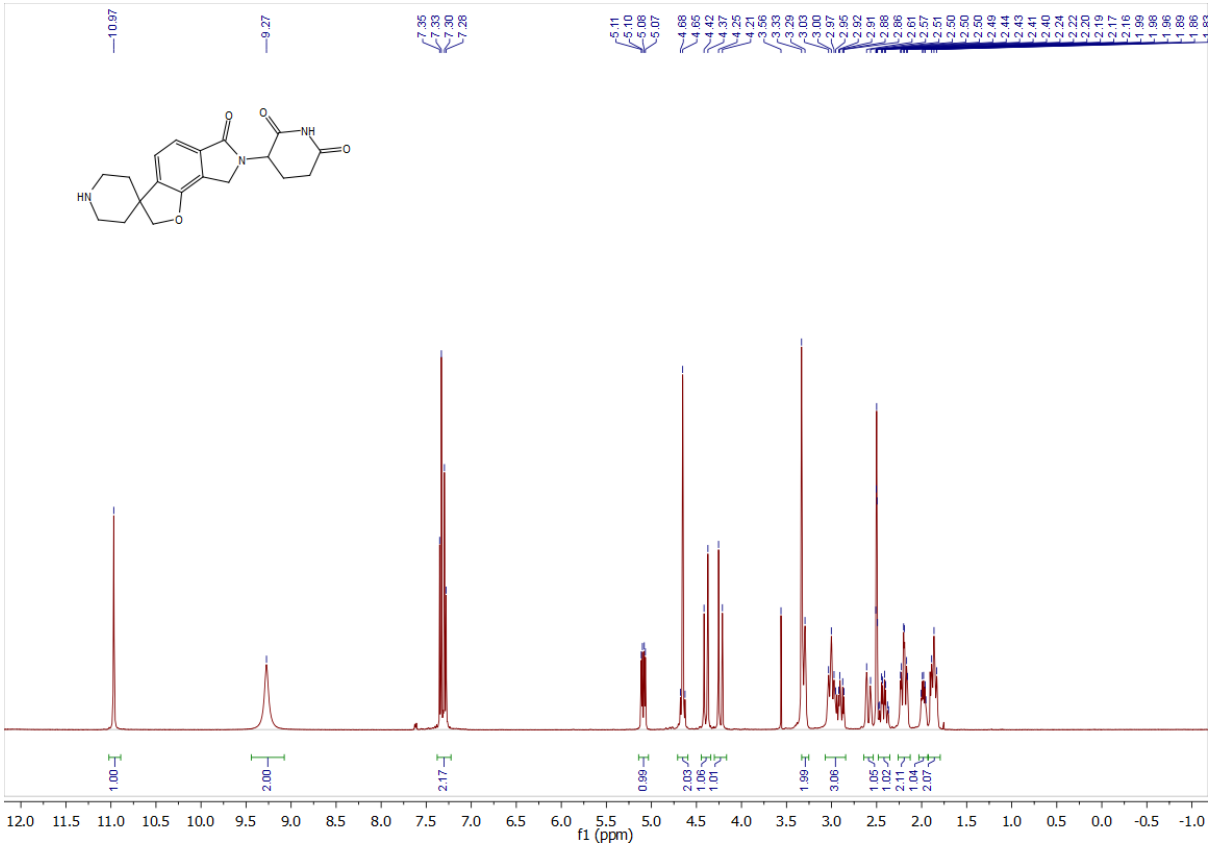
[1]H-NMR spectrum for the final compound **16b**



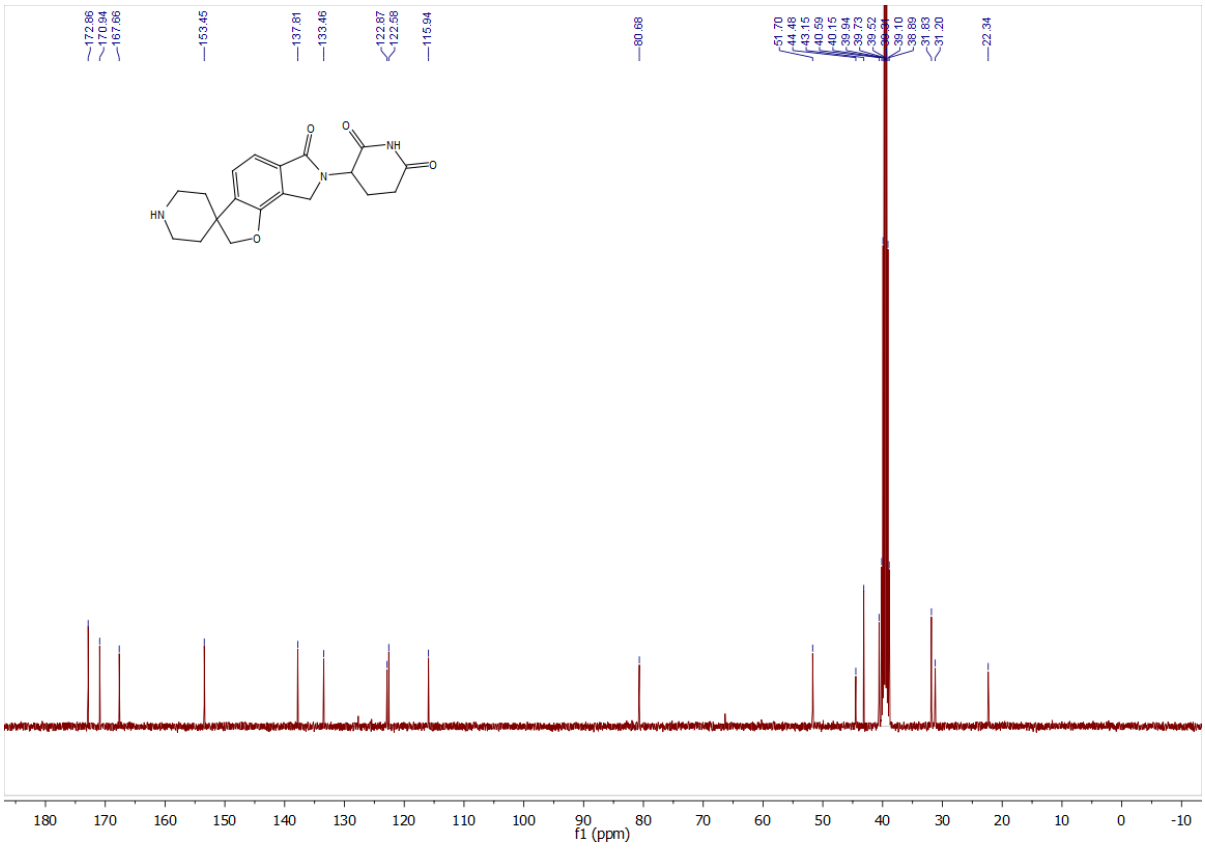
[13]C-NMR spectrum for the final compound **16b**



