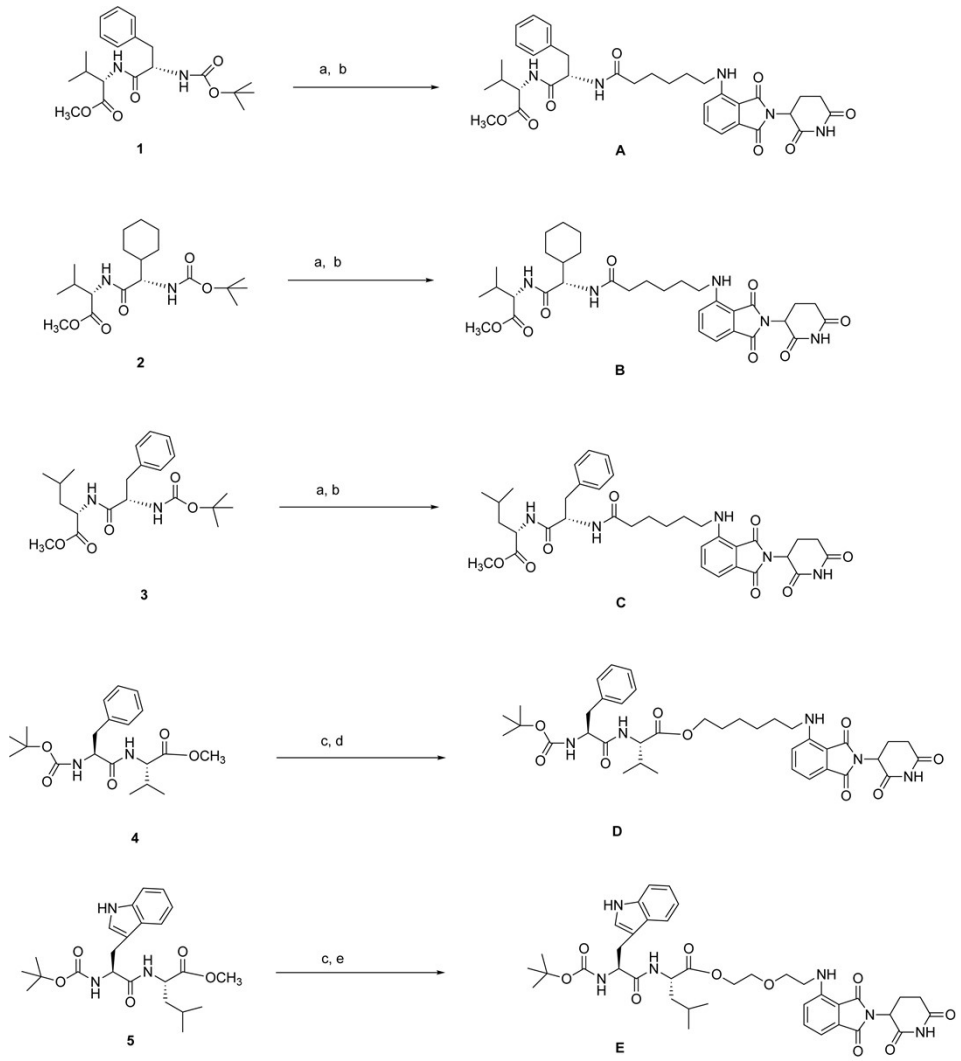
Supplementary Information

Synthesis of PROTACs(A-E)

All reagents and solvents were purchased from commercial suppliers and used without further purification. Analytical thin layer chromatography was performed on precoated silica gel F254 TLC plates with visualization under UV light or by iodine staining. Column chromatography was conducted on silica (Merck Silica Gel 40–63 m). NMR spectra were recorded using a Bruker 600 MHz NMR spectrometer.

Synthesis procedures of A, B, C, D and E, see scheme 1. We adopted a convergent synthesis method to prepare target molecules. Firstly, dipeptide intermediates 1-5 and thalidomide with linkers 6-8 were obtained respectively. Compound 1-5 were hydrolyzed and then condensed with 6-



8 to prepare A-E.

Scheme 1 a. HCl, dioxane; b. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), 4-Dimethylaminopyridine, CH_2Cl_2, **6**; c. LiOH, H_2O_2, Tetrahydrofuran（THF）, H_2O; d. EDCI, N-Hydroxy succinimide (NHS), CH_2Cl_2, N, N-Diisopropylethylamine (DIPEA), **7**; e EDCI, NHS, CH_2Cl_2, DIPEA, **8**.

