Supporting information for

Degradation of Intracellular TGF-β1 by PROTACs Efficiently Reverses M2 Macrophage Induced Malignant Pathological Events †

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1. Supplementary Figures

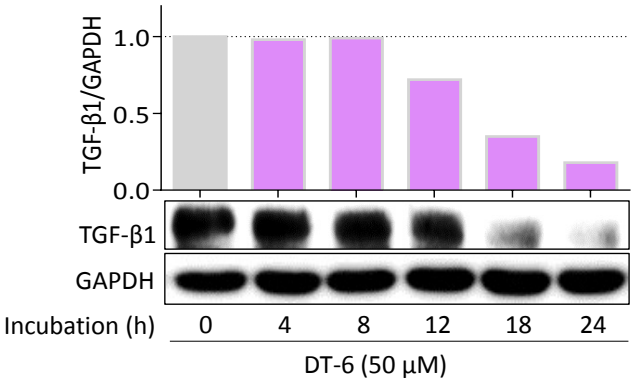


Figure S1. DT-6 time- dependently promotes the degradation of TGF-β1 in THP-1 cells by Western blot analysis.

2. Supplementary Methods

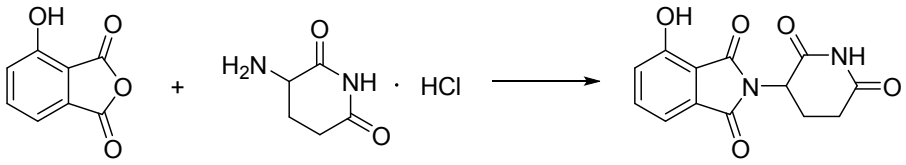
2.1 General

All commercial reagents and solvents were purchased from vendors and used without further purification or distillation. Chemical reagents were purchased from bidepharm(shanghai)Ltd. Resins, protected amino acids, and coupling regents were purchased from GL Biochem (shanghai)Ltd. ^{1}H NMR spectra were recorded with a Burker BioSpin Ultrashield 600 or 400 NMR system. HRMS spectra were recorded with a ThermoFisher Orbitrap-Fusion-Lumos system. The purity of peptides and PROTACs used for biological evaluation (>95%) were determined on a DIONEX Ultimate 3000 HPLC system (Chromeleon SR9 Build 2673): column, SHISEIDO C18, 5 μm, 4.6 × 250 mm. Separation conditions were: 1.0 mL min^{-1} flow rate, a linear gradient of 5% to 100% MeCN with 0.1% TFA in 40 min, washed with 100% for 5 min, and then calibrated at 5% for 10 min. All peptides and PROTACs were lyophilized and stored at -20°C before use. Compounds were dissolved in DMSO and diluted to the indication concentration by using the culture medium. MG132 (cat. No. BD140100)

was purchased from bidepharm(shanghai)Ltd, Galunisertib (cat. No. HY-13226) was purchased from MedChemExpress, thalidomide (cat. No. T126856) was purchased from Aladdin. Recombinant Human TGF-β1 (cat. No. HZ-1011) was purchased from Proteintech Group. Primary antibodies against TGF-β1 (Rabbit mAb, cat. No. AF1027), E-cadherin (Rabbit mAb, cat. No. AF0131), vimentin (Rabbit mAb, cat. No. AF7013), and GAPDH (Mouse mAb, cat. No. T0004) were purchased from Affinity. Primary antibodies against IKZF1 (Rabbit mAb, cat. No. 12016-1-AP), IKZF3 (Rabbit mAb, cat. No. 19055-1-AP) and CRBN (Mouse mAb, cat. No. 66336-1-Ig) were purchased from Proteintech Group. Primary antibody against SMAD2 (Mouse mAb, cat. No. sc-101153) was purchased from santa cruz biotechnology. Primary antibodies against p-SMAD2 (Rabbit mAb, cat. No. AB3849-1) and Ubiquitin (Mouse mAb, cat. No. MABS451) were purchased from Millipore. Primary antibody against TGF-β1 (Mouse mAb, cat. No. ab27969) was purchased from Abcam. Primary antibody against CRBN (Rabbit mAb, cat. No. 71810S) was purchased from cell signaling technology. Anti-Rabbit IgG (cat. No. A7016) and protein A+G agarose beads (cat. No. P2012) were purchased from Beyotime. Human Transforming Growth factor β1 (TGF-β1) ELISA kit was purchased from CUSABIO (cat. No. CSB E04725h). RIPI1640 and DMEM Medium, PBS, and fetal bovine serum (FBS) were purchased from GIBCO (cat. No. 42F8481K). Total RNA Kit was purchased from Beyotime (cat. No. R0016). The PCR primer sequences were purchased from Sangon Biotech. Cell lines including MCF-7: human breast adenocarcinoma cell line MCF7, human monocytic cell line THP-1, human lung adenocarcinoma cells line A549, human glioblastoma cell line U87, and human hepatocellular carcinoma cell line HepG2, were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA); mouse immortalized microglial cell line BV2 was obtained from the Cell Resource Center, Peking Union Medical College.

2.2 Synthesis of thalidomide derivate CAT[1]

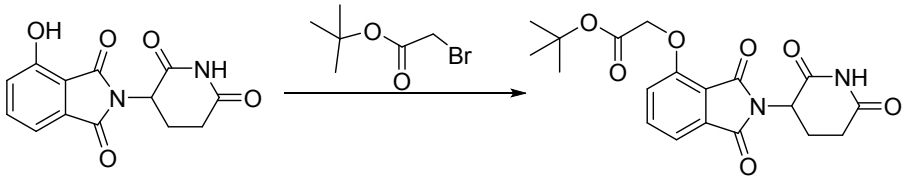
Synthesis of intermediate A:



A

In a round-bottom flask, 3-hydroxyphthalic anhydride (200 mg, 1.2 mmol, 1.0 *eq.*) and 3-aminoperidine-2, 6-dione hydrochloride (200 mg, 1.2 mmol, 1.0 *eq.*) were mixed in toluene (10 mL). TEA (183 µL, 1.32 mmol, 1.1 *eq.*) was added. The resulting reaction mixture was heated to reflux for 15 h with Dean-Stark trap equipment. After cooling to ambient temperature, evaporation of most of the solvent afforded a crude product, which was purified by flash column chromatography with DCM/MeOH to obtain the desired intermediate A as a slightly yellow solid (206 mg, 63% yield). ^{1}H NMR (400 MHz, DMSO) δ 11.16 (s, 1H), 11.08 (s, 1H), 7.66 (t, *J* = 7.8 Hz, 1H), 7.33 (d, *J* = 7.2 Hz, 1H), 7.26 (d, *J* = 8.4 Hz, 1H), 5.08 (dd, *J* = 12.9, 5.3 Hz, 1H), 2.94-2.85 (m, 1H), 2.62-2.47 (m, 2H), 2.06-2.00 (m, 1H).