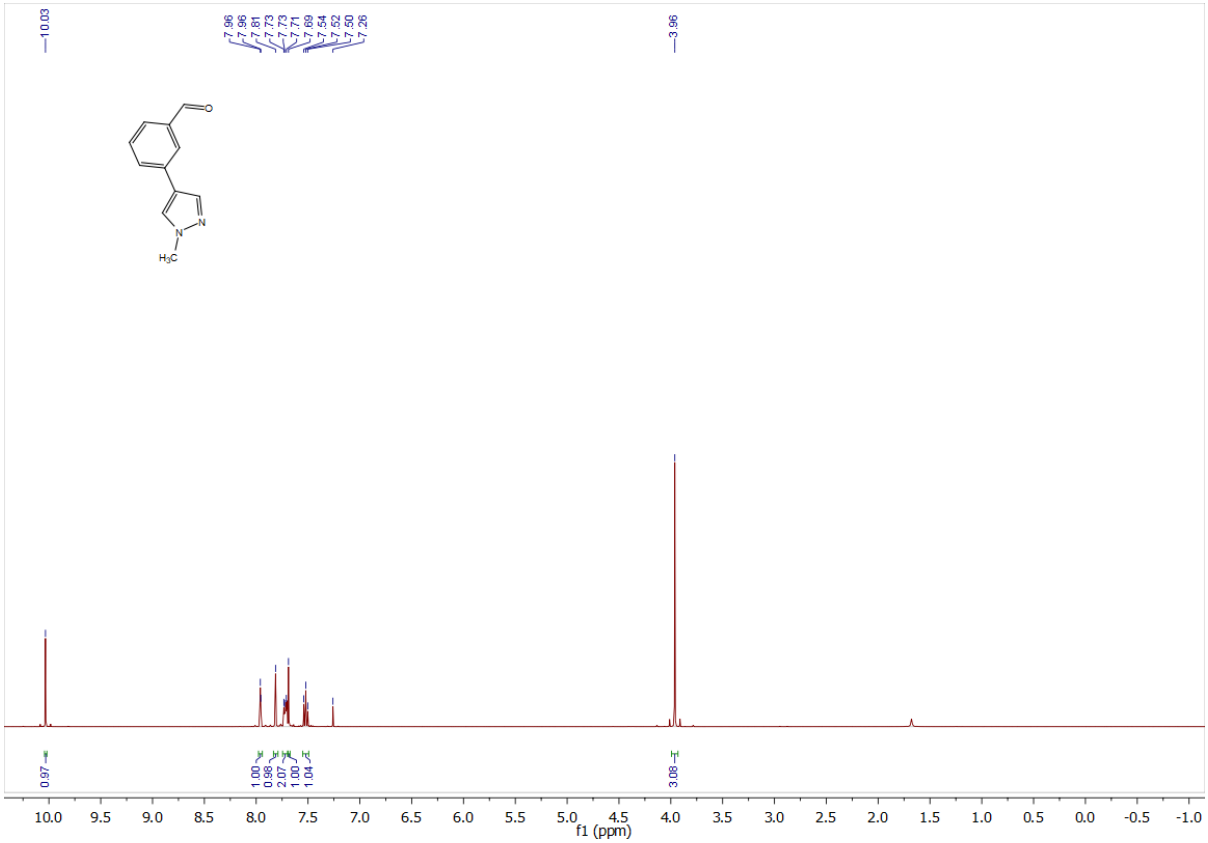
^{1}H-NMR spectrum for the key intermediate **29a**



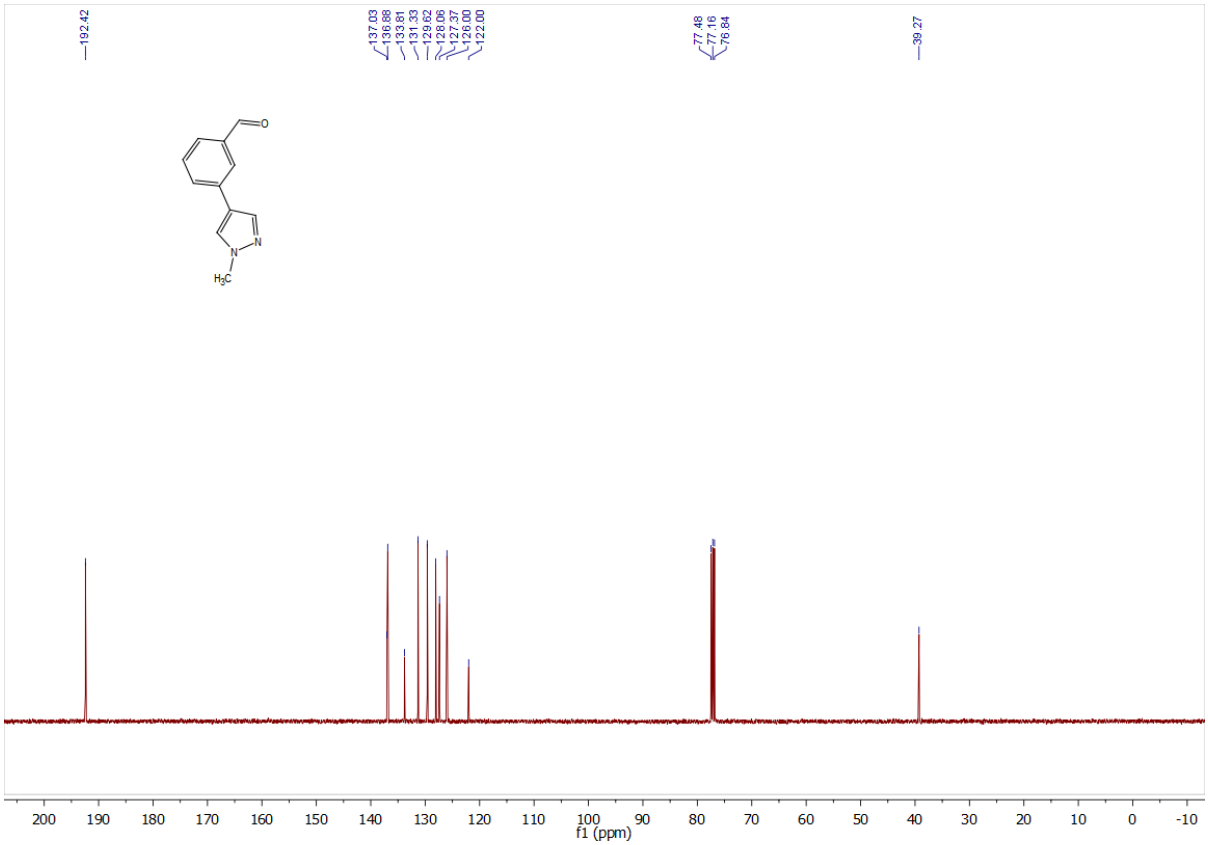
^{13}C-NMR Spectrum for the Key Intermediate **29a**



^{1}H-NMR spectrum for the key intermediate **39**

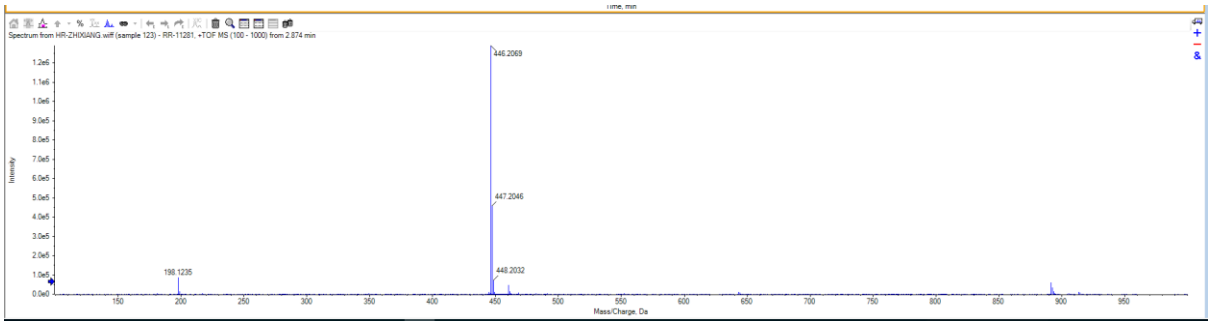


^{13}C-NMR spectrum for the key intermediate **39**

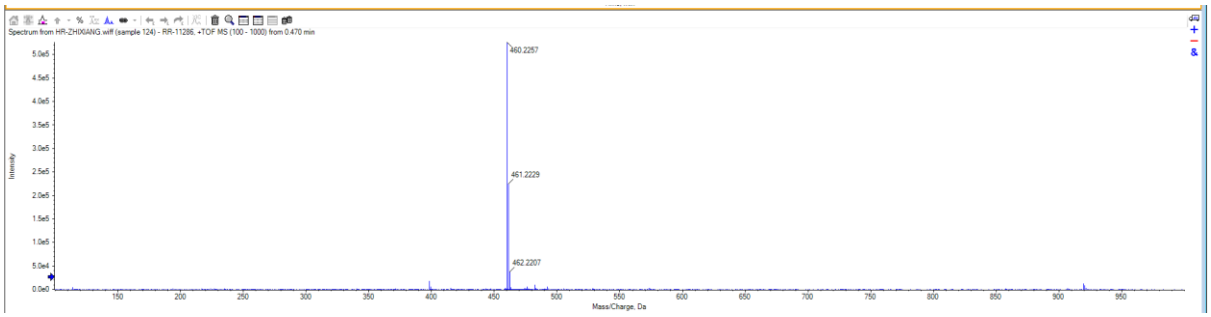


HRMS spectra for the final compounds and key intermediates

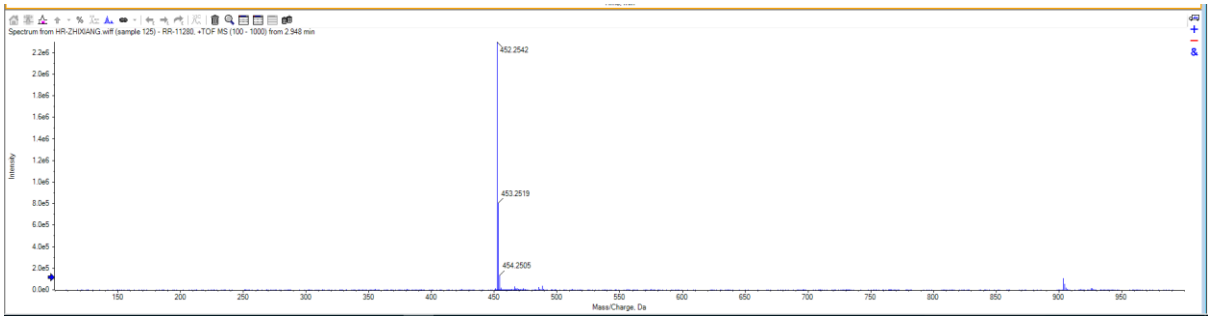
HRMS spectrum for the final compound **5**