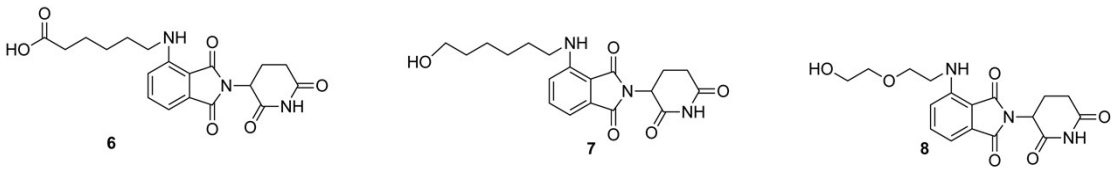


Fig. S1. Structures of Key intermedia 6-8

PROTAC A, ^{1}H NMR (600 MHz, DMSO) δ 11.12 (s, 1H), 8.78 – 8.47 (m, 1H), 8.22 (d, *J* = 7.9 Hz, 1H), 8.04 (d, *J* = 8.2 Hz, 1H), 7.58 (t, *J* = 7.6 Hz, 1H), 7.26 – 7.24 (m, 4H), 7.06 – 7.02 (m, 2H), 5.07 – 5.05 (m, 1H), 4.68 – 4.65 (m, 1H), 4.20 (t, *J* = 6.6 Hz, 1H), 3.63 (s, 3H), 3.23 – 3.17 (m, 2H), 3.00 – 2.98 (m, 1H), 2.91 – 2.87 (m, 1H), 2.76 – 2.70 (m, 1H), 2.61 – 2.58 (m, 1H), 2.05 – 2.03 (m, 4H), 1.53 – 1.44 (m, 2H), 1.44 – 1.37 (m, 2H), 1.15 (bs, 2H), 0.90 – 0.87 (m, 8H). **^{13}C NMR (151 MHz, DMSO)** δ 173.28, 172.45, 172.38, 172.29, 172.12, 170.56, 169.41, 167.77, 161.70, 151.54, 146.83, 138.38, 136.73, 132.64, 129.64, 128.36, 126.59, 121.16, 117.55, 110.84, 109.47, 57.84, 56.21, 53.79, 52.16, 49.01, 42.20, 37.91, 35.49, 31.46, 30.40, 28.85, 26.14, 25.39, 22.63, 19.37, 18.66. **HR-MS(ESI) *m/z*:** Calcd for $C_{34}H_{41}N_5O_8Na$ [M+Na^+] 678.2853, found 670.2857.