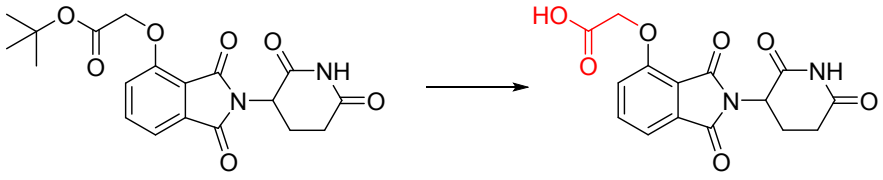
Synthesis of intermediate B:



A B

In a round-bottom flask, intermediate A (196 mg, 0.71 mmol, 1.0 *eq.*) was dissolved in DMF (5 mL). KI (11.6 mg, 0.07 mmol, 0.1 *eq.*) and $KHCO_3$ (106.6 mg, 1.06 mmol, 1.5 *eq.*) were added to the stirred solution. Then tert-butyl bromoacetate (124 µL, 0.85 mmol, 1.2 *eq.*) was added dropwise. The resulting mixture was stirred at 60°C for 4 h. The reaction solution was extracted three times with EtOAc/saturated brine, and the combined organic layer was dried over Na_2SO_4. After filtration and evaporation, the residue was purified by flash column chromatography with PE/EA to get the intermediate B as a white solid (201 mg, 73% yield). ^{1}H NMR (400 MHz, DMSO) δ 11.10 (s, 1H), 7.81 (t, *J* = 7.7 Hz, 1H), 7.49 (d, *J* = 7.2 Hz, 1H), 7.39 (d, *J* = 8.6 Hz, 1H), 5.11 (dd, *J* = 12.8, 5.2 Hz, 1H), 4.97 (s, 2H), 2.95-2.86 (m, 1H), 2.62-2.54 (m, 2H), 2.09-2.03(m, 1H), 1.44 (s, 9H).

Synthesis of intermediate thalidomine derivate CAT:



B CAT

The intermediate B (195 mg, 0.5 mmol, 1.0 *eq.*) was dissolved in TFA (2.0 mL). The reaction mixture was stirred at the room temperature for 2 h. After evaporation of the solvent, the residue was freeze-dried on a lyophilizer to afford thalidomine derivate CAT as a white solid (182 mg, 90% yield), which was used in the following steps without the further purification. ^{1}H NMR (400 MHz, DMSO) δ 11.10 (s, 1H), 7.80 (t, *J* = 7.9 Hz, 1H), 7.48 (d, *J* = 7.2 Hz, 1H), 7.40 (d, *J* = 8.6 Hz, 1H), 5.11 (dd, *J* = 12.8, 5.3 Hz, 1H), 5.00 (s, 2H), 2.95-2.86 (m, 1H), 2.62-2.54 (m, 2H), 2.06-1.99 (m, 1H).

2.3 Solid-phase synthesis of the peptides and PROTACs[2]

General Procedure for Peptide Synthesis:

The peptides were synthesized using standard Fmoc solid phase synthesis (SPSS) upon 2-Cl-Trityl resin, Thr, Ser, Leu, Asp, Ala, IIe, Trp, Met, Gln, and Asn were used with following protection form respectively: Fmoc-Thr(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Leu-OH, Fmoc-Asp(otBu)-OH, Fmoc-Ala-OH, Fmoc-IIe-OH, Fmoc-Trp(Boc)-OH, Fmoc-Met-OH, Fmoc-Gln(Trt)-OH,Fmoc-Asn(Trt)-OH. 1-Hydroxybenzotriazole (HOBt), 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate (HBTU), 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU), N,N-Diisopropylethylamine (DIEA) and Trifluoroacetic Acid (TFA) were used to obtain the peptides.

Attachment of the first amino acid: Prior to the first coupling step, 2-Chlorotrityl chloride resin (500 mg, loading 0.95 mmol/g, 1.0 *eq.*) was swollen in CH_2Cl_2 (DCM) for 30 min. The resin was added to a 25 mL round-bottle flask with a solution of Fmoc-protected amino acid (3.0 *eq.*), DIEA (10 *eq.*) and DCM (5 mL). The mixture was drained and washed with DCM (5×5 mL) after gently agitated for 2 h. The unreacted site on the resin were capped by a mixture of DCM /MeOH/DIEA (16:3:1, 10 mL) for

40 min. The resin was then washed with DMF (5×5 mL), and followed by Fmoc deprotection using 5% piperidine and 2% DBU in DMF for 20 min.

Stepwise Elongation: Chain elongation was performed with the solution of the Fmoc protected amino acid (3.0 *eq.*), HBTU (3.0 *eq.*), HOBt (3.0 *eq.*) and DIEA (6.0 *eq.*) for 2 h. Each deprotection and coupling step was followed by washing the resin with DMF (5×5 mL). The completeness of each step was checked with Kaiser test. Finally, the N-terminal amine was liberated by Fmoc deprotection using 5% piperidine and 2% DBU in DMF for 20 min, and followed by washing 3 times with DMF, MeOH, and DCM (5 mL, 3 min) respectively.

Cleavage from the Resin: Resin-bound peptide was treated with TFA/Phenol/i-Pr_3SiH/H_2O (88:5:5:2 10 mL) for 3 h. The filtrate was dried in vacuo to obtain the crude product. The crude product was then precipitated by cold ether and purified by reverse-phase HPLC and lyophilized to give the final product with purity >95%.

Synthesis of P144: Following the procedures described above, P144 [NH_2-Thr-Ser-Leu-Asp-Ala-Ser-Ile-Ile-Trp-Ala-Met-Met-Gln-Asn] was prepared, purified by reverse-phase HPLC and lyophilized to give a white powder. HPLC purity: 96.20%, t_R=17.834 min.^{1}H NMR (600 MHz, DMSO) δ 10.76 (s, 1H), 8.64 (d, *J* = 7.0 Hz, 1H), 8.24-8.21 (m, 2H), 8.11-7.97 (m, 8H), 7.90 (d, *J* = 6.8 Hz, 1H), 7.79 (d, *J* = 6.1 Hz, 2H), 7.70 (d, *J* = 6.6 Hz, 1H), 7.54 (d, *J* = 7.9 Hz, 1H), 7.40 (s, 1H), 7.31 (d, *J* = 8.1 Hz, 1H), 7.18 (s, 1H), 7.12 (s, 1H), 7.04 (t, *J* = 7.4 Hz, 1H), 6.96 (t, *J* = 7.5 Hz 1H), 6.92 (s, 1H), 6.76 (s, 1H), 5.56 (s, 1H), 5.17 (s, 1H), 5.01 (s, 1H), 4.53-4.48 (m, 3H), 4.42 (dd, *J* = 13.3, 6.2 Hz, 1H), 4.38-4.19 (m, 7H), 4.17-4.15 (m, 1H), 4.09-4.05 (m, 1H), 3.83-3.81 (m, 1H), 3.66-3.58 (m, 5H), 3.10-3.09 (m, 1H), 2.98-2.94 (m, 1H), 2.72 (dd, *J* = 16.3, 5.0 Hz 1H), 2.56-2.39 (m, 4H), 2.12-2.09 (m, 2H), 2.02 (s, 3H), 2.01 (s, 3H), 1.93-1.60 (m, 10H), 1.46-1.34 (m, 4H), 1.24 (s, 1H), 1.20-1.16 (m, 9H), 1.05-1.01 (m, 3H), 0.87-0.86 (d, *J* = 6.5, 3H), 0.83-0.82 (d, *J* = 6.5, 3H), 0.79-0.71 (m, 12H). HRMS: calcd for $C_{68}H_{109}N_{17}O_{22}S_2$, $[M+2H]^{2+}$ m/z 790.8687, found, 790.8738.