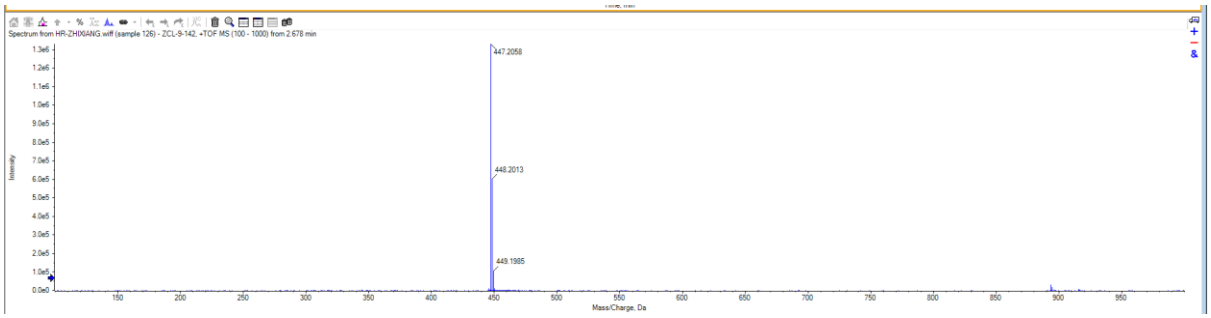
HRMS spectrum for the final compound **10**



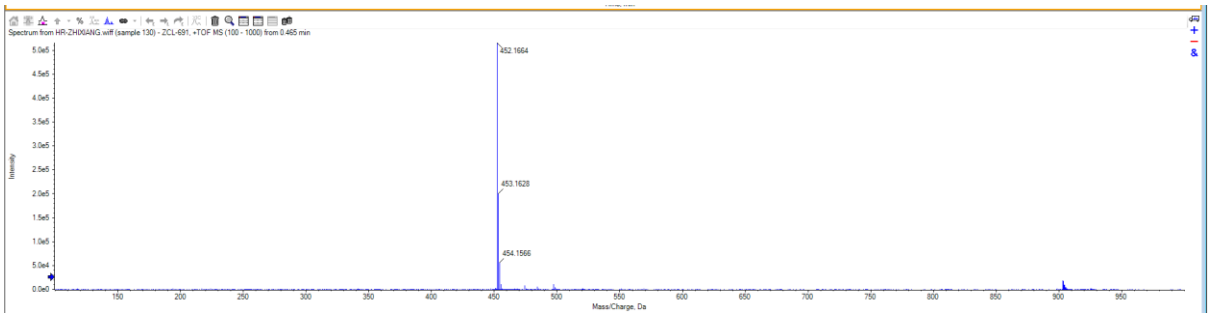
HRMS spectrum for the final compound **13**



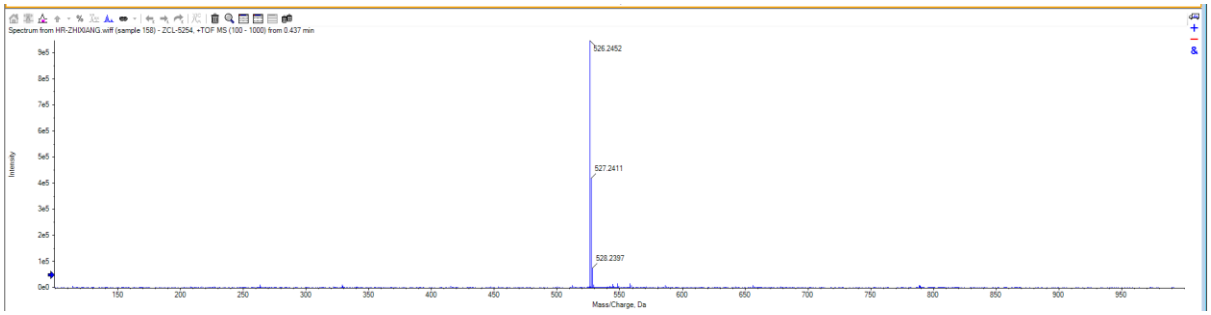
HRMS spectrum for the final compound **14**



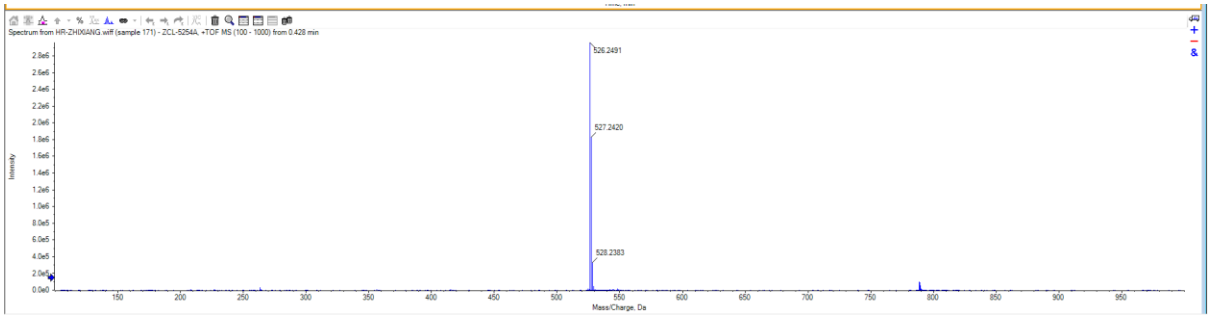
HRMS spectrum for the final compound **15**



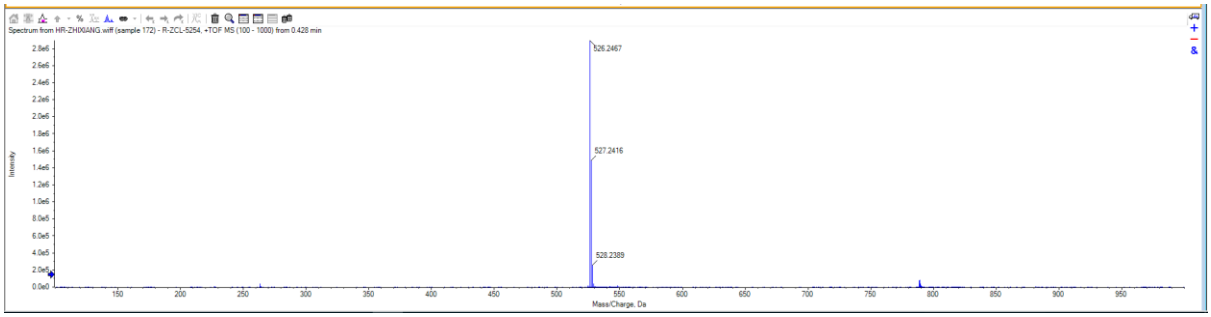
HRMS spectrum for the final compound **16**