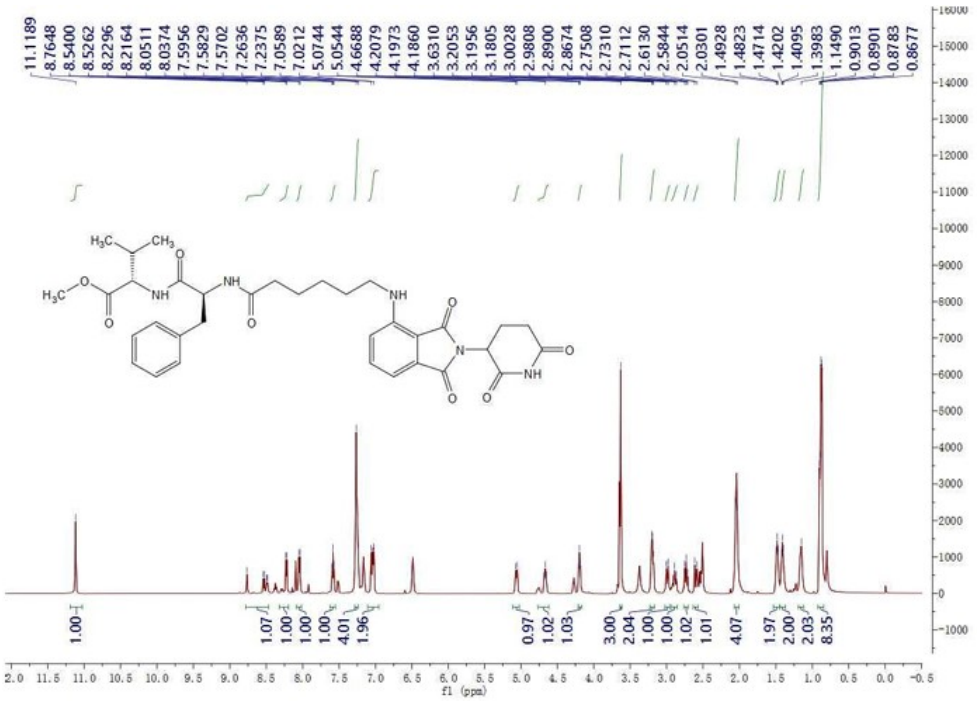


Fig. S2.^{1}H NMR of A

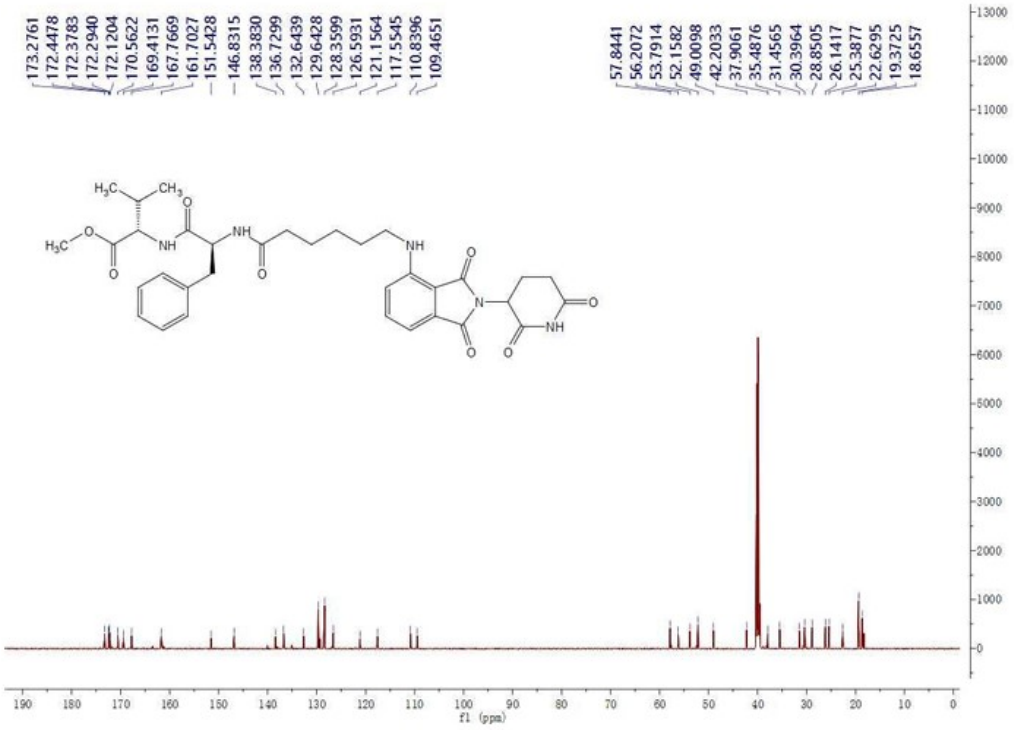


Fig. S3. ^{13}C NMR of A

PROTAC B, ^{1}H NMR (600 MHz, DMSO) δ 11.11 (s, 1H), 8.12 (d, *J* = 7.7 Hz, 1H), 7.80 (d, *J* = 8.8 Hz, 1H), 7.58 (t, *J* = 7.7 Hz, 1H), 7.08 (d, *J* = 8.6 Hz, 1H), 7.02 (d, *J* = 7.0 Hz, 1H), 5.06 (dd,

J = 12.8, 5.3 Hz, 1H), 4.29 (t, *J* = 7.9 Hz, 1H), 4.12 (t, *J* = 6.9 Hz, 1H), 3.61 (s, 3H), 2.92 – 2.86 (m, 1H), 2.61 – 2.53 (m, 2H), 2.21 – 2.10 (m, 2H), 2.02 (dd, *J* = 12.9, 6.3 Hz, 2H), 1.67 – 1.50 (m, 10H), 1.34 – 1.29 (m, 2H), 1.16 – 0.93 (m, 6H), 0.87 (dd, *J* = 17.1, 6.7 Hz, 6H). **^{13}C NMR (151 MHz, DMSO)** δ 173.27, 172.42, 172.24, 172.03, 170.55, 169.40, 167.76, 146.86, 136.74, 132.64, 117.60, 110.83, 109.45, 57.89, 57.02, 51.99, 48.99, 42.27, 40.32, 35.43, 31.45, 30.08, 29.40, 28.90, 28.64, 26.36, 26.05, 25.61, 22.63, 19.34, 18.76. **HR-MS(ESI) *m/z*:** Calcd for $C_{33}H_{45}N_5O_8Na$ [M+Na$^+$] 662.3166, found 662.3168.