Synthesis of conjugates: Following the attachment and elongation procedures described above, we first obtained the resin-bound, side chain protected peptide: NH_2-Thr(tBu)-Ser(tBu)-Leu-Asp(OtBU)-Ala-Ser(tBu)-Ile-Ile-Trp(Boc)-Ala-Met-Met-

Gln(Trt)-Asn(Trt)-Resin; then this resin-bound peptide was treated as following to yield different conjugates.

For **DT-0:** The resin-bound peptide was added a solution of thalidomine derivate CAT (3.0 *eq.*), HBTU (3.0 *eq.*), HOBt (3.0 *eq.*) and DIEA (10 *eq.*) for 6 h, then it was cleaved from the resin and purified by reverse-phase HPLC and lyophilized to give DT-0 as a white powder. HPLC purity: 99.18%, t_R=20.033 min. ^{1}H NMR (600 MHz, DMSO) δ 11.13 (s, 1H), 10.75 (s, 1H), 8.24-7.92 (m, 10H), 7.89 (d, *J* = 7.6 Hz, 1H), 7.84-7.74 (m, 3H), 7.69 (d, *J* = 7.7 Hz, 1H), 7.54 (d, *J* = 7.9 Hz, 1H), 7.49 (d, *J* = 7.2 Hz, 1H), 7.44 (d, *J* = 8.6 Hz, 1H), 7.39 (s, 1H), 7.31 (d, *J* = 8.1 Hz, 1H), 7.17 (s, 1H), 7.12 (s, 1H), 7.04 (t, *J* = 7.5 Hz, 1H), 6.96 (t, *J* = 7.4 Hz, 1H), 6.92 (s, 1H), 6.75 (s, 1H), 5.12 (dd, *J* = 12.8, 5.4 Hz, 1H), 4.90 (d, *J* = 3.7 Hz, 2H), 4.55-4.46 (m, 3H), 4.39-4.21 (m, 9H), 4.18-3.97 (m, 3H), 3.68-3.55 (m, 5H), 3.12-3.06 (m, 1H), 2.99-2.87 (m, 2H), 2.72 (dd, *J* = 16.7, 5.3 Hz, 1H), 2.64-2.55 (m, 2H), 2.54-2.36 (m, 4H), 2.13-2.09 (m, 2H), 2.07-1.98 (m, 7H), 1.97-1.55 (m, 10H), 1.49-1.32 (m, 4H), 1.30-1.19 (m, 8H), 1.09-0.99 (m, 4H), 0.86 (d, 3H), 0.82 (d, *J* = 6.3 Hz, 3H), 0.78-0.71 (m, 12H). HRMS: calcd for $C_{83}H_{119}N_{19}O_{28}S_2$, $[M+2H]^{2+}$ m/z 947.8956, found, 948.4108.

For **DT-3:** The resin-bound peptide was added a solution of Fmoc-β-Ala-OH (3.0 *eq.*), HBTU (3.0 *eq.*), HOBt (3.0 *eq.*) and DIEA (10 *eq.*) for 2 h, and then the N-terminal amine was liberated by Fmoc deprotection. Subsequently, the resin was added a solution of thalidomine derivate CAT (3.0 *eq.*), HBTU (3.0 *eq.*), HOBt (3.0 *eq.*) and DIEA (10 *eq.*) for 6 h. Finally, the peptide was cleaved from the resin and purified by reverse-phase HPLC and lyophilized to give DT-3 as a white powder. HPLC purity: 96.69% , t_R=19.625 min.^{1}H NMR (600 MHz, DMSO) δ 11.13 (s, 1H), 10.76 (s, 1H), 8.16-7.70 (m, 16H), 7.52 (dd, *J* = 25.1, 7.5 Hz, 2H), 7.40-7.36 (m, 2H), 7.31 (d, *J* = 8.1 Hz, 1H), 7.17 (s, 1H), 7.13 (s, 1H), 7.04 (t, *J* = 7.5 Hz, 1H), 6.96 (t, *J* = 7.4 Hz, 1H), 6.92 (s, 1H), 6.75 (s, 1H), 5.12 (dd, *J* = 12.9, 5.4 Hz, 1H), 4.78 (s, 2H), 4.52-4.48 (m, 3H), 4.31-4.23 (m, 9H), 4.14-3.95 (m, 3H), 3.68-3.36 (m, 5H), 3.11 (d, *J* = 11.2 Hz, 1H), 2.99-2.87 (m, 2H), 2.74-2.70 (m, 1H), 2.62-2.55 (m, 2H), 2.51-2.35 (m, 6H), 2.13-2.09 (m, 2H), 2.07-1.98 (m, 7H), 1.96-1.58 (m, 10H), 1.48-1.31 (m, 5H), 1.24-1.14 (m, 8H), 1.08-0.97 (m, 5H), 0.86 (d, *J* = 6.4 Hz, 3H), 0.82 (d, *J* = 6.4 Hz, 3H), 0.79-0.71 (m, 12H).

HRMS: calcd for $C_{86}H_{124}N_{20}O_{29}S_2$, $[M+2H]^{2+}$ m/z 983.4142, found, 983.9197.

For **DT-6:** The resin-bound peptide was added a solution of Fmoc-N-6-aminohexanoic acid (3.0 *eq.*), HBTU (3.0 *eq.*), HOBt (3.0 *eq.*) and DIEA (10 *eq.*) for 6 h, and then the N-terminal amine was liberated by Fmoc deprotection. Subsequently, the resin was added a solution of thalidomine derivate CAT (3.0 *eq.*), HBTU (3.0 *eq.)*, HOBt (3.0 *eq.*) and DIEA (10 *eq.*) for 6 h. Finally, the peptide was cleaved from the resin and purified by reverse-phase HPLC and lyophilized to give DT-6 as a white powder. HPLC purity: 98.29% , t_R=20.058 min.^{1}H NMR (600 MHz, DMSO) δ 11.13 (s, 1H), 10.76 (s, 1H), 8.14-7.69 (m, 16H), 7.52 (dd, *J* = 22.4, 7.6 Hz, 2H), 7.39 (d, *J* = 8.7 Hz, 2H), 7.31 (d, *J* = 8.1 Hz, 1H), 7.17 (s, 1H), 7.13 (s, 1H), 7.04 (t, *J* = 7.5 Hz, 1H), 6.96 (t, *J* = 7.4 Hz, 1H), 6.92 (s, 1H), 6.75 (s, 1H), 5.13 (dd, *J* = 12.9, 5.4 Hz, 1H), 4.77 (s, 2H), 4.52-4.48 (m, 3H), 4.34-4.23 (m, 9H), 4.16-3.97 (m, 3H), 3.68-3.56 (m, 3H), 3.15-3.10 (m, 3H), 2.99-2.87(m, 2H), 2.72 (dd, *J* = 16.5, 5.0 Hz, 1H), 2.62-2.55 (m, 2H), 2.53-2.38 (m, 4H), 2.23-2.17 (m, 2H), 2.14-2.09 (m, 2H), 2.06-2.00 (m, 7H), 1.97-1.56 (m, 9H), 1.52-1.34 (m, 9H), 1.23 (m, 11H), 1.05-1.00 (m, 5H), 0.86 (d, *J* = 6.6 Hz, 3H), 0.82 (d, *J* = 6.5 Hz, 3H), 0.80-0.67 (m, 12H). HRMS: calcd for $C_{89}H_{130}N_{20}O_{29}S_2$, $[M+2H]^{2+}$ m/z 1004.4377, found, 1004.9431.