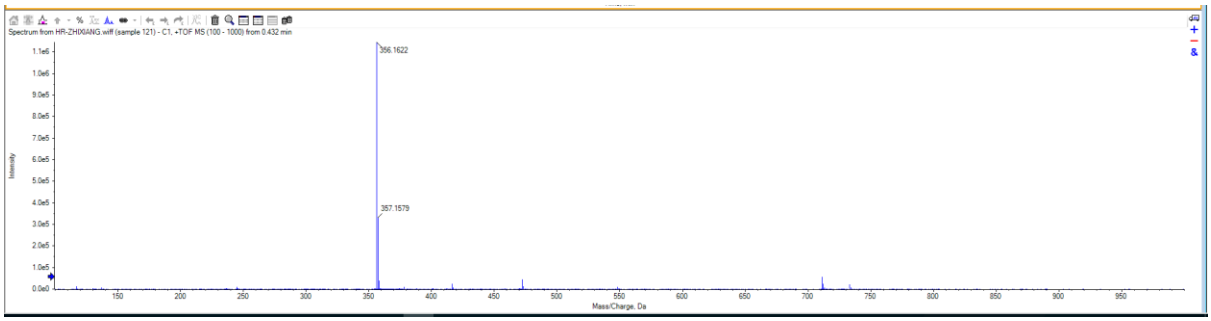
HRMS spectrum for the final compound **16a** (PVTX-405)



HRMS Spectrum for the Final Compound **16b**



HRMS spectrum for the key intermediate **29a**

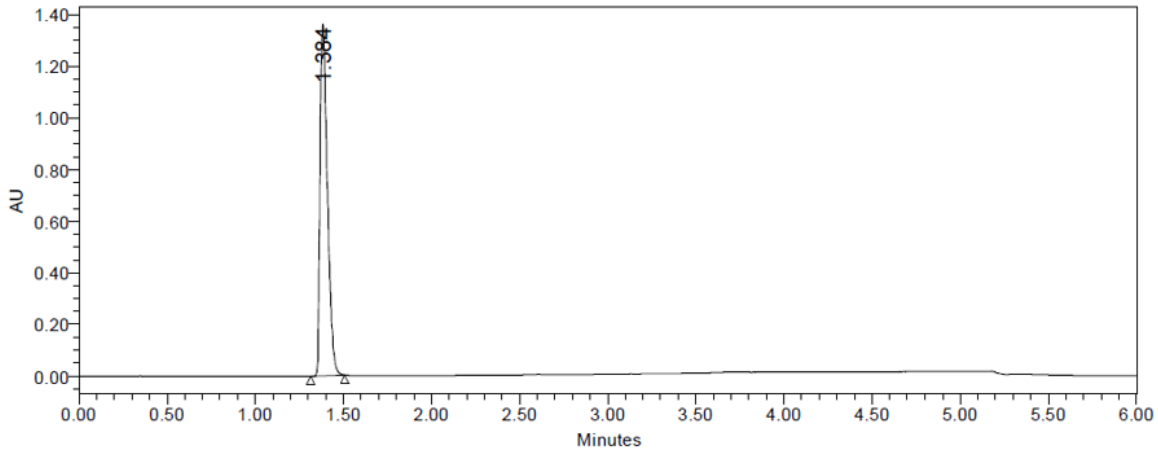


UPLC spectra for the final compounds

UPLC spectra for the final compound **5**

SAMPLE INFORMATION

Sample Name:	ZXC-9-139-P-3	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	3
Vial:	1:B,8	Acq. Method Set:	10to100Bin6min_noDelay
Injection #:	1	Processing Method:	New_Processing_Method
Injection Volume:	5.00 ul	Channel Name:	254.0nm
Run Time:	6.0 Minutes	Proc. Chnl. Descr.:	PDA Spectrum PDA 254.0 nm
Date Acquired:	9/14/2023 11:59:11 PM EDT		
Date Processed:	9/15/2023 12:55:11 AM EDT		



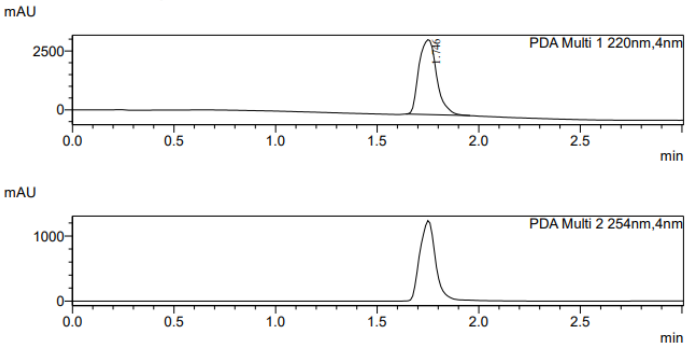
	RT	Area	% Area	Height
1	1.384	3920527	100.00	1360088

LC-MS and UPLC spectra for the final compound **6**

<LC-MS Condition>

Instrument	: LCMS2020 (E-LCMS 021)
Column	: Shim-Pack Scepter C18, 33*3.0mm, 3um
Mobile Phase	: Solvent A: H2O/MeCN/FA = 90:10:0.05 Solvent B: CH3CN
Temperature	: 40°C
Flow Rate	: 1.2mL/min
Method	: positive-negative 3min-1.lcm
Run Time	: 0.01min @ 20% B, 1.79min gradient (20-95% B), then 0.7min @ 95% B
Injection volume	: 8uL
Detector	: UV
Data Filename	: PR-WP-0138-P1-LCMS.lcd
Batch Filename	: 20220613.lcb
Date Acquired	: 6/15/2022 10:16:41 AM
Date Processed	: 6/15/2022 11:01:22 AM

<PDA Chromatogram>



<Peak Table>

PDA Ch1 220nm

Peak#	Ret. Time	Area	Area%
1	1.746	19309663	100.000
Total		19309663	100.000

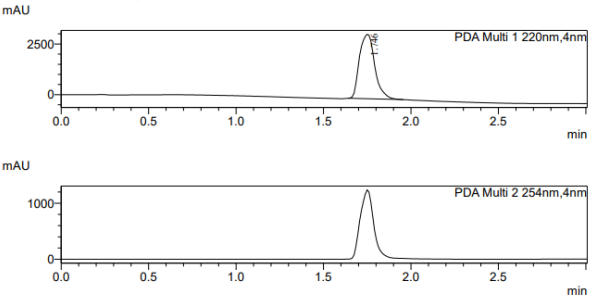
PDA Ch2 254nm

Peak#	Ret. Time	Area	Area%
Total			

<LC-MS Condition>

Instrument	: LCMS2020 (E-LCMS 021)
Column	: Shim-Pack Scepter C18, 33*3.0mm, 3um
Mobile Phase	: Solvent A: H2O/MeCN/FA = 90:10:0.05 Solvent B: CH3CN
Temperature	: 40°C
Flow Rate	: 1.2mL/min
Method	: positive-negative 3min-1.lcm
Run Time	: 0.01min @ 20% B, 1.79min gradient (20-95% B), then 0.7min @ 95% B
Injection volume	: 8uL
Detector	: UV
Data Filename	: PR-WP-0138-P1-LCMS.lcd
Batch Filename	: 20220613.lcb
Date Acquired	: 6/15/2022 10:16:41 AM
Date Processed	: 6/15/2022 11:01:22 AM

<PDA Chromatogram>



<Peak Table>

PDA Ch1 220nm

Peak#	Ret. Time	Area	Area%
1	1.746	19309663	100.000
Total		19309663	100.000

PDA Ch2 254nm

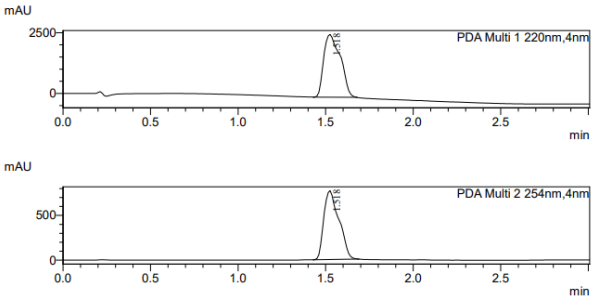
Peak#	Ret. Time	Area	Area%
Total			

LC-MS and UPLC spectra for the final compound **7**

<LC-MS Condition>

Instrument	: LCMS2020 (E-LCMS 021)
Column	: Shim-Pack Scepter C18, 33*3.0mm, 3um
Mobile Phase	: Solvent A: H2O/MeCN/FA = 90:10:0.05 Solvent B: CH3CN
Temperature	: 40°C
Flow Rate	: 1.2mL/min
Method	: positive-negative 3min-1.lcm
Run Time	: 0.01min @ 20% B, 1.79min gradient (20-95% B), then 0.7min @ 95% B
Injection volume	: 20uL
Detector	: UV
Data Filename	: PR-WP-0139-P1-LCMS.lcd
Batch Filename	: 20220613.lcb
Date Acquired	: 6/15/2022 7:31:20 PM
Date Processed	: 6/16/2022 9:38:28 AM