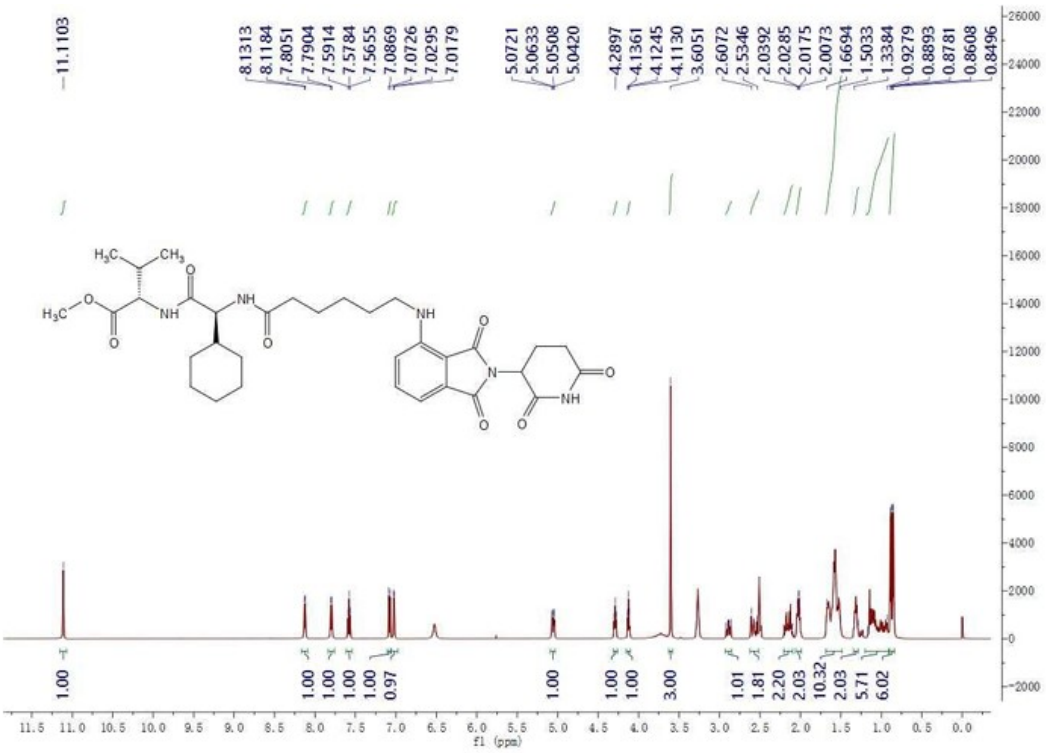


Fig. S4. ^{1}H NMR of B

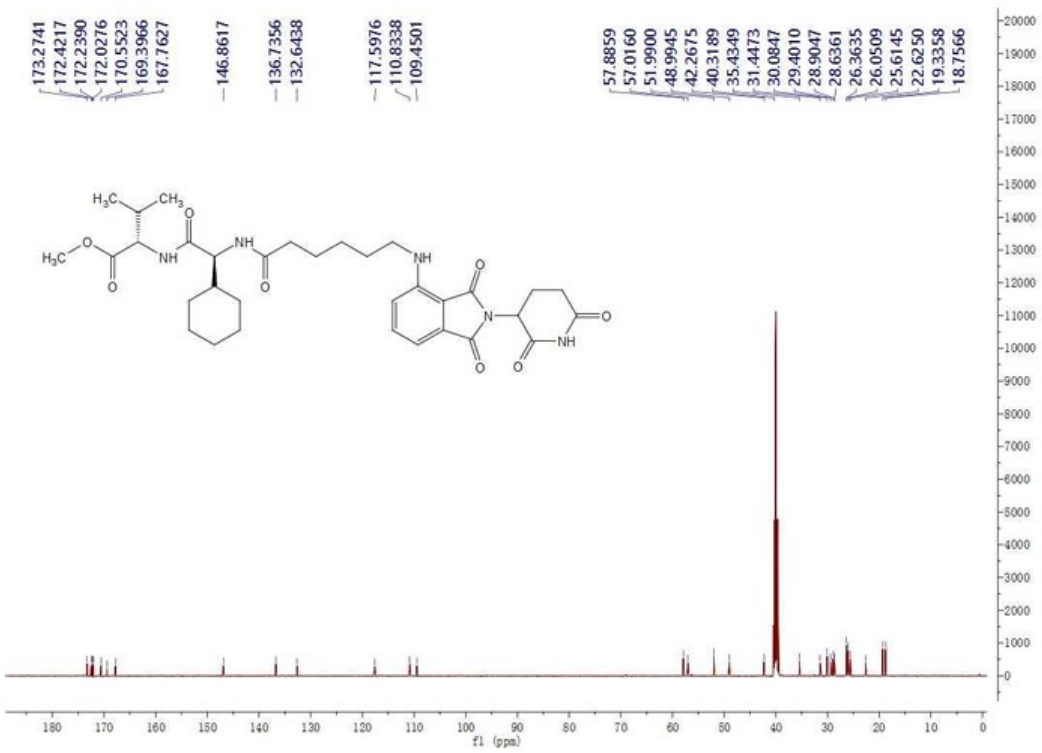


Fig. S5. ^{13}C NMR of B

PROTAC C, ^{1}H NMR (600 MHz, DMSO) δ 11.10 (s, 1H), 8.35 (d, *J* = 7.6 Hz, 1H), 8.00 (d, *J* = 8.6 Hz, 1H), 7.59 (t, *J* = 7.8 Hz, 1H), 7.25 (d, *J* = 4.3 Hz, 4H), 7.17 (s, 1H), 7.05 (dd, *J* = 20.3, 7.8 Hz, 2H), 6.49 (t, *J* = 5.6 Hz, 1H), 5.06 (dd, *J* = 12.8, 5.4 Hz, 1H), 4.58 (td, *J* = 9.4, 4.3 Hz, 1H), 4.30 (td, *J* = 8.8, 6.4 Hz, 1H), 3.62 (s, 3H), 3.21 (dd, *J* = 13.3, 6.6 Hz, 2H), 3.00 (dd, *J* = 13.8, 4.1 Hz, 1H), 2.92 – 2.86 (m, 1H), 2.72 (dd, *J* = 13.8, 10.5 Hz, 1H), 2.61 – 2.52 (m, 2H), 2.03 (t, *J* = 7.2 Hz, 3H), 1.66 – 1.55 (m, 2H), 1.53 – 1.46 (m, 3H), 1.43 – 1.37 (m, 2H), 1.18 – 1.14 (m, 2H), 0.87 (dd, *J* = 32.3, 6.4 Hz, 6H). **^{13}C NMR (151 MHz, DMSO)** δ 173.26, 172.33, 172.18, 170.55, 169.42, 167.77, 146.85, 138.41, 136.75, 132.66, 129.62, 128.39, 126.60, 117.58, 110.85, 109.48, 53.79, 52.30, 50.74, 49.01, 42.21, 40.17, 38.01, 35.50, 31.46, 28.86, 26.14, 25.37, 24.64, 23.22, 22.63, 21.77. **HR-MS(ESI) *m/z*:** Calcd for $C_{35}H_{43}N_5O_8Na$ [M+Na$^+$] 684.3009, found 684.3007.