2.4 Cell Culture[3, 4]

THP-1 cells, HepG2 cells, A549 cells, U87 cells, BV2 cells and MCF-7 cells were cultured in RPMI1640 or DMEM medium supplemented with 100 μg/mL penicillin, 100 μg/mL streptomycin and 10% heat-inactivated fetal bovine serum (FBS). Then, the cells were incubated in a 5% CO_2 humidified incubator at 37°C typically passaged with sub-cultivation ratio of 1:3 every 2-3 days.

2.5 Western blot assay[5]

HepG2 cells, A549 cells, U87 cells, BV2 cells and MCF-7 cells were seeded in 6-well plates at a density of 2 x 10^5 per well, and THP-1 cells were cultured on 6-well plates at a density of 5 x 10^5 per well. Cells were washed twice with PBS after being treated

with different conditions, and then cells were lysed on ice using 50 μL radio immunoprecipitation assay (RIPA) buffer containing protease inhibitors. The samples were centrifuged (12000 rpm, 4°C) for 30 min, and then supernatants were collected to obtain the total protein. Total protein was quantified using BCA protein Assay Kit. Protein was solubilized with 4 μL 5x loading buffer containing 5% β-mercaptoethanol and boiled for 10 min. Samples were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, and then transferred to polyvinylidene difluoride membranes. The membranes were blocked for 2 h at room temperature in Tris-buffered saline containing 0.1% Tween 20 (TBST) and 5% non-fat milk, and then incubated overnight at 4°C with primary antibodies against TGF-β1 (1:1000), E-cadherin (1:1000), vimentin (1:1000), IKZF1 (1:1000), IKZF3 (1:1000), SMAD2 (1:800), p-SMAD2 (1:800), CRBN (1:1000), Ubiquitin (1:1000) and GAPDH (1:5000, as the internal reference for equality of sample loading) in Western primary antibodies diluent (Beyotime, AZ050). After that, the membranes were detected with the secondary antibody (1:10000, DingGuo BioTechnology, IH-0031/IH-0011). The images were obtained with an ELC imager, and signal intensities were quantified using the Image J softwar. The quantification results were normalized to the control or the group without CM.

2.6 Co-immunoprecipitation (Co-IP)[6]

$1x10^6$ HepG2 cells were treated with 5 μM DT-6, P144 and Thal for 4 h, then cells were lysed on ice using 500 μL radio immunoprecipitation assay (RIPA) buffer containing protease inhibitors. The samples were centrifuged (12000 rpm, 4°C) for 30 min, and then supernatants were collected to obtain the total protein. Total protein was quantified using BCA protein Assay Kit. Cellular lysate (1 mg) was used to immunoprecipitate. After pre-clearing with protein A+G agarose beads for 1 h, the cell lysate was incubated with anti-CRBN or anti-TGF-β1 overnight at 4℃, then protein A+G agarose beads were added for another 3 h. The beads were washed with PBS at least 5 times, and boiled with SDS loading buffer for 5 min, then Western

blotting was conducted to examine CRBN, TGF-β1 or ubiquitin.

2.7 M2 macrophage induction[7]

THP-1 cells were seeded and incubated with 25 ng/mL PMA for 36 h, followed by washing-out for 24 h with the drug-free medium. Cells were further cultured with 20 ng/mL interleukin (IL)-4 for 36 h to induce M2 polarization. The mRNA levels of M2 macrophage biomarkers CD68 and CD163 were greatly up-regulated as determined by qRT-PCR, suggesting that M2 phenotype macrophage was successfully induced.

2.8 Quantitative real-time PCR (qRT-PCR)[8]

$5x10^6$ induced THP-1 cells were collected. Total RNA was extracted using the TRIzol method, and then the concentration of RNA was quantified using nanodrop 2000. Total RNA (2 μg) was reverse transcribed into cDNA using TransScript® Reverse Transcriptase kit (YEASEN Biotech), and amplification and detection were implemented using with an iCycler (Bio-Rad, Hercules, USA) using primers and SYBR® Green based qPCR kit (YEASEN Biotech). The threshold cycle (CT) was used to quantify the transcript levels of genes, and results were normalized according to the GAPDH mRNA expression. The quantification results of CD68 and CD163 were normalized to the group of THP-1 cells. The primers sequences are as follows:

Gene	Forward Primer	Reverse Primer
CD68	5’-GGGAATGACTGTCCTCACAAA-3’	5’-GTGGTTTTGTGGCTCTTGGTA-3’
CD163	5’-ACTTGAAGACTCTGGATCTGCT-3’	5’-CTGGTGACAAAACAGGCACTG-3’
GAPDH	5’-GCACCGTCAAGGCTGAGAAC-3’	5’-TGGTGAAGACGCCAGTGGA-3’

2.9 Enzyme-linked immunosorbent assay (ELISA)[8]

M2 macrophages were seeded in 6-well plates at a density of 4×10^6 per well, and then treated with different compounds or not for 24 h. The secreted TGF-β1 in the

medium was detected by ELISA kit (CUSABIO) according to the instructions.

2.10 Wound healing assay[9]

The scratch was made across the monolayer in HepG2 cells. Cells were cultured on 6-well plates at a density of $5x10^5$ per well, then a 10-μL sterile plastic pipette tip was used to wound cell monolayers when cells reaching about 85% confluent. The cells were washed twice with phosphate-buffered saline (PBS), then cultured for different times in the presence of different CM or TGF-β1 (10 ng/mL). For each scratch, photographs were taken at 0, 24, and 48 h using EVOS FL Color Imaging System. The quantification results of migration ratio in every time point were normalized to the control group without addition of CM and TGF-β1.

3. Supplementary References

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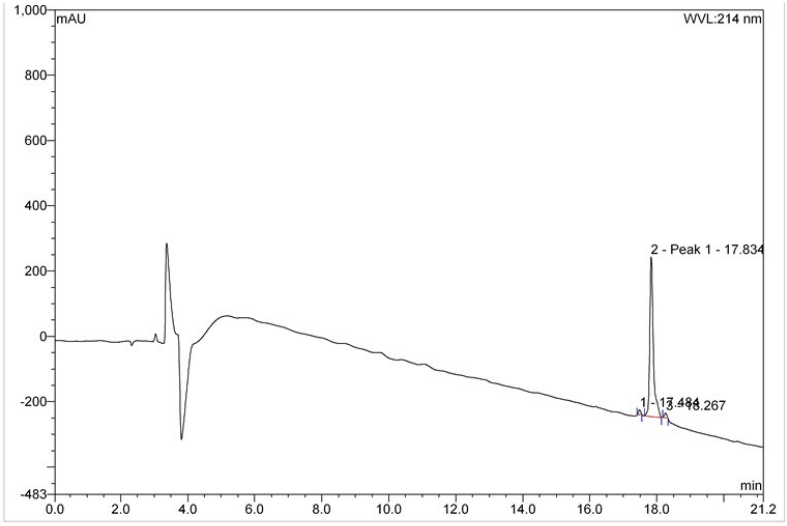
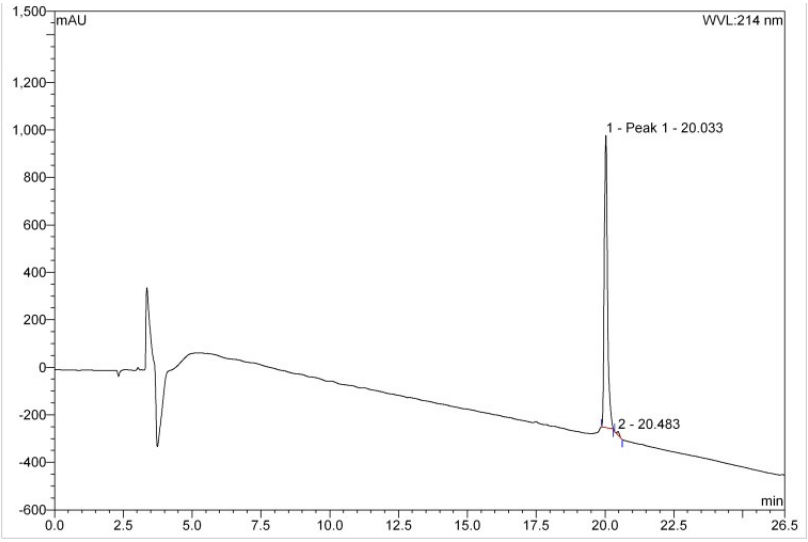
4. Supplementary HPLC, ^{1}HNMR and HRMS spectra