<PDA Chromatogram>



<Peak Table>

PDA Ch1 220nm

Peak#	Ret. Time	Area	Area%
1	1.518	17091638	100.000
Total		17091638	100.000

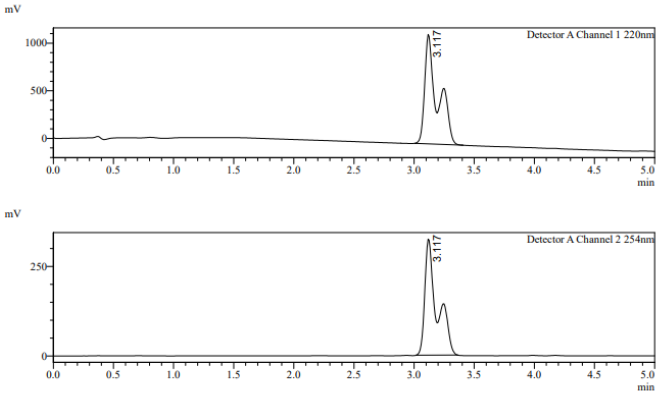
PDA Ch2 254nm

Peak#	Ret. Time	Area	Area%
1	1.518	4652530	100.000
Total		4652530	100.000

<LC Condition>

Instrument	: LC-20AD (E-LC 016)
Column	: YMC-Triart C18, 50×4.6mm, 5um
Mobile phase	: Solvent C: H2O/MeCN/TFA = 90/10/0.1 Solvent D: H2O/MeCN/TFA = 10/90/0.1
Flow Rate	: 2.5ml/min
Method	: 20-95%-MeCN.lcm
Run Time	: 0.4min @ 20% D, 3.4min gradient (20-95% D), then 0.8min @ 95% D
Temperature	: 40°C
Detector	: UV
Injection Volume	: 20uL
Date File Name	: PR-WP-0139-P1-HP.lcd
Bath File	: 20220614.lcb
Date Acquired	: 6/15/2022 5:29:38 PM
Date Processed	: 6/16/2022 9:47:51 AM

<LC Chromatogram>



<Peak Table>

Detector A Channel 1 220nm

Peak#	Ret. Time	Area	Area%
1	3.117	8494775	100.000
Total		8494775	100.000

Detector A Channel 2 254nm

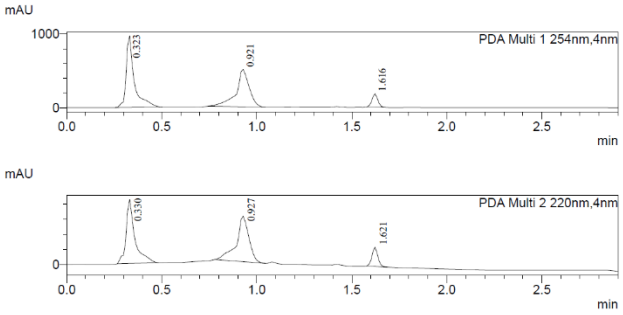
Peak#	Ret. Time	Area	Area%
1	3.117	2288801	100.000
Total		2288801	100.000

LC-MS and UPLC Spectrum for Final Compound **8**

<LC-MS Condition>

Instrument	: LCMS2020 (E-LCMS 012)
Column	: YMC-Triart C18, 50*4.6mm, 5um
Mobile Phase	: Solvent A: H2O/MeCN/FA = 90:10:0.05 Solvent B: CH3CN
Temperature	: 40°C
Flow Rate	: 2.3mL/min
Method	: 0-70%-positive-negative.lcm
Run Time	: 0.01min @ 0% B, 1.79min gradient (0-70% B), then 0.7min @ 70% B
Injection volume	: 5uL
Detector	: UV
Data Filename	: N211712-057-LCMS.lcd
Batch Filename	: 20211220.lcb
Date Acquired	: 12/21/2021 1:38:22 PM
Date Processed	: 12/21/2021 1:54:57 PM

<PDA Chromatogram>



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	0.323	2847160	50.371
2	0.921	2425161	42.905
3	1.616	380028	6.723
Total		5652349	100.000

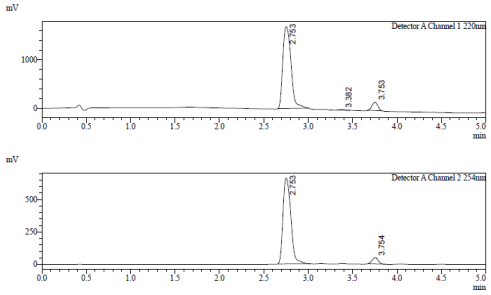
PDA Ch2 220nm

Peak#	Ret. Time	Area	Area%
1	0.330	6756219	45.039
2	0.927	6919651	46.128
3	1.621	1325049	8.833
Total		15000920	100.000

<LC Condition>

Instrument	: LC-20AD (E-LC 009)
Column	: YMC-Triart C18, 50x4.6mm, 5um
Mobile phase	: Solvent C: H2O/MeCN/TFA = 90/10/0.1 Solvent D: H2O/MeCN/TFA = 10/90/0.1
Flow Rate	: 2.5ml/min
Method	: 0-70%-MeCN.lcm
Run Time	: 0.4min @ 0% D, 3.4min gradient (0-70% D), then 0.8min @ 70% D
Temperature	: 40°C
Detector	: UV
Injection Volume	: 20uL
Data File Name	: N211712-057-HPLC-MeCN.lcd
Bath File	: 20211219.lcb
Date Acquired	: 12/20/2021 11:20:13 AM
Date Processed	: 12/20/2021 11:27:34 AM

<LC Chromatogram>



<Peak Table>

Detector A Channel 1 220nm

Peak#	Ret. Time	Area	Area%
1	2.753	10774301	92.843
2	3.382	56930	0.491
3	3.753	773673	6.667
Total		11604903	100.000

Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Area%
1	2.753	4048802	94.666
2	3.754	228127	5.334

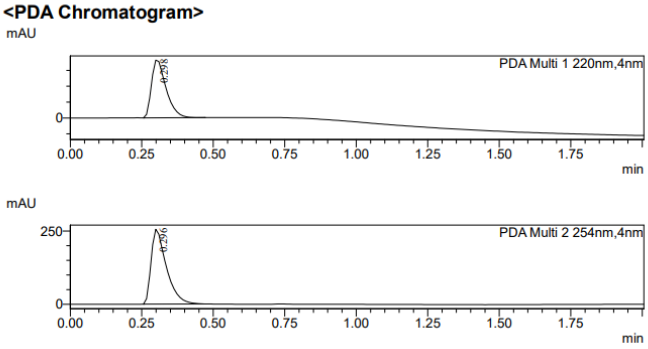
Peak#	Ret. Time	Area	Area%
Total		4276928	100.000

LC-MS and UPLC spectra for the final compound **9**

<LC-MS Condition>

Instrument	: LCMS2020 (E-LCMS 013)
Column	: Shim-Pack Scepter C18, 33*3.0mm, 3um
Mobile Phase	: Solvent A: H2O/MeCN/FA = 90:10:0.05 Solvent B: CH3CN
Temperature	: 40°C
Flow Rate	: 1.2mL/min
Method	: positive-negative 2min.lcm
Run Time	: 0.01min @ 40% B, 1.09min gradient (40-95% B), then 0.4min @ 95% B
Injection volume	: 5uL
Detector	: UV
Data Filename	: N211658-073-LCMS.lcd
Batch Filename	: 20211223.lcb
Date Acquired	: 12/24/2021 3:35:44 PM
Date Processed	: 12/28/2021 12:07:43 PM