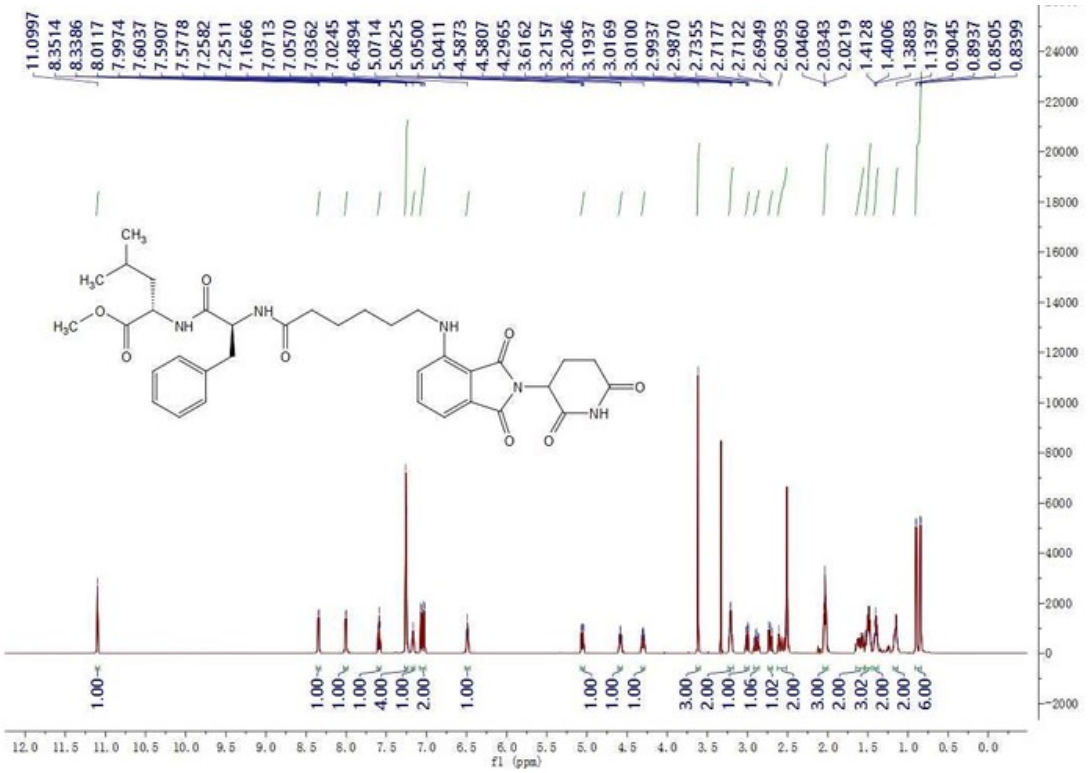


Fig. S6. ^{1}H NMR of C

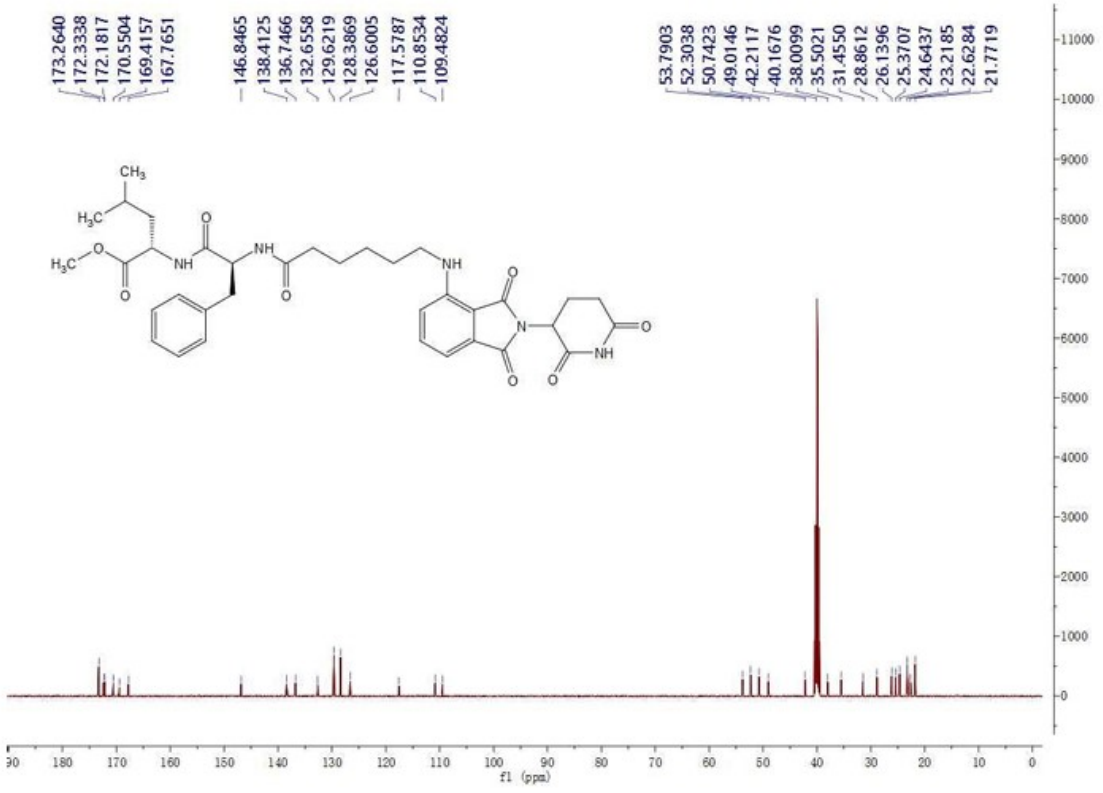


Fig. S7. ^{13}C NMR of C

PROTAC D, ^{1}H NMR (600 MHz, DMSO) δ 12.70 (s, 1H), 8.52 (s, 1H), 8.18 (s, 1H), 7.96 (s, 0.5H), 7.95 (s, 0.5H), 7.51 (s, 1H), 7.23 (m, 5H), 6.48 (s, 1H), 6.47 (s, 1H), 4.57 – 4.13 (m, 3H), 4.06 (d, J = 6.1 Hz, 2H), 2.95 (s, 1H), 2.79 (s, 0.5H), 2.78 (s, 0.5H), 2.04 (s, 0.5H), 2.03 (s, 0.5H), 1.56 (s, 5H), 1.39 – 1.20 (m, 17H), 0.87 (s, 3H), 0.86 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 172.88 (s), 171.93 (s), 169.86 (s), 168.01 (s), 162.09 (s), 155.24 (s), 146.13 (s), 138.17 (s), 135.82 (s), 132.48 (s), 129.21 (s), 127.97 (s), 126.14 (s), 116.72 (s), 110.03 (s), 109.40 (s), 78.05 (s), 63.22 (s), 60.64 (s), 56.95 (s), 55.63 (s), 41.82 (s), 37.22 (s), 36.86 (s), 32.36 (s), 30.19 (s), 28.58 (s), 28.11 (s), 26.23 (s), 26.23 (s), 25.87 (s), 25.87 (s), 25.08 (s), 19.04 (s), 17.86 (s). **HR-MS(ESI) *m/z*:** Calcd for $C_{38}H_{49}N_5O_9Na$ [M+Na+] 742.3428, found 742.3430