HPLC purity spectra of P144:



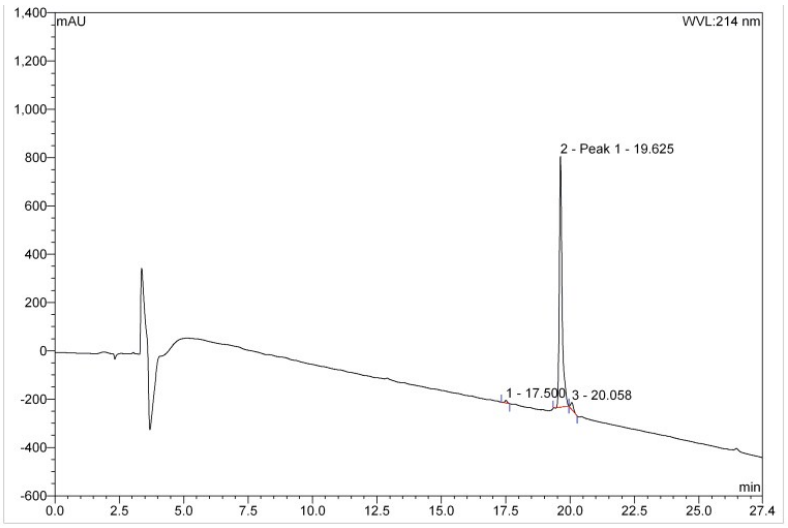
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	17.48	n.a.	14.807	1.155	1.85	n.a.	BMB*
2	17.83	Peak 1	488.797	60.052	96.20	n.a.	BMB*
3	18.27	n.a.	14.440	1.220	1.95	n.a.	BMB*
Total:			518.044	62.427	100.00	0.000	

HPLC purity spectra of DT-0:



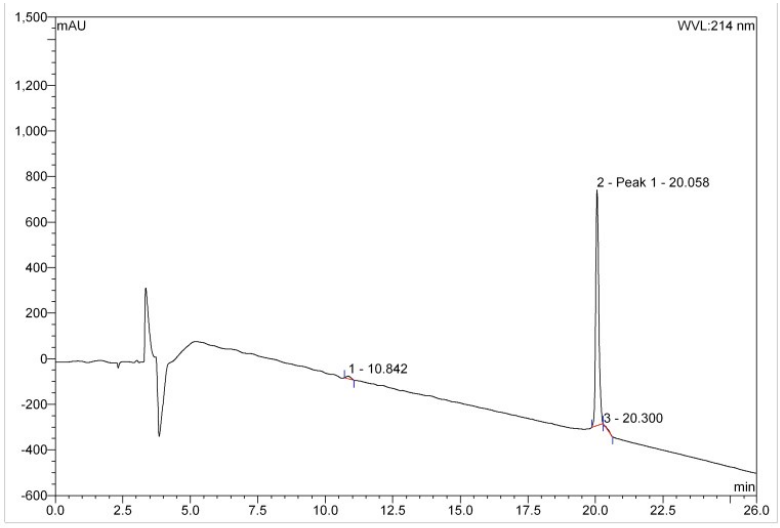
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	20.03	Peak 1	1230.079	163.363	99.18	n.a.	BMB*
2	20.48	n.a.	15.306	1.348	0.82	n.a.	BMB*
Total:			1245.385	164.711	100.00	0.000	

HPLC purity spectra of DT-3:



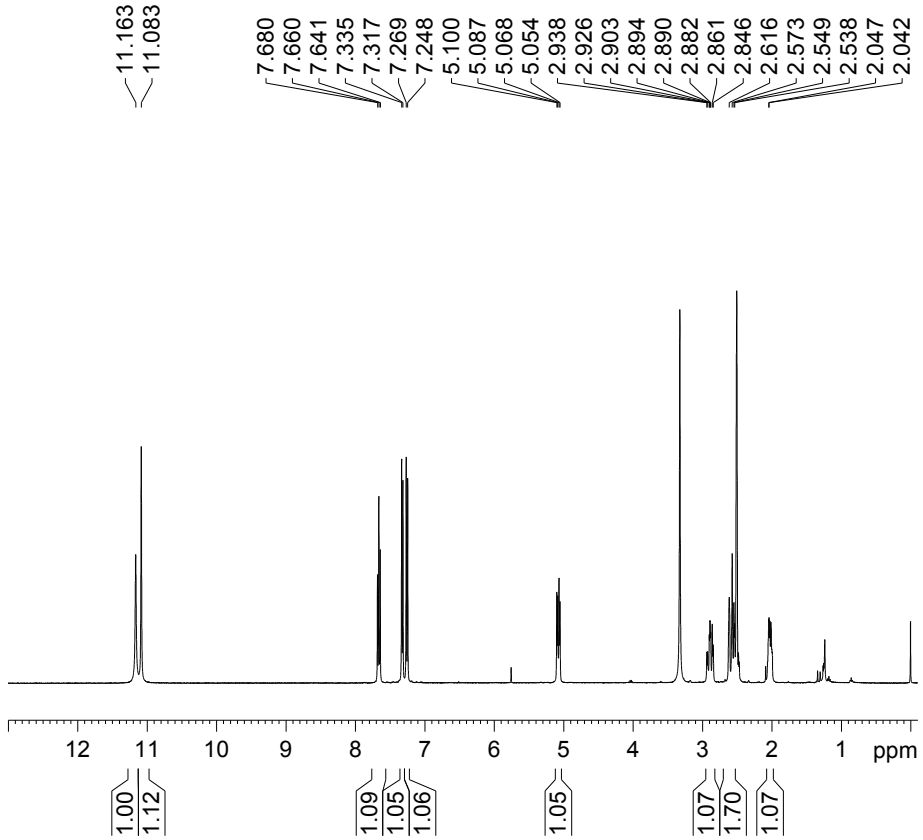
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	17.50	n.a.	10.889	1.089	0.76	n.a.	BMB*
2	19.63	Peak 1	1038.819	138.226	96.69	n.a.	BMb*
3	20.06	n.a.	29.206	3.643	2.55	n.a.	bMB*
Total:			1078.914	142.958	100.00	0.000	