<PDA Chromatogram>



<Peak Table>

PDA Ch1 220nm

Peak#	Ret. Time	Area	Area%
1	0.298	2592117	100.000
Total		2592117	100.000

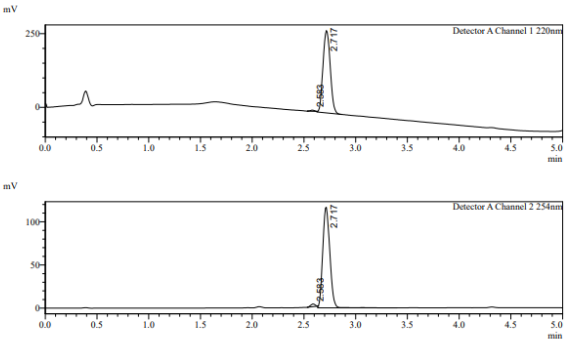
PDA Ch2 254nm

Peak#	Ret. Time	Area	Area%
1	0.296	953262	100.000
Total		953262	100.000

<LC Condition>

Instrument	: LC-20AD (E-LC 008)
Column	: YMC-Triart C18, 50×4.6mm, 5um
Mobile phase	: Solvent C: H2O/MeCN/TFA = 90/10/0.1 Solvent D: H2O/MeCN/TFA = 10/90/0.1
Flow Rate	: 2.5ml/min
Method	: 0-70%-MeCN
Run Time	: 0.4min @ 0% D, 3.4min gradient (0-70% D), then 0.8min @ 70% D
Temperature	: 40°C
Detector	: UV
Injection Volume	: 10uL
Date File Name	: N211658-073-HPLC-MeCN.lcd
Bath File	: 20211223.lcb
Date Acquired	: 12/24/2021 4:00:41 PM
Date Processed	: 12/28/2021 12:05:19 PM

<LC Chromatogram>



<Peak Table>

Detector A Channel 1 220nm

Peak#	Ret. Time	Area	Area%
1	2.583	11035	0.806
2	2.717	1357963	99.194
Total		1368998	100.000

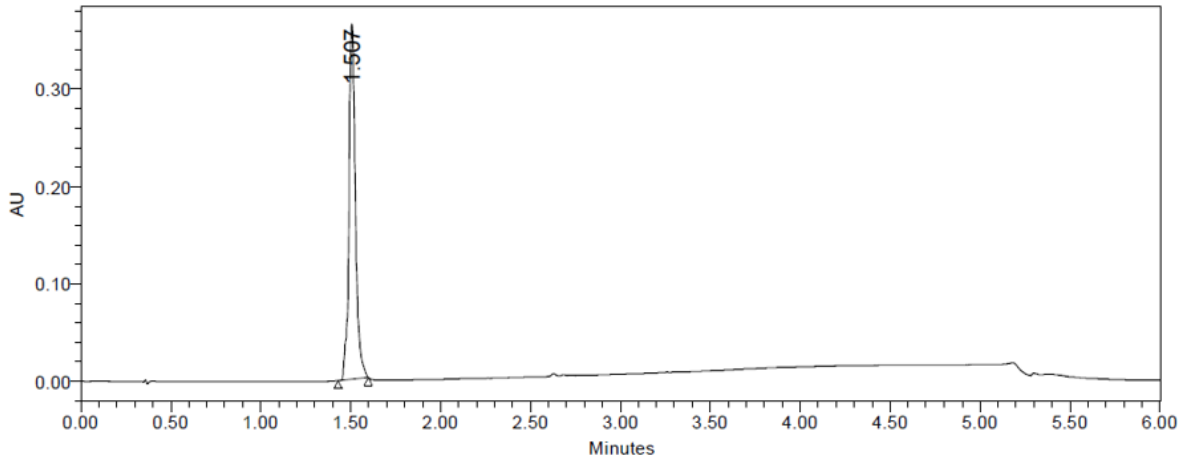
Detector A Channel 2 254nm

Peak#	Ret. Time	Area	Area%
1	2.583	9258	1.642
2	2.717	554596	98.358
Total		563853	100.000

UPLC spectra for the final compound **10**

SAMPLE INFORMATION

Sample Name:	ZXC-9-140-P	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	RA
Vial:	1:C,1	Acq. Method Set:	10to100Bin6min_noDelay
Injection #:	1	Processing Method:	LC PQ ApexTrack
Injection Volume:	5.00 ul	Channel Name:	254.0nm
Run Time:	6.0 Minutes	Proc. Chnl. Descr.:	PDA Spectrum PDA 254.0 nm
Date Acquired:	9/14/2023 7:19:46 PM EDT		
Date Processed:	9/14/2023 10:15:32 PM EDT		



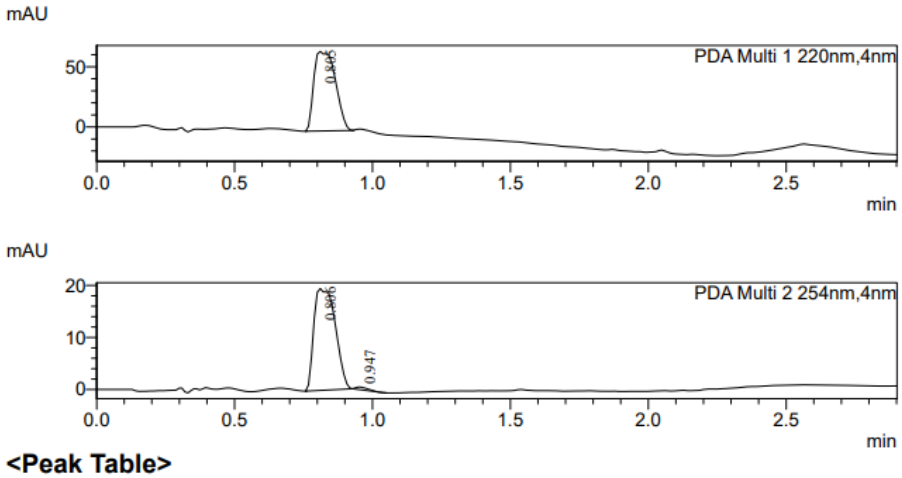
	RT	Area	% Area	Height
1	1.507	911650	100.00	364225

LC-MS and UPLC Spectrum for Final Compound **11**

<LC-MS Condition>

Instrument	: LCMS2020 (E-LCMS 017)
Column	: Shim-pack C18, 50*4.6mm, 5um
Mobile Phase	: Solvent A: H2O/MeCN/FA = 90:10:0.05 Solvent B: CH3CN
Temperature	: 40°C
Flow Rate	: 2.5mL/min
Method	: positive-negative 3min-1.lcm
Run Time	: 0.01min @ 20% B, 1.79min gradient (20-95% B), then 0.7min @ 95% B
Injection volume	: 1uL
Detector	: UV
Data Filename	: PR-ZH-0093-LCMS-P2.lcd
Batch Filename	: 20220531.lcb
Date Acquired	: 5/31/2022 9:54:30 AM
Date Processed	: 6/1/2022 9:32:13 AM

<PDA Chromatogram>



<Peak Table>

PDA Ch1 220nm

Peak#	Ret. Time	Area	Area%
1	0.805	357213	100.000
Total		357213	100.000

PDA Ch2 254nm

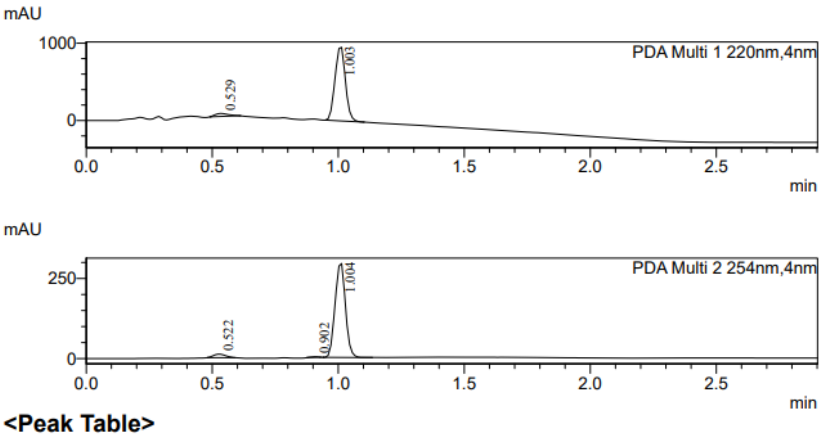
Peak#	Ret. Time	Area	Area%
1	0.806	105390	98.839
2	0.947	1238	1.161
Total		106628	100.000

UPLC Spectrum for Final Compound **12**

<LC-MS Condition>

Instrument	: LCMS2020 (E-LCMS 017)
Column	: Shim-pack C18, 50*4.6mm, 5um
Mobile Phase	: Solvent A: H2O/MeCN/FA = 90:10:0.05 Solvent B: CH3CN
Temperature	: 40°C
Flow Rate	: 2.5mL/min
Method	: positive-negative 3min-1.lcm
Run Time	: 0.01min @ 20% B, 1.79min gradient (20-95% B), then 0.7min @ 95% B
Injection volume	: 3uL
Detector	: UV
Data Filename	: PR-YY-0091-LCMS-P1.lcd
Batch Filename	: 20220629.lcb
Date Acquired	: 6/29/2022 6:59:22 PM
Date Processed	: 6/30/2022 10:20:07 AM

<PDA Chromatogram>



<Peak Table>

PDA Ch1 220nm

Peak#	Ret. Time	Area	Area%
1	0.529	144452	4.988
2	1.003	2751405	95.012
Total		2895857	100.000

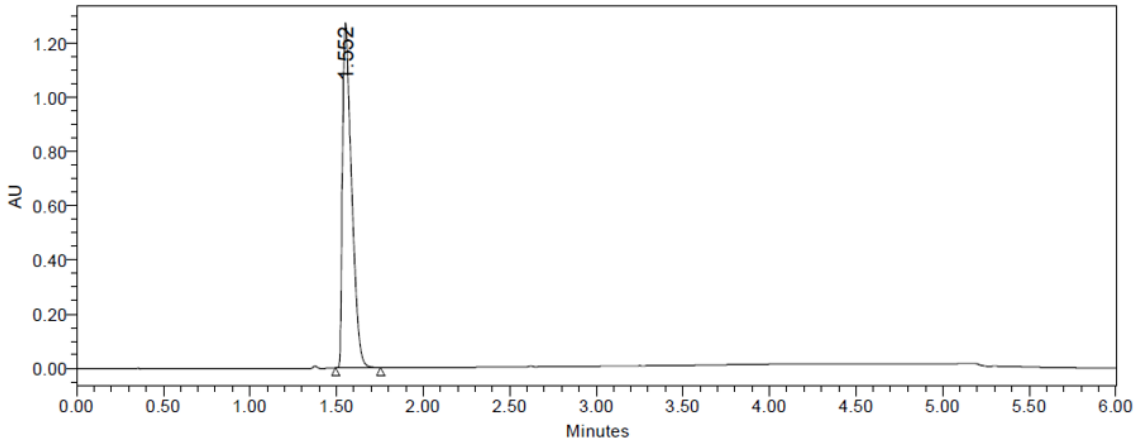
PDA Ch2 254nm

Peak#	Ret. Time	Area	Area%
1	0.522	36524	4.107
2	0.902	4529	0.509
3	1.004	848198	95.383
Total		889251	100.000

UPLC Spectrum for Final Compound **13**

SAMPLE INFORMATION

Sample Name:	ZXC-9-141-P	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	RA
Vial:	1:C,2	Acq. Method Set:	10to100Bin6min_noDelay
Injection #:	1	Processing Method:	MS_spec
Injection Volume:	5.00 ul	Channel Name:	254.0nm
Run Time:	6.0 Minutes	Proc. Chnl. Descr.:	PDA Spectrum PDA 254.0 nm
Date Acquired:	9/14/2023 7:26:52 PM EDT		
Date Processed:	9/14/2023 10:48:42 PM EDT		

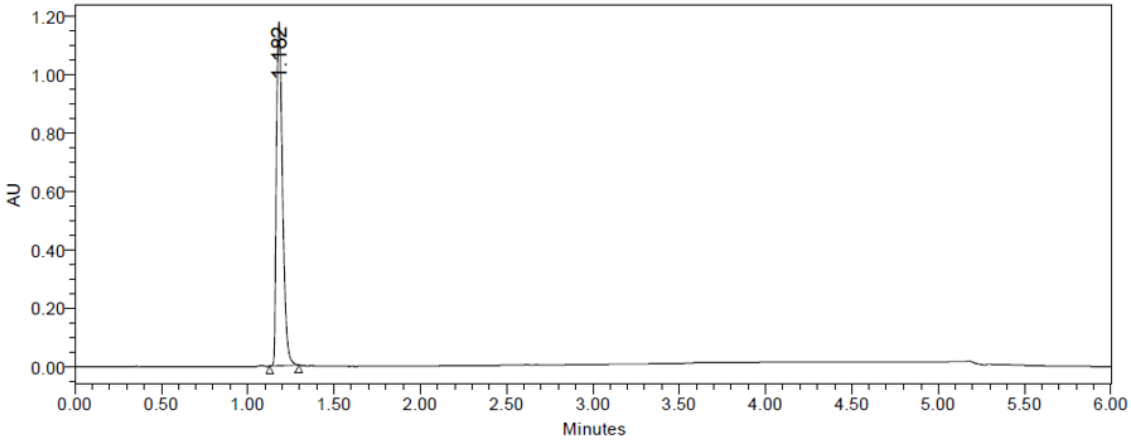


	RT	Area	% Area	Height
1	1.552	4659344	100.00	1269351

UPLC Spectrum for Final Compound **14**

SAMPLE INFORMATION

Sample Name:	ZXC-9-142-P	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	3
Vial:	1:C,5	Acq. Method Set:	10to100Bin6min_noDelay
Injection #:	1	Processing Method:	MS_spec
Injection Volume:	5.00 ul	Channel Name:	254.0nm
Run Time:	6.0 Minutes	Proc. Chnl. Descr.:	PDA Spectrum PDA 254.0 nm
Date Acquired:	9/15/2023 12:32:41 AM EDT		
Date Processed:	9/15/2023 12:57:08 AM EDT		



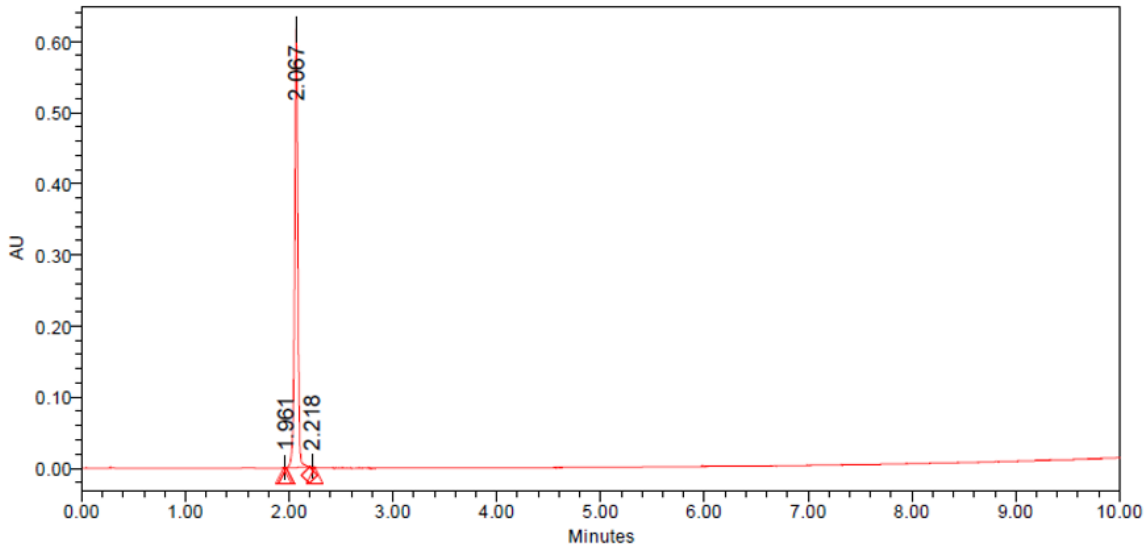
	RT	Area	% Area	Height
1	1.182	2946959	100.00	1177052

UPLC Spectrum for Final Compound **16**

SAMPLE INFORMATION

Sample Name:	ZXC-5-254-P	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	1
Vial:	1:A,2	Acq. Method Set:	10to10%Bin10Minutes_Delay5min
Injection #:	1	Processing Method	Default
Injection Volume:	9.00 ul	Channel Name:	254.0nm
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	PDA Spectrum PDA 254.0 nm
Date Acquired:	9/28/2023 2:22:03 PM EDT		
Date Processed:	9/28/2023 2:50:29 PM EDT		

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1		1.961	283	277		
2		2.067	1280810	617643		
3		2.218	2346	1073		

PDA Result Table

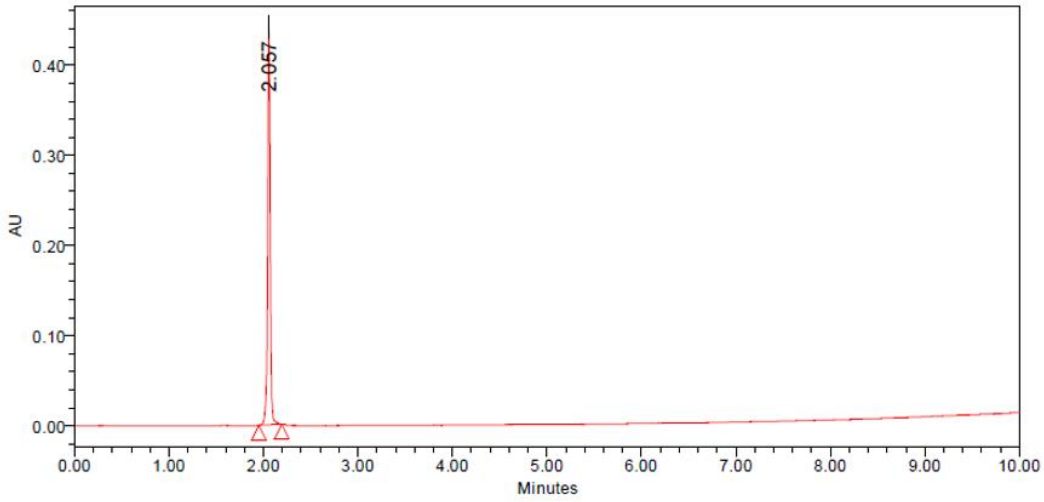
	Name	RT	Purity1 Angle	Purity1 Threshold	Match1 Spect. Name	Match1 Angle	Match1 Threshold
1		1.961					
2		2.067					
3		2.218					

UPLC Spectrum for Final Compound **16a** (PVTX-405)

SAMPLE INFORMATION

Sample Name:	ZXC-5-254A-P	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	1
Vial:	1:A,3	Acq. Method Set:	10to10%Bin10Minutes_Delay5min
Injection #:	1	Processing Method	Default
Injection Volume:	6.00 ul	Channel Name:	254.0nm
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	PDA Spectrum PDA 254.0 nm
Date Acquired:	9/28/2023 2:37:42 PM EDT		
Date Processed:	9/28/2023 6:28:38 PM EDT		

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1		2.057	827186	441994		

PDA Result Table

	Name	RT	Purity1 Angle	Purity1 Threshold	Match1 Spect. Name	Match1 Angle	Match1 Threshold
1		2.057					

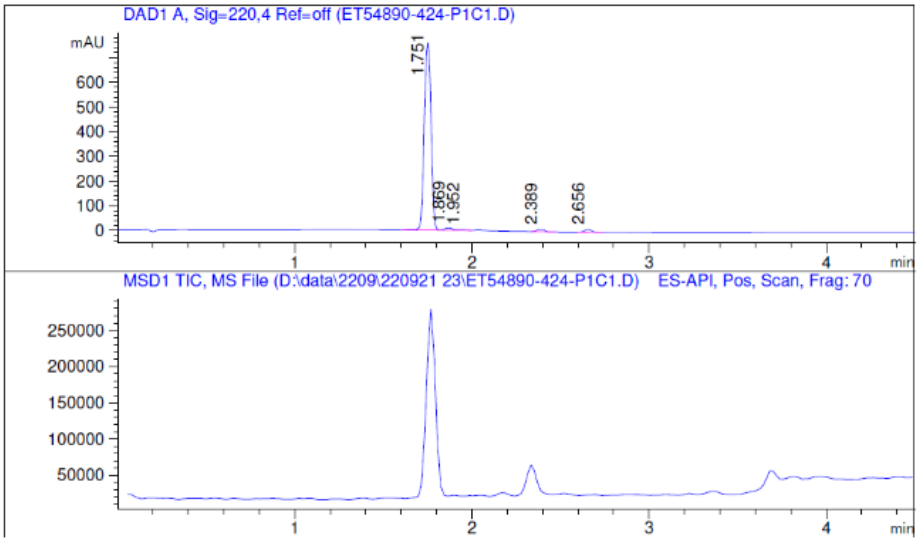
LC-MS Spectrum for Final Compound **16b**

LCMS REPORT

Compound ID : PVT-0005018
Sample ID : ET54890-424-P1C1
Injection Date : 21. Sep. 2022
Location : P1-A-02
Inj. Vol. : 1.00 uL
Acq Method : D:\data\2209\220921 23\5_95AB_6min-220.M
Data Filename : D:\data\2209\220921 23\ET54890-424-P1C1.D
Instrument : CR

Method Info : Instrument:Agilent 1260 HPLC MSD:6135B single quadrupole MSD
Column: Luna C18,2.0*50mm, 5μm
Column Temp: 40℃
Mobile Phase:A: 0.04%TFA in H2O
Mobile Phase:B: 0.02%TFA in ACN
Flow Rate: 0.8 ml/min

Time	B%	Flow(ml/min)
0.01	5	0.8
0.40	5	0.8
3.00	95	0.8
4.00	95	0.8
4.01	5	0.8
4.50	5	0.8



Report

Signal 1 : DAD1 A, Sig=220,4 Ref=off

Peak #	RT [min]	Height	Height %	Width [min]	Area	Area %
1	1.751	760.142	95.895	0.043	2009.173	95.201
2	1.869	9.856	1.243	0.051	32.949	1.561
3	1.952	3.284	0.414	0.054	12.347	0.585
4	2.389	9.189	1.159	0.044	25.540	1.210
5	2.656	10.215	1.289	0.047	30.447	1.443

UPLC Spectrum for Final Compound **16b**

HPLC REPORT

Compound ID : PVT-0005018
Filename/Sample ID: ET54890-424-P1C
Injection Date : 2022/9/21 13:39:01

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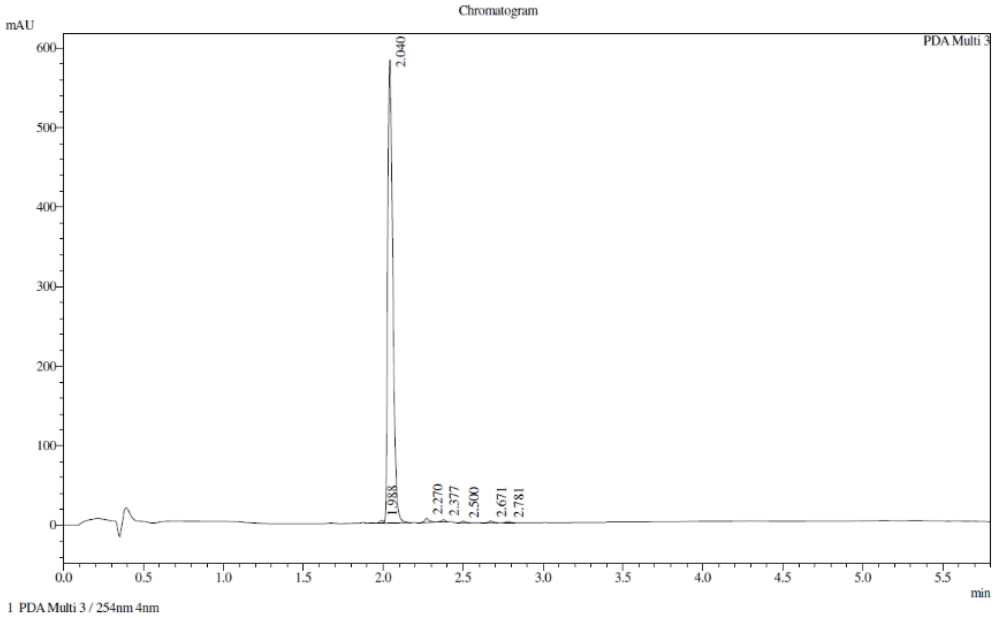
Method Info : Instrument: Shimadzu LC-20AB
Column:Kinetex C18 5um 50*2.1mm
Column Temp:40◆C
Mobile Phase:A:0.04%TFA in H2O
Mobile Phase:B:0.02%TFA in ACN
Flow Rate:0.5 ml/min

Time	B%	Flow(ml/min)
0.01	10	0.5
4.50	80	0.5
5.40	80	0.5
5.41	10	1.0
6.00	10	1.0



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Integration Result

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PDA Ch3 254nm 4nm

Peak#	Ret. Time	Height	Height %	USP Width	Area	Area %
1	1.988	3699	0.618	0.048	5531	0.422
2	2.040	581988	97.204	0.061	1281406	97.751
3	2.270	5247	0.876	0.052	10369	0.791
4	2.377	2906	0.485	0.054	5341	0.407
5	2.500	1783	0.298	0.047	2846	0.217
6	2.671	1972	0.329	0.048	3322	0.253
7	2.781	1135	0.189	0.054	2074	0.158