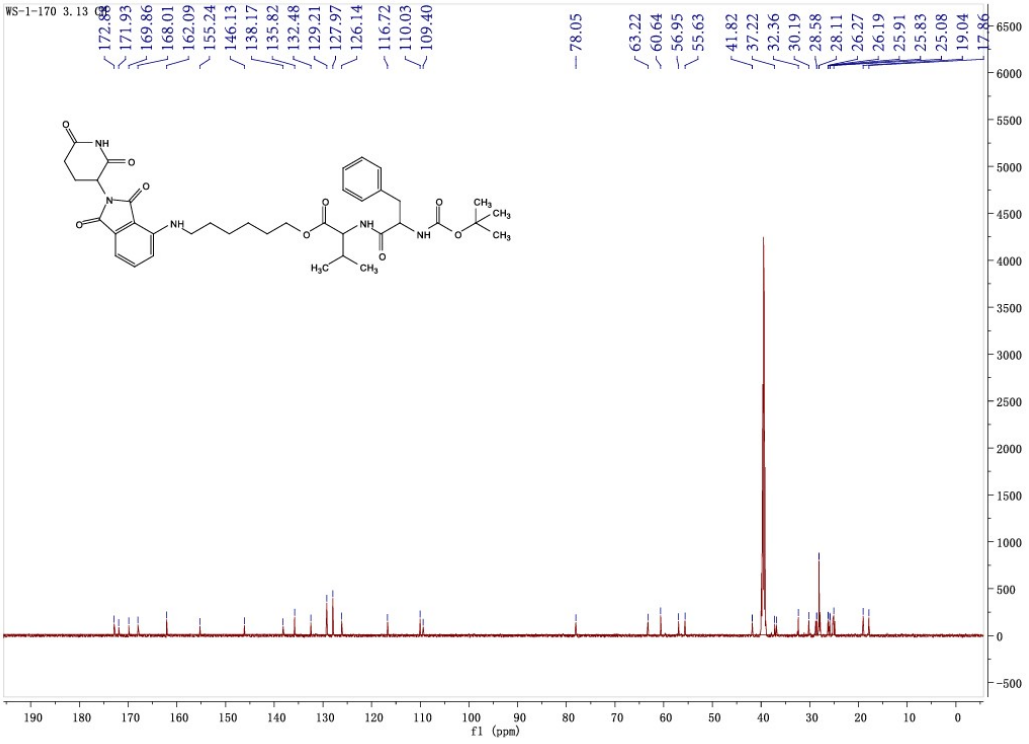


Fig. S8. ^{1}H NMR of D

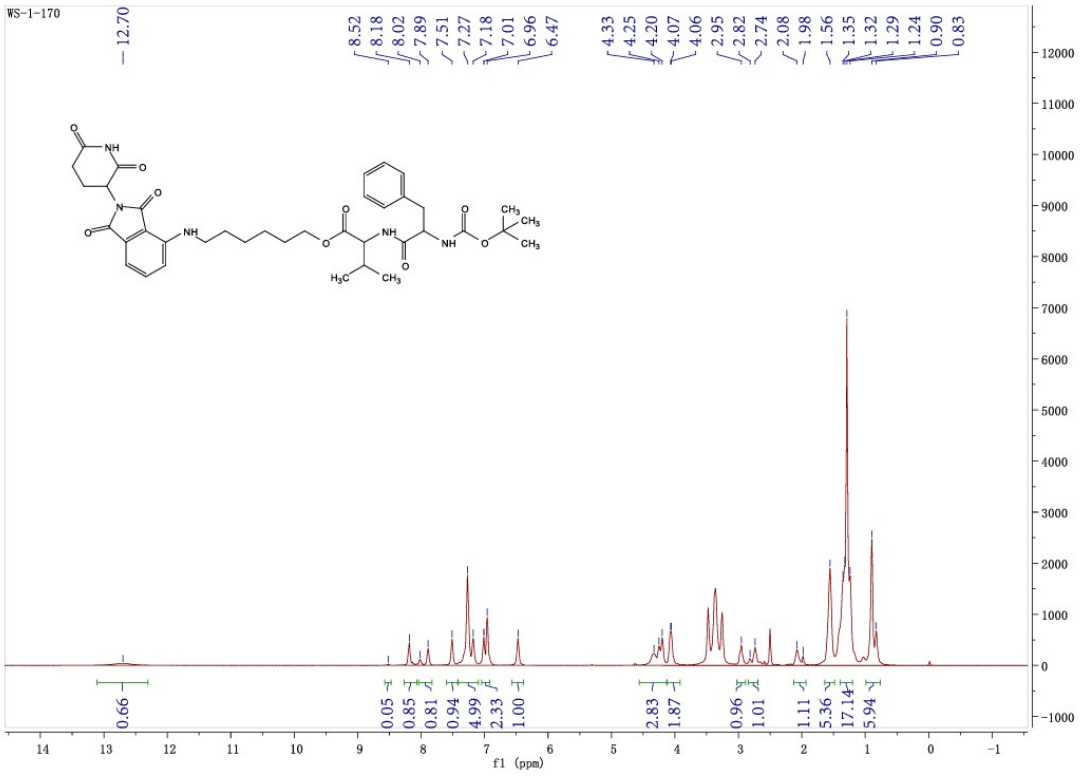


Fig. S9. ^{13}C NMR of D

PROTAC E, ^{1}H NMR (600 MHz, DMSO) δ 11.11 (s, 1H), 10.81 (s, 1H), 8.40 (s, 1H), 8.21 (s, 1H), 7.91 (m, 2H), 7.59 (d, *J* = 6.5 Hz, 1H), 7.31 (d, *J* = 7.9 Hz, 1H), 7.18 – 7.01 (m, 3H), 6.97 (d, *J* = 7.2 Hz, 1H), 6.61 (s, 1H), 5.06 (d, *J* = 5.3 Hz, 1H), 4.37 – 4.08 (m, 3H), 3.62 (m, 6H), 3.48 (d, *J* = 5.4 Hz, 1H), 3.30 (d, *J* = 6.4 Hz, 1H), 3.25 – 3.18 (m, 1H), 3.06 (d, *J* = 9.4 Hz, 1H), 2.88 (d, *J* = 9.6 Hz, 1H), 2.58 (d, *J* = 11.2 Hz, 1H), 2.42 (t, *J* = 6.7 Hz, 2H), 1.99 (s, 1H), 1.37 – 1.27 (m, 10H), 0.84 (d, *J* = 6.4 Hz, 6H). ^{13}C NMR (151 MHz, DMSO) δ 172.82 (s), 171.93 (s), 171.66 (s), 167.29 (s), 162.12 (s), 161.17 (s), 155.18 (s), 146.38 (s), 136.23 (s), 136.01 (s), 132.08 (s), 123.66 (s), 120.79 (s), 118.50 (s), 118.13 (s), 117.47 (s), 111.23 (s), 110.72 (s), 110.15 (s), 109.28 (s), 96.33 (s), 78.08 (s), 68.80 (s), 68.05 (s), 62.59 (s), 55.20 (s), 51.37 (s), 50.85 (s), 48.55 (s), 41.59 (s), 34.70 (s), 33.50 (s), 28.09 (s), 23.99 (s), 23.00 (s), 22.14 (s), 21.70 (s). **HR-MS(ESI) *m/z*:** Calcd for $C_{39}H_{48}N_6O_{10}Na$ [M+Na$^+$] 783.3330, found 783.3327.

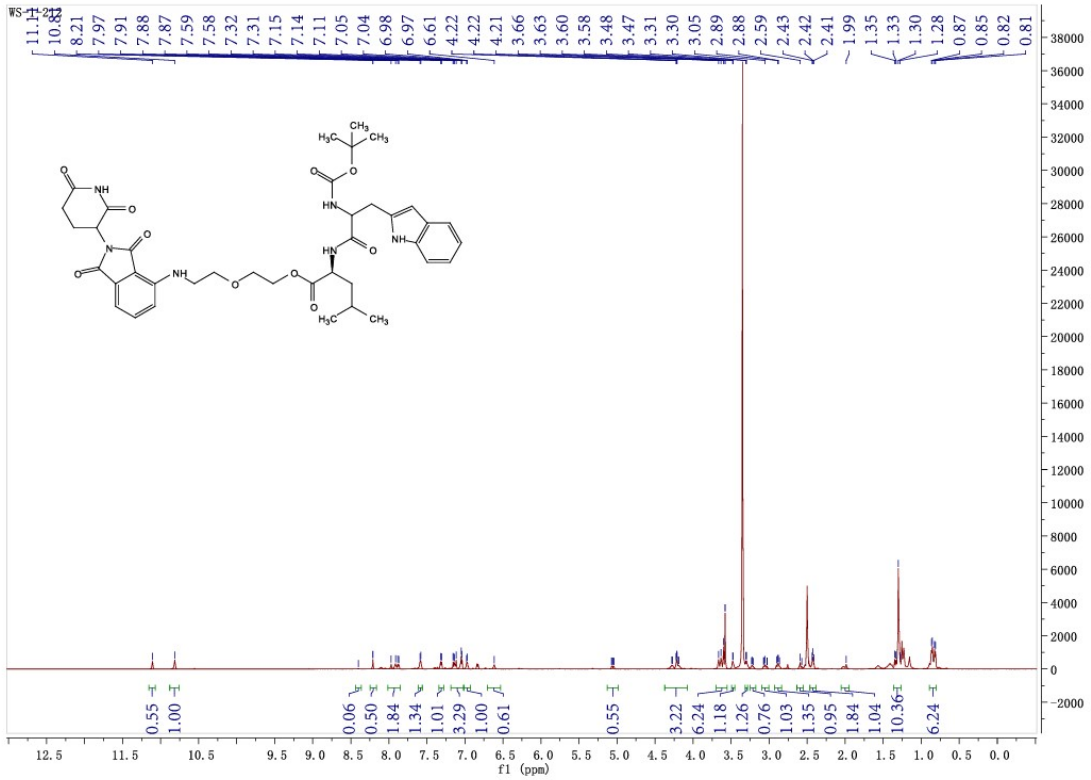


Fig. S10. ^{1}H NMR of E

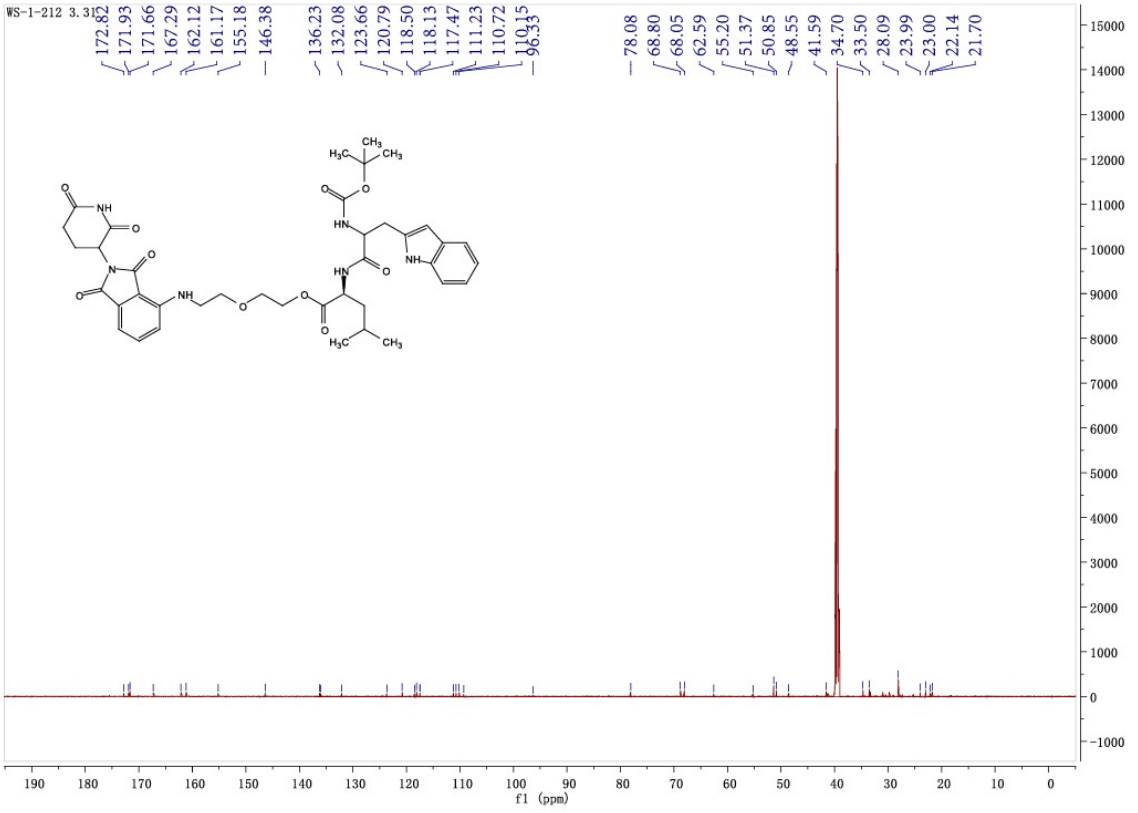


Fig. S11. ^{13}C NMR of E

Supplemental figures

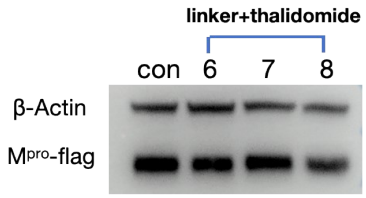


Fig. S12. M^{pro} degradation by control compounds 6, 7, and 8. Immunoblot analysis showing the concentration-dependent degradation of M^{pro} in HEK293 stable cells after 72-hour treatment with each compound. Compounds 6, 7, and 8 are linker-E3 ligand conjugates lacking the M^{pro}-targeting warhead. β-Actin serves as a loading control.