HPLC purity spectra of DT-6:

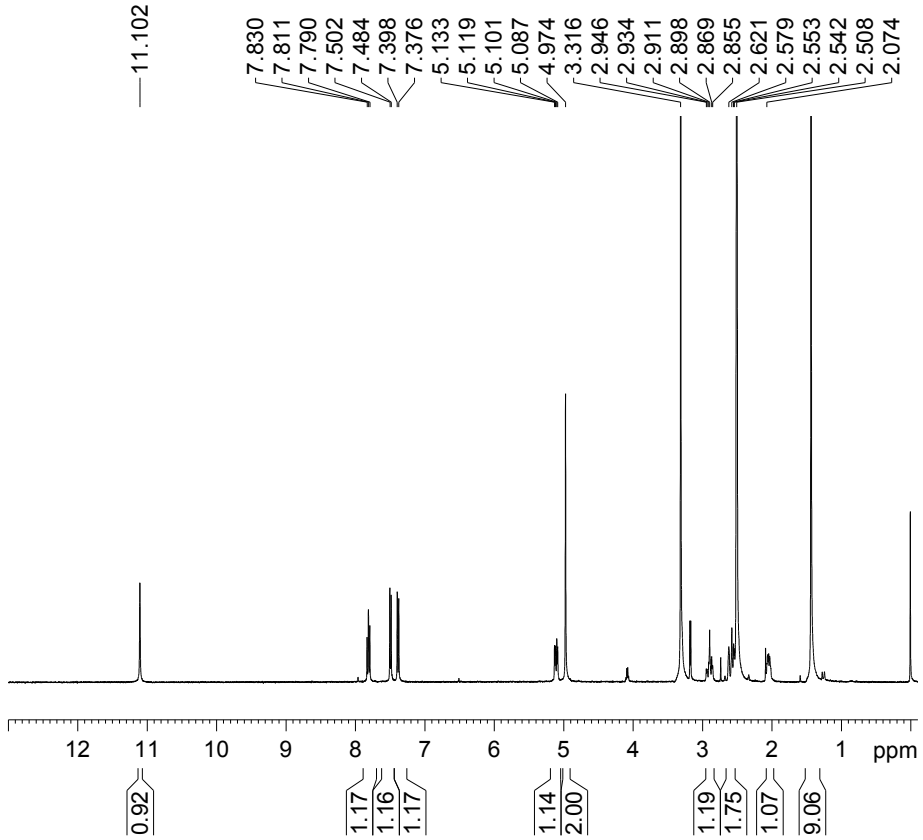


No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	10.84	n.a.	10.523	2.164	1.57	n.a.	BMB*
2	20.06	Peak 1	1032.898	135.362	98.29	n.a.	BMb*
3	20.30	n.a.	5.851	0.185	0.13	n.a.	bMB*
Total:			1049.272	137.710	100.00	0.000	

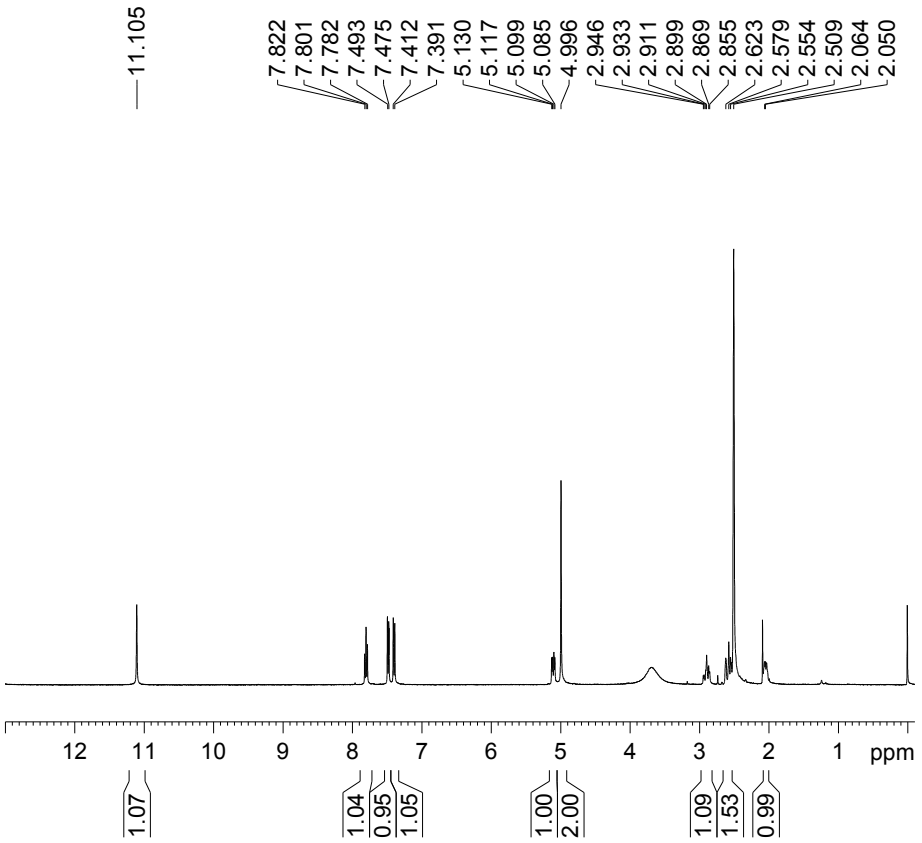
^{1}HNMR for intermediate A:



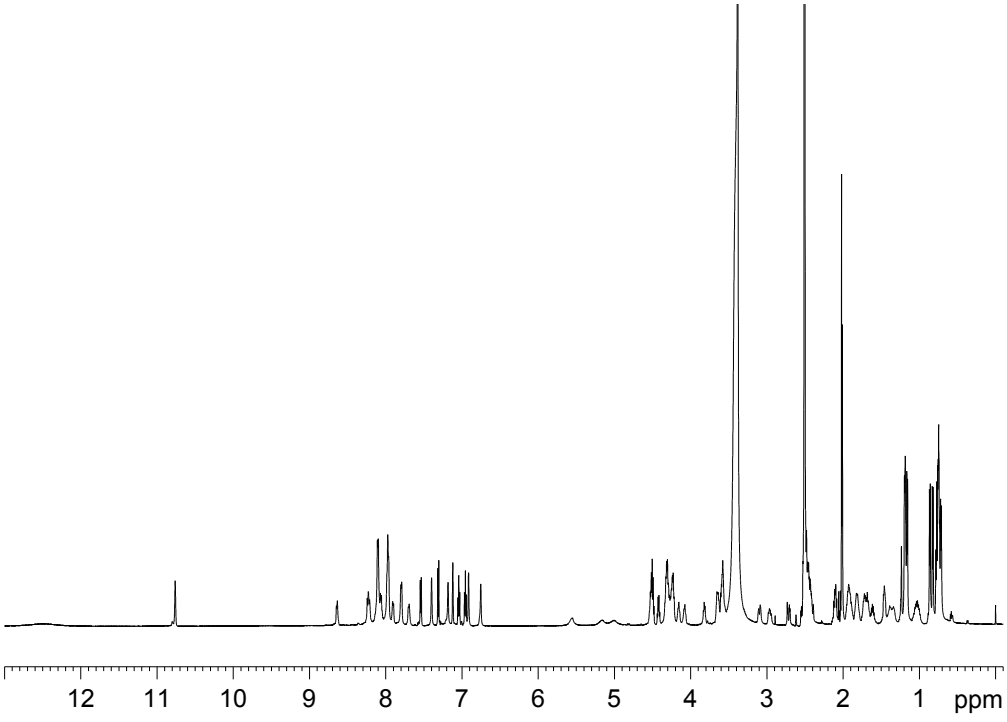
^{1}HNMR for intermediate B:



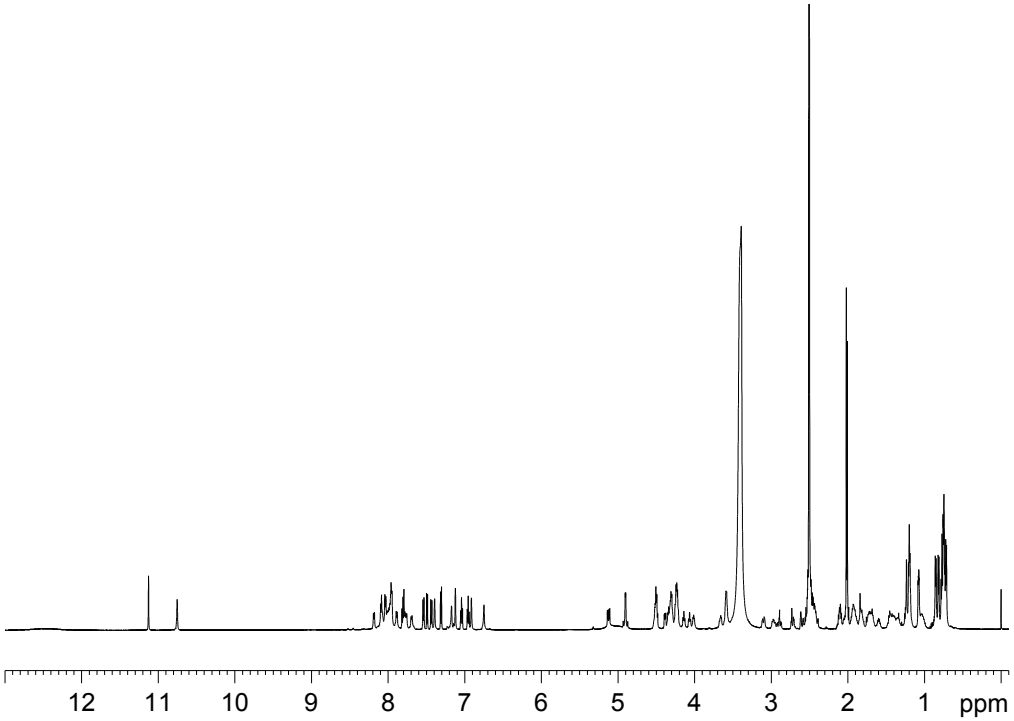
[1]HNMR for thalidomine derivate CAT:



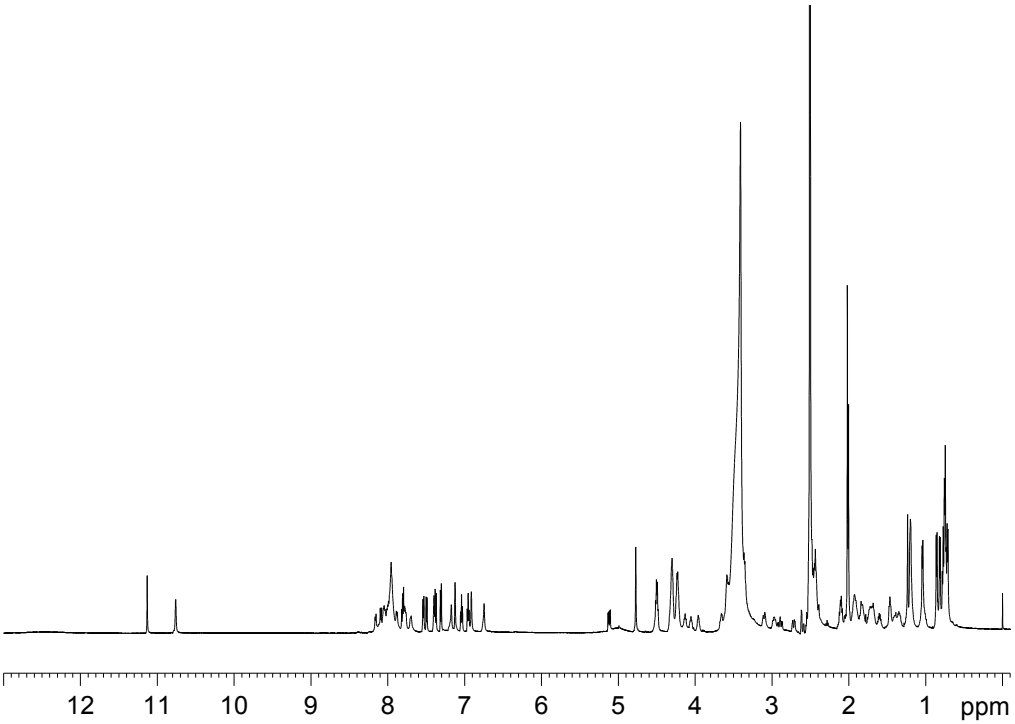
[1]HNMR for P144:



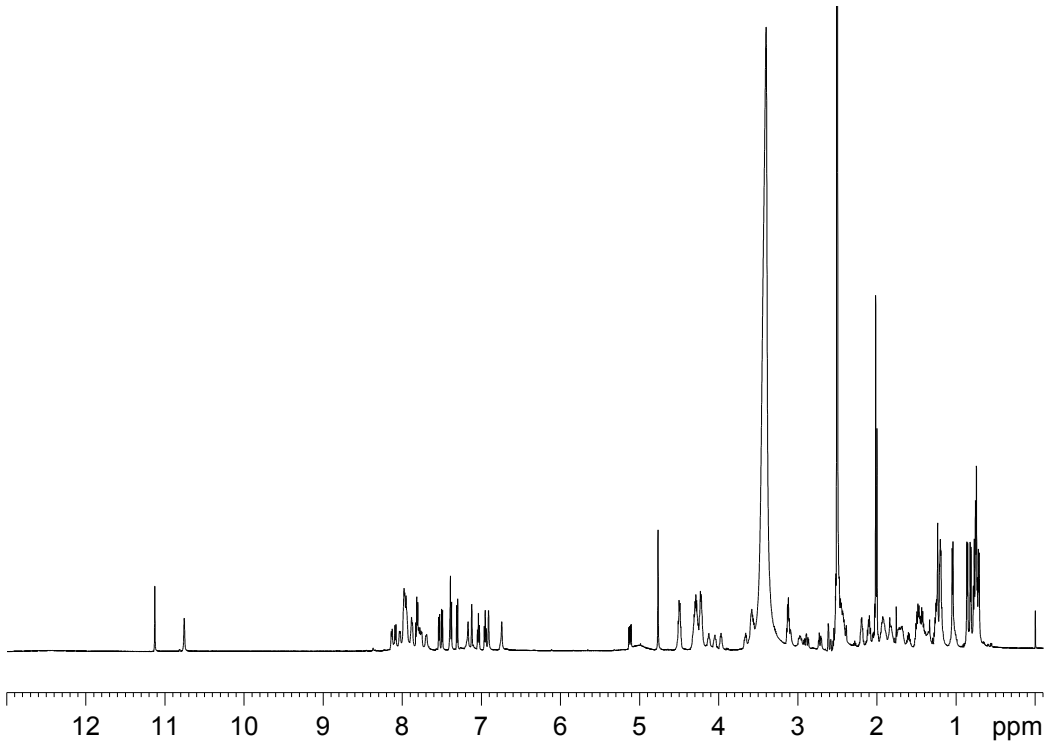
^{1}HNMR for DT-0:



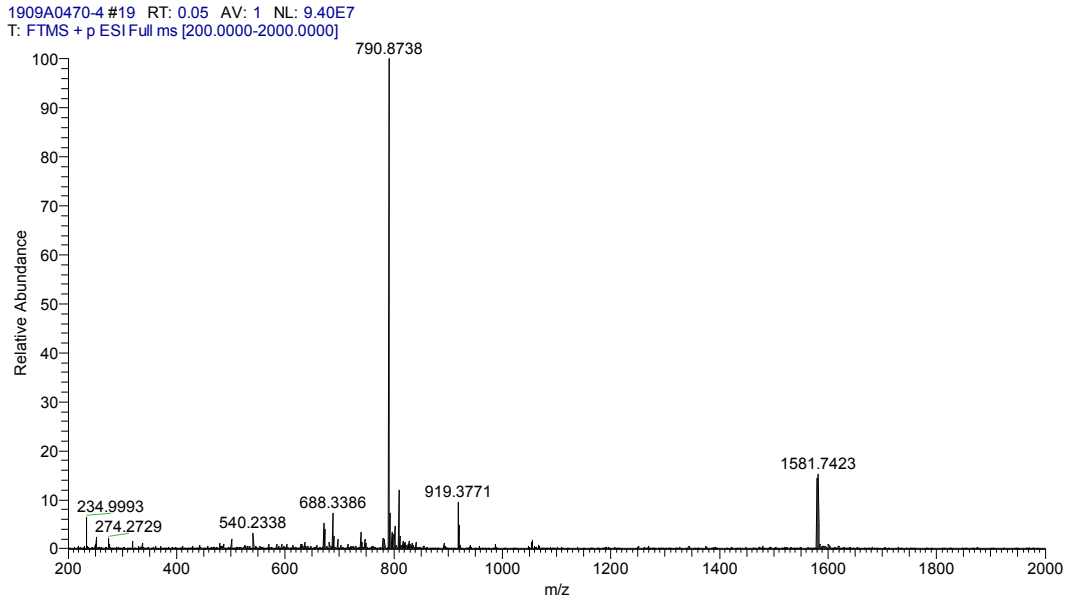
^{1}HNMR for DT-3:



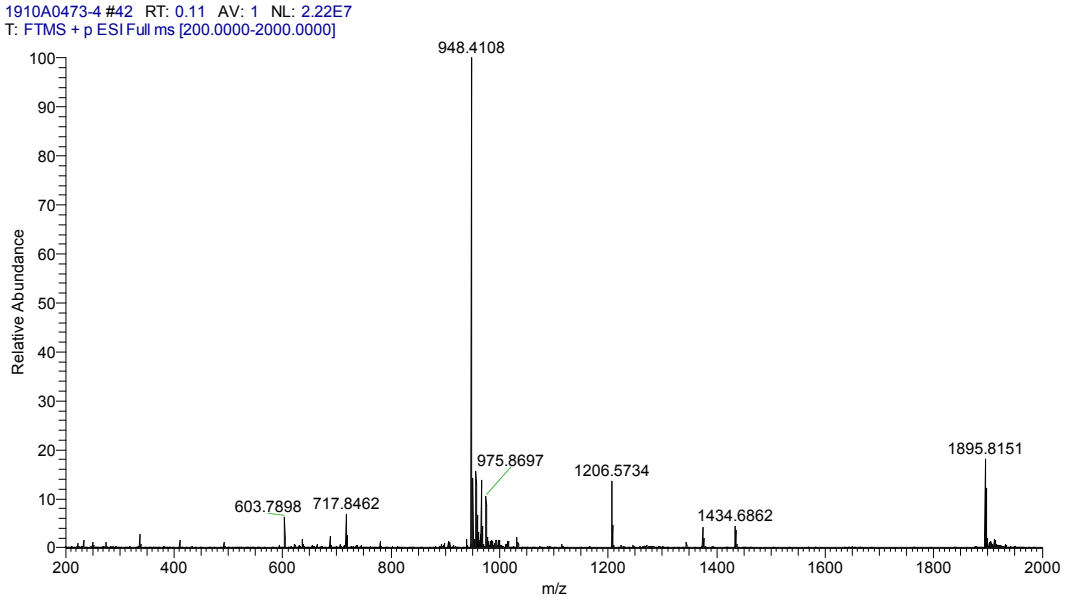
^{1}HNMR for DT-6:



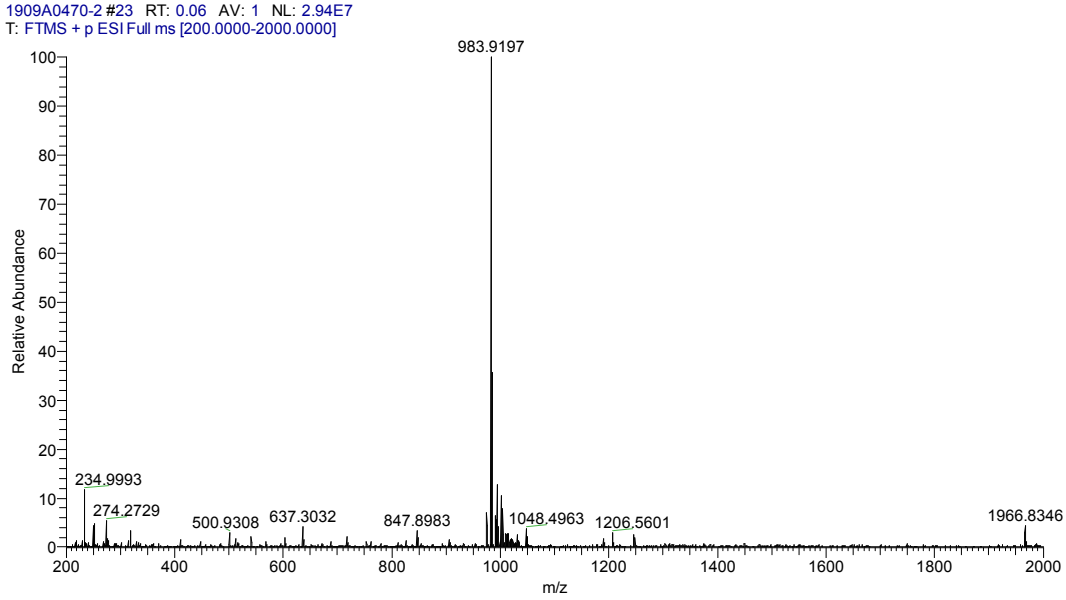
ESI mass spectra of P144:



ESI mass spectra of DT-0:



ESI mass spectra of DT-3:



ESI mass spectra of DT-6: