pharmaceuticals

Supplementary Materials: Synthesis and Evaluation of AS1411-Lenalidomide Targeted Degradation Chimera in Antitumor Therapy

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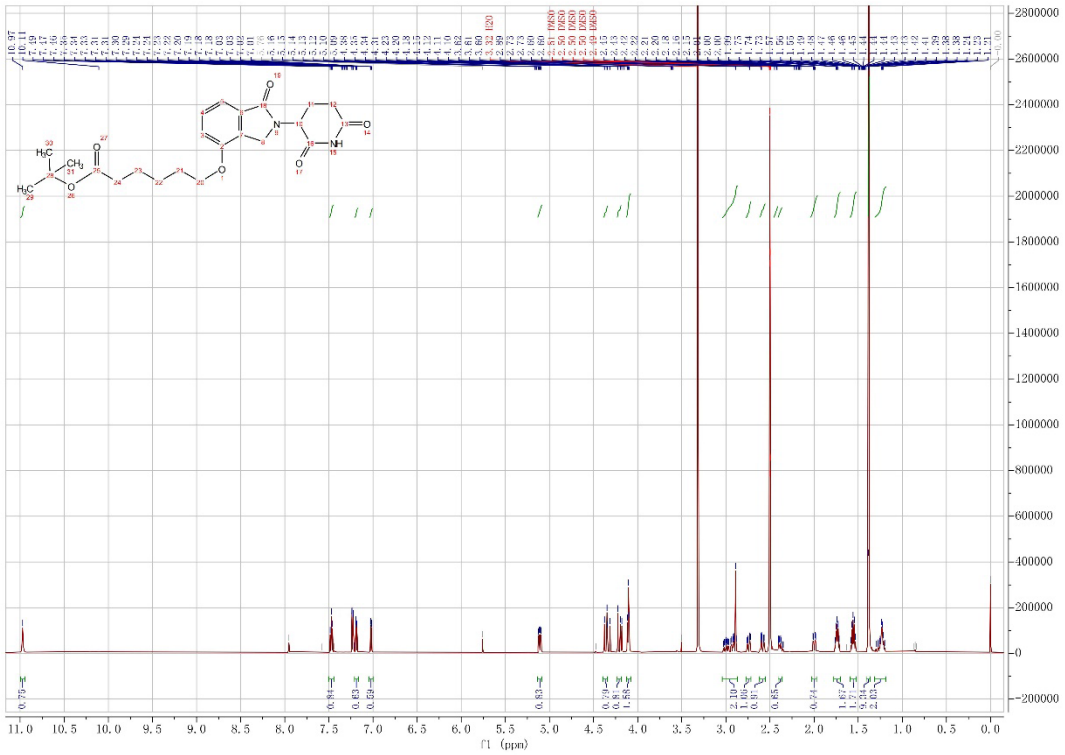
Synthesis of compounds

Compound 1a. 2 g 3-(4-hydroxy-1-oxo-1,3-dihydroisoindolin-2-yl)piperidine-2,6-dione (0.0077 mol, 1 eq) was dissolved in 40 mL DMF. Then 2.22 g *tert*-butyl 6-bromohexanoate (0.0092 mol, 1.2 eq) and 3.2 g K_2CO_3 (0.0231 mol, 3 eq) were added, and the mixture was stirred at 80 °C for 24 h. After reaction, the solution was diluted with 20 mL H_2O, extracted three times with 50 mL EtOAc, and the combined organic layer was washed three times with 40 mL saturated NaCl, then dried over anhydrous Na_2SO_4 overnight. After filtration, vacuum concentration and drying, the crude product was purified by fast column chromatography (DCM : MeOH = 50 : 1). Concentration and drying gave white powder product 1a (yield 75%).

^{1}H NMR (600 MHz, DMSO - d_6): δ 10.97 (s, 1H), 7.47 (t, J = 7.8 Hz, 1H), 7.19 (dd, J = 7.5, 0.8 Hz, 1H), 7.02 (dd, J = 8.0, 0.9 Hz, 1H), 5.11 (dd, J = 13.4, 5.2 Hz, 1H), 4.36 (d, J = 17.2 Hz, 1H), 4.21 (d, J = 17.3 Hz, 1H), 4.11 (t, J = 6.3 Hz, 2H), 3.04 – 2.87 (m, 2H), 2.77 – 2.72 (m, 1H), 2.62 – 2.55 (m, 1H), 2.41 – 2.36 (m, 1H), 2.00 (dd, J = 13.1, 4.5 Hz, 1H), 1.74 (p, J = 6.5 Hz, 2H), 1.56 (p, J = 7.3 Hz, 2H), 1.38 (d, J = 2.5 Hz, 9H), 1.31 – 1.19 (m, 2H).

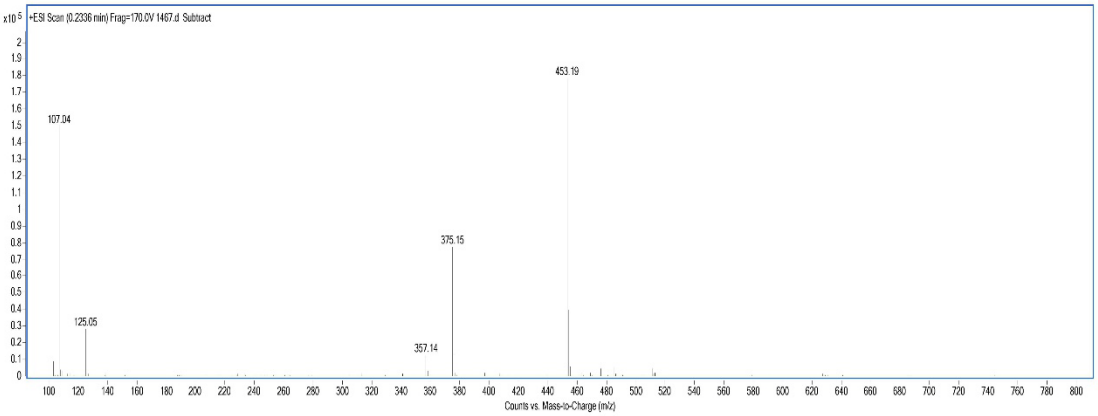
ESI-MS, $[C_{23}H_{30}N_2O_6]^+$. Theoretical value: 430.21. Measured value: 453.19 (M + Na^+).

^{1}H NMR of compound **1a**



 https://doi.org/10.3390/ph18121867

ESI-MS of compound **1a**

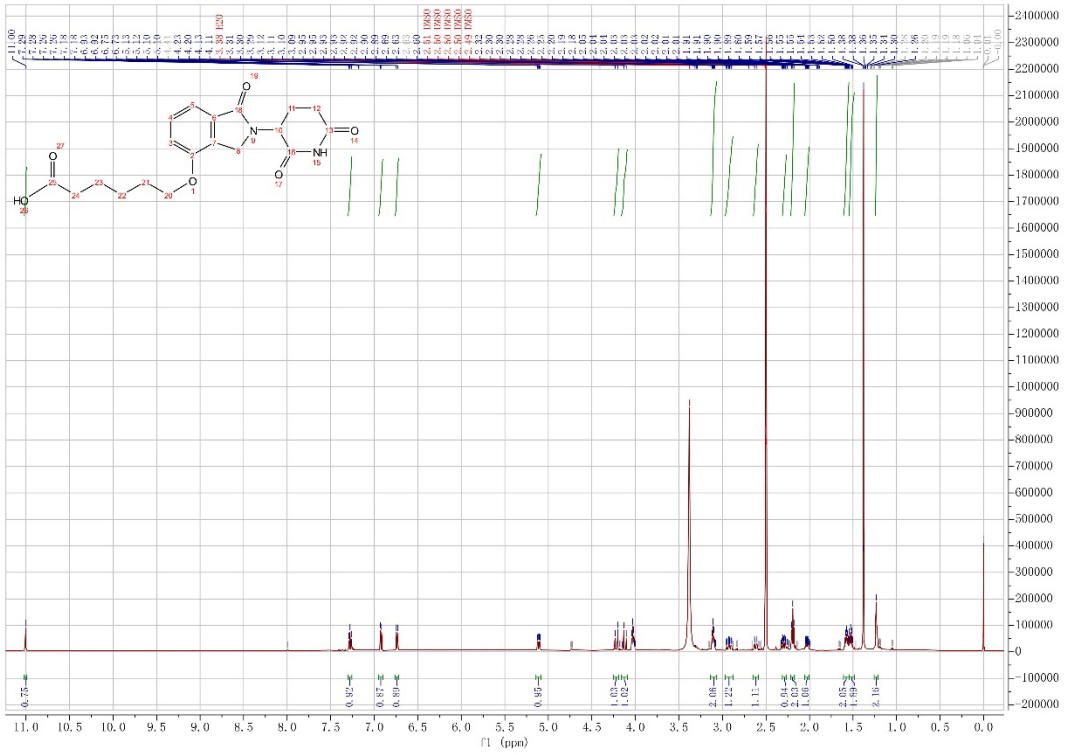


Compound 1b. 0.5 g 1a (0.0012 mol, 1 eq) was dissolved in 2 mL DCM. 6 g TFA (0.0524 mol, 45 eq) was added, and the mixture was stirred at room temperature for 2 h. After reaction, the solvent was evaporated, 5 mL Et_2O was added. The mixture was filtered, and the precipitate was collected to obtain white powder product 1b (yield 99 %).

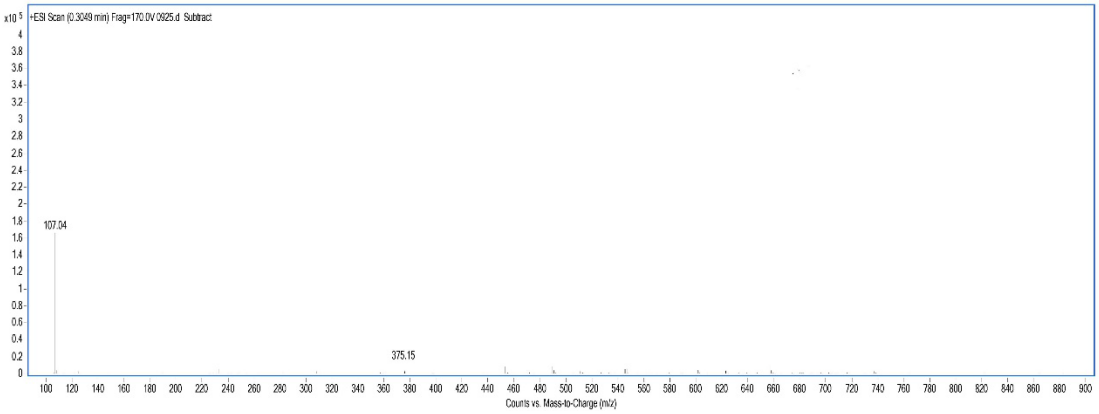
^{1}H NMR (600 MHz, DMSO - d_6): δ 11.00 (s, 1H), 7.28 (t, J = 7.7 Hz, 1H), 6.92 (d, J = 7.4 Hz, 1H), 6.74 (d, J = 8.0 Hz, 1H), 5.11 (dd, J = 13.3, 5.1 Hz, 1H), 4.22 (d, J = 17.0 Hz, 1H), 4.12 (d, J = 17.1 Hz, 1H), 4.03 (q, J = 6.4, 5.8 Hz, 2H), 2.92 (ddd, J = 17.3, 13.6, 5.4 Hz, 1H), 2.62 (d, J = 17.5 Hz, 1H), 2.29 (dd, J = 13.3, 4.5 Hz, 1H), 2.19 (t, J = 7.3 Hz, 2H), 2.03 (ddq, J = 10.8, 5.6, 3.0, 2.5 Hz, 1H), 1.57 (p, J = 7.1 Hz, 2H), 1.52 (q, J = 7.5 Hz, 2H), 1.23 (d, J = 6.0 Hz, 2H).

ESI-MS, $[C_{19}H_{22}N_2O_6]^+$. Theoretical value: 374.15. Measured value: 375.15 ($M + H^+$).

^{1}H NMR of compound **1b**



ESI-MS of compound **1b**



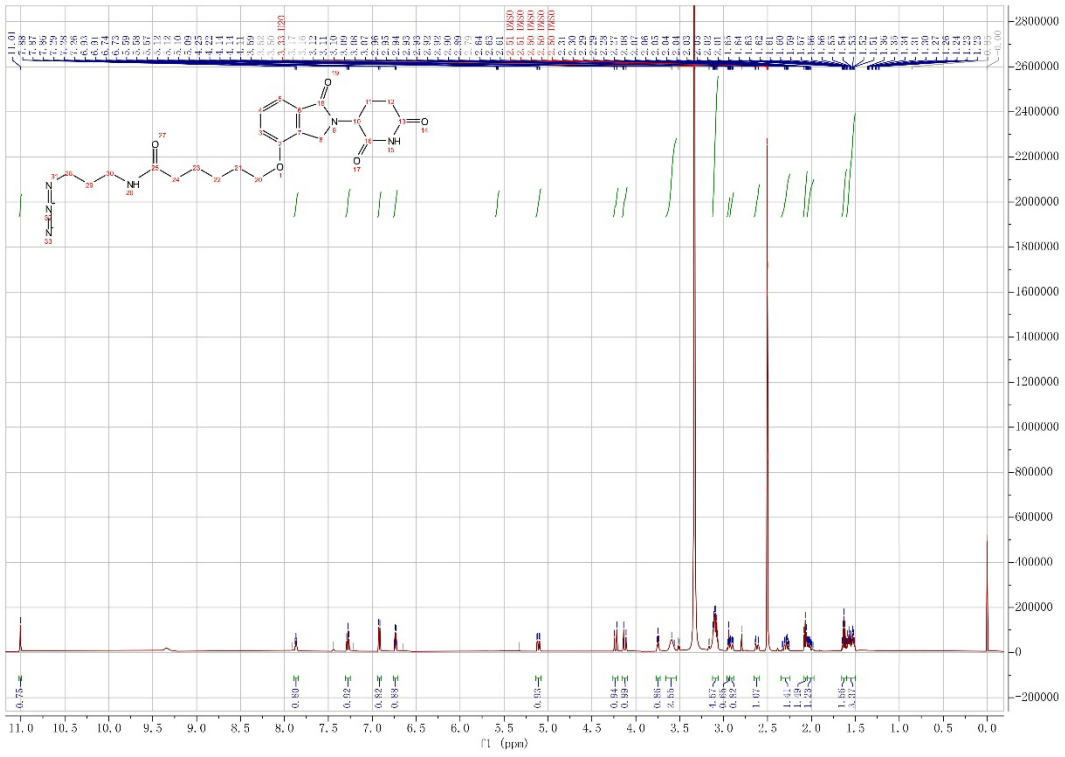
Compound 1c. Solution A: 0.1 g 1b (0.00027 mol, 1 eq) was dissolved in 3 mL DMF. Then 0.102 g HBTU (0.00027 mol, 1 eq), 0.036 g HOBt (0.00027 mol, 1 eq), and 0.172 g DIPEA (0.0013 mol, 5 eq) were added. Solution B: 0.027 g 3-azidopropylamine (0.00027 mol, 1 eq) was mixed with TFA ($V_{3\text{-}axi}d_{opropy}y_{lamine}$: V_TFA = 1 : 1). Solution A was stirred for 10 min before adding Solution B. The reaction mixture was stirred at room temperature for 12 h. The crude product was purified by thin-layer chromatography (DCM : MeOH = 10 : 1) to obtain light yellow powder product 1c (yield 43 %).

^{1}H NMR (600 MHz, DMSO - d_6): δ 11.01 (s, 1H), 7.87 (t, J = 5.6 Hz, 1H), 7.28 (t, J = 7.7 Hz, 1H), 6.92 (d, J = 7.4 Hz, 1H), 6.73 (d, J = 8.1 Hz, 1H), 5.11 (dd, J = 13.3, 5.1 Hz, 1H), 4.23 (d, J = 17.1 Hz, 1H), 4.12 (d, J = 17.1 Hz, 1H), 3.75 (t, J = 5.5 Hz, 1H), 3.58 (d, J = 18.2 Hz, 3H), 3.09 (dq, J = 12.8, 6.6 Hz, 5H), 2.94 (q, J = 4.9, 4.5 Hz, 1H), 2.93 – 2.89 (m, 1H), 2.65 – 2.59 (m, 1H), 2.29 (qd, J = 13.0, 4.6 Hz, 1H), 2.07 (t, J = 7.4 Hz, 1H), 2.05 – 1.97 (m, 1H), 1.63 (p, J = 6.8 Hz, 2H), 1.55 (dp, J = 26.0, 7.4 Hz, 3H).

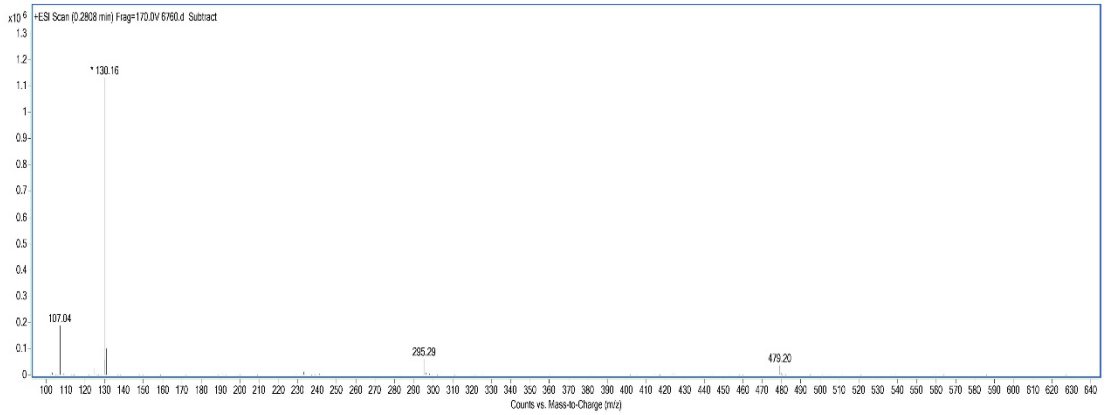
ESI-MS, $[C_{22}H_{28}N_6O_5]^+$. Theoretical value: 456.21. Measured value: 479.20 (M + Na^+).

HRMS, $[C_{22}H_{28}N_6O_5]^+$. Theoretical value: 456.2121. Measured value: 457.2191 & 479.2012 (M + H^+ & M + Na^+).

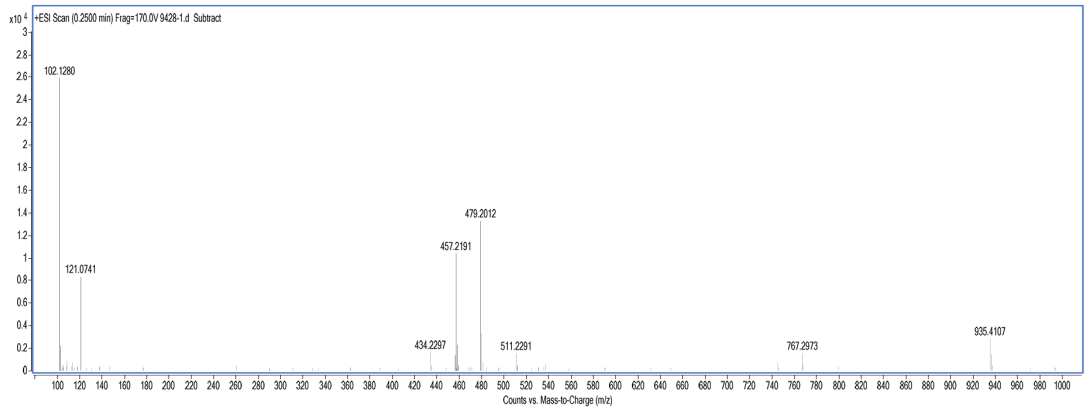
^{1}H NMR of compound **1c**



ESI-MS of compound **1c**



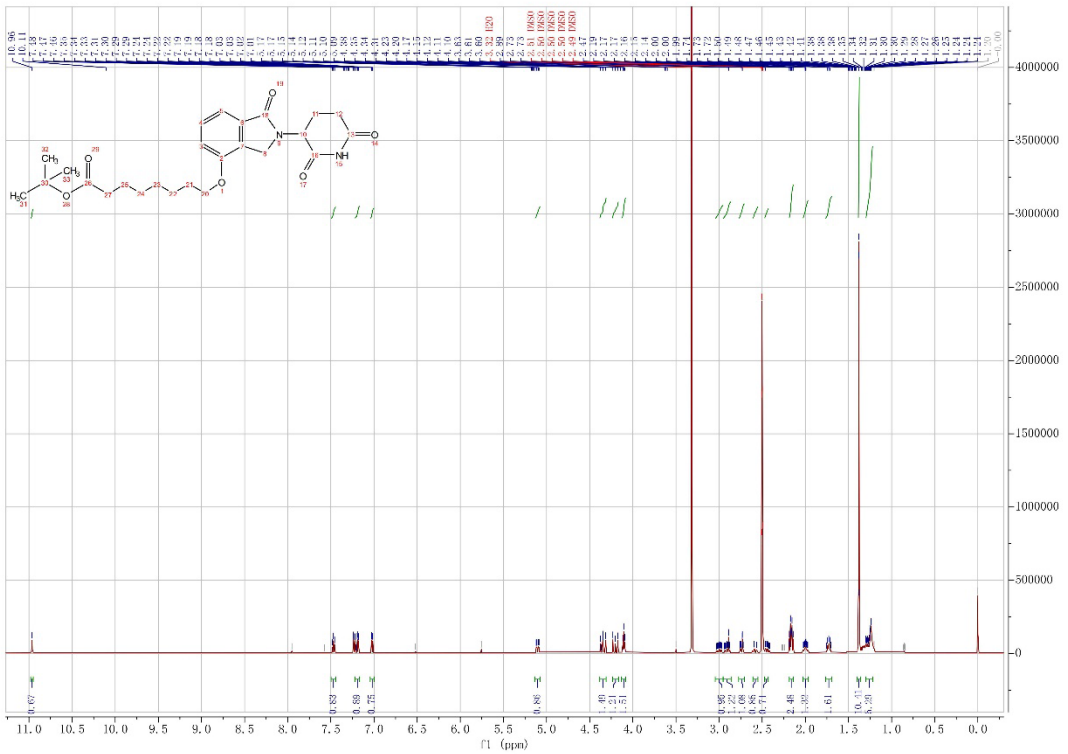
HRMS of compound **1c**



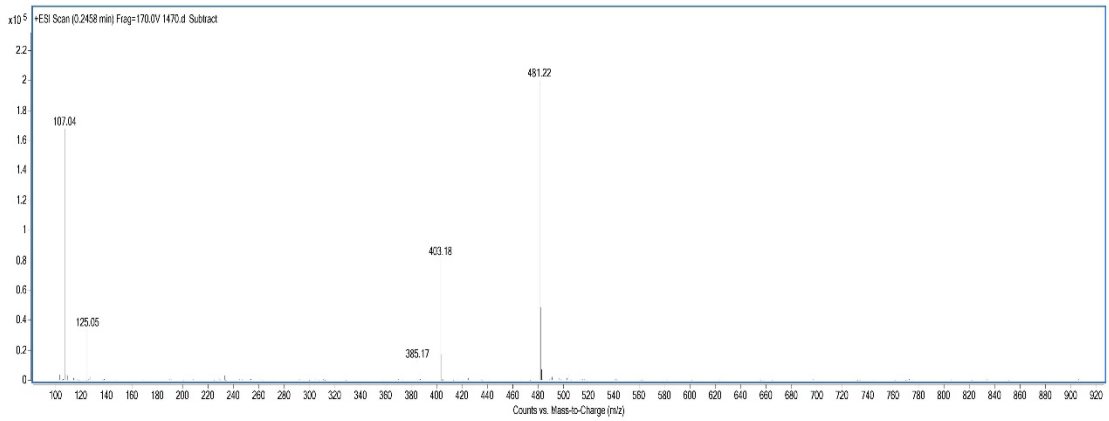
Compound 2a. 2 g 3-(4-hydroxy-1-oxo-1,3-dihydroisoindolin-2-yl)piperidine-2,6-dione (0.0077 mol, 1 eq) was dissolved in 40 mL DMF. Then 2.58 g *tert*-butyl 8-bromooctanoate (0.0092 mol, 1.2 eq) and 3.2 g K_2CO_3 (0.0231 mol, 3 eq) were added, and the mixture was stirred at 80 °C for 24 h. Subsequent operations were consistent with the procedure for 1a. White powder product 2a was obtained (yield 45 %).

^{1}H NMR (600 MHz, DMSO - d_6): δ 10.96 (s, 1H), 7.47 (t, J = 7.8 Hz, 1H), 7.19 (dd, J = 7.4, 0.9 Hz, 1H), 7.02 (dd, J = 8.0, 0.9 Hz, 1H), 5.10 (dd, J = 13.3, 5.1 Hz, 1H), 4.35 (dd, J = 20.1, 17.2 Hz, 1H), 4.24 – 4.16 (m, 1H), 4.11 (t, J = 6.4 Hz, 2H), 3.00 (ddd, J = 17.3, 13.7, 5.4 Hz, 1H), 2.95 – 2.86 (m, 1H), 2.77 – 2.71 (m, 1H), 2.58 (d, J = 17.3 Hz, 1H), 2.45 (dd, J = 13.1, 4.5 Hz, 1H), 2.16 (dt, J = 11.4, 7.3 Hz, 2H), 2.03 – 1.97 (m, 1H), 1.73 (p, J = 6.5 Hz, 2H), 1.38 (d, J = 1.5 Hz, 10H), 1.30 – 1.22 (m, 5H). ESI-MS, $[C_{25}H_{34}N_2O_6]^+$. Theoretical value: 458.24. Measured value: 481.22 ($M + Na^+$).

^{1}H NMR of compound **2a**



ESI-MS of compound **2a**

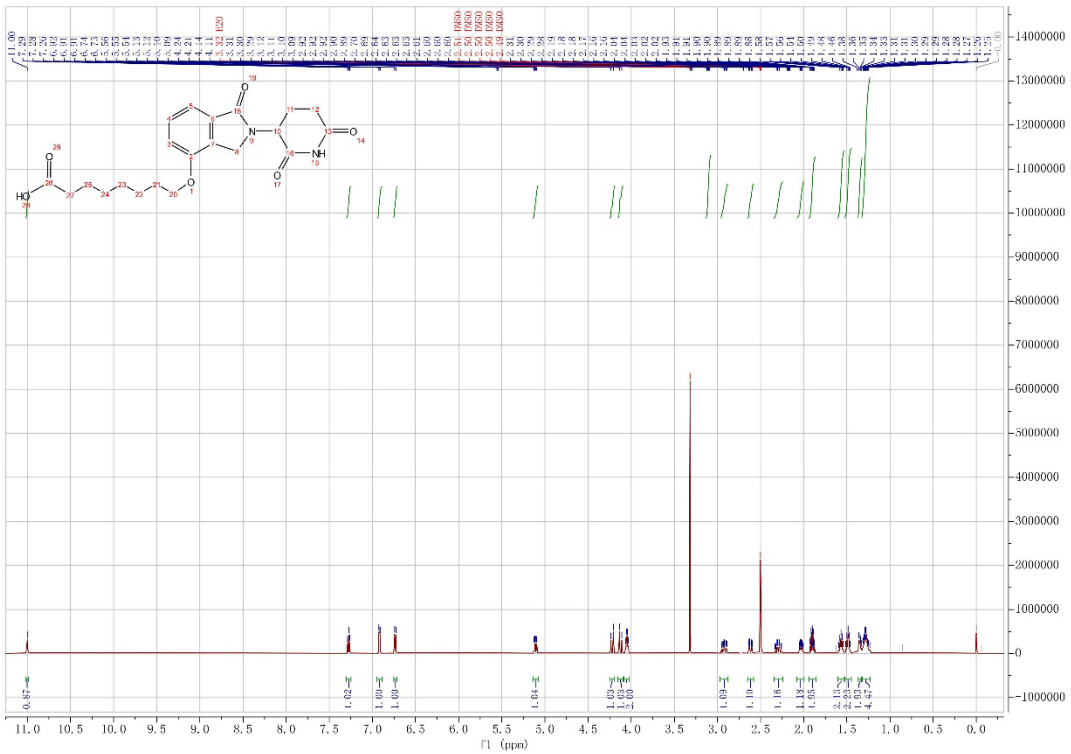


Compound 2b. 0.5 g 2a (0.0011 mol, 1 eq) was dissolved in 2 mL DCM. 5.61 g TFA (0.0492 mol, 45 eq) was added, and the mixture was stirred at room temperature for 2 h. Subsequent operations were consistent with the procedure for 1b. White powder product 2b was obtained (yield 98 %).

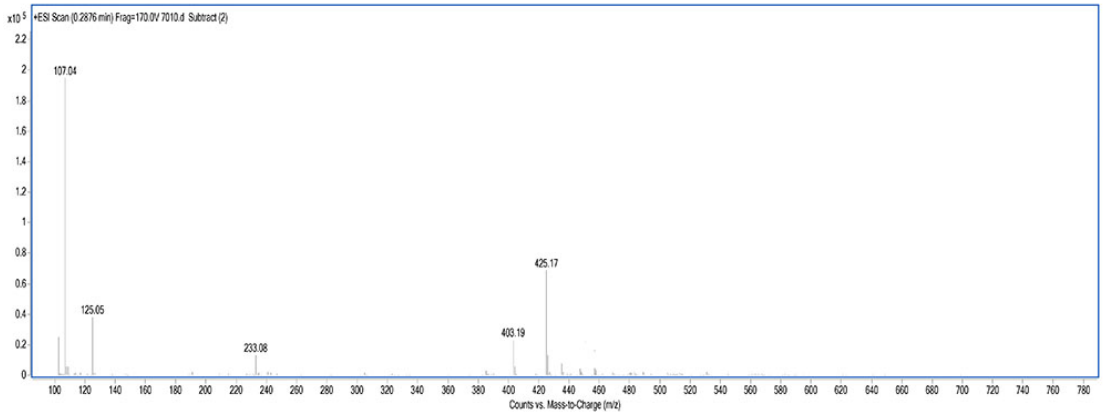
^{1}H NMR (600 MHz, DMSO - d_6): δ 11.00 (s, 1H), 7.28 (t, J = 7.7 Hz, 1H), 6.95 – 6.89 (m, 1H), 6.74 (d, J = 8.0 Hz, 1H), 5.11 (dd, J = 13.3, 5.2 Hz, 1H), 4.22 (d, J = 17.1 Hz, 1H), 4.12 (d, J = 17.1 Hz, 1H), 4.01 (q, J = 6.6 Hz, 2H), 2.92 (ddd, J = 17.3, 13.6, 5.4 Hz, 1H), 2.62 (ddd, J = 17.3, 4.5, 2.4 Hz, 1H), 2.29 (qd, J = 13.3, 4.4 Hz, 1H), 2.03 (dtd, J = 12.6, 5.2, 2.2 Hz, 1H), 1.94 – 1.86 (m, 2H), 1.57 (p, J = 7.2 Hz, 2H), 1.49 (p, J = 7.3 Hz, 2H), 1.37 – 1.32 (m, 2H), 1.33 – 1.23 (m, 4H).

ESI-MS, $[C_{21}H_{26}N_2O_6]^+$. Theoretical value: 402.18. Measured value: 403.19 & 425.17 ($M + H^+$ & $M + Na^+$).

^{1}H NMR of compound **2b**



ESI-MS of compound **2b**



Compound 2c. Solution A: 0.1 g 2b (0.00025 mol, 1 eq) was dissolved in 3 mL DMF. Then 0.095 g HBTU (0.00025 mol, 1 eq), 0.034 g HOBt (0.00027 mol, 1 eq), and 0.162 g DIPEA (0.00125 mol, 5 eq) were added. Solution B: 0.025 g 3-azidopropylamine (0.00025 mol, 1 eq) was mixed with TFA ($V_{3\text{-}axi}d_{opropy}l_{amine}$: V_TFA = 1 : 1). Subsequent operations were consistent with the procedure for 1c. White powder product 2c was obtained (yield 40 %).

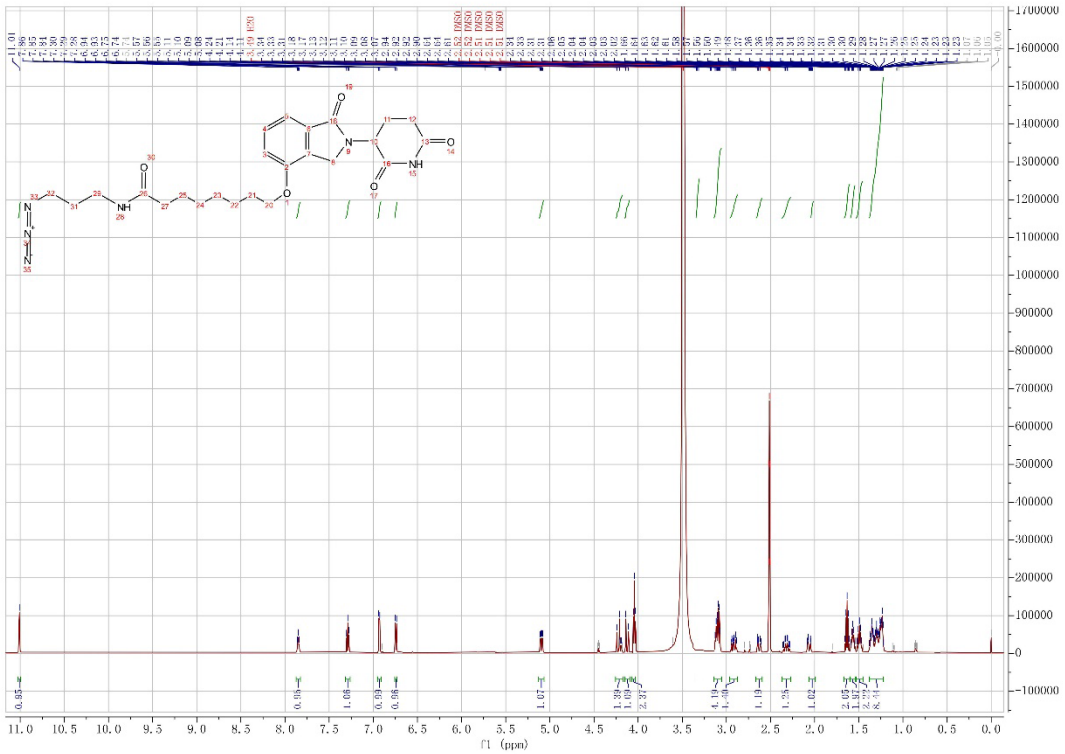
^{1}H NMR (600 MHz, DMSO - d_6): δ 11.01 (s, 1H), 7.85 (t, J = 5.8 Hz, 1H), 7.29 (t, J = 7.7 Hz, 1H), 6.93 (d, J = 7.4 Hz, 1H), 6.74 (d, J = 8.1 Hz, 1H), 5.10 (dd, J = 13.3, 5.1 Hz, 1H), 4.26 – 4.17 (m, 1H), 4.12 (d, J = 17.1 Hz, 1H), 4.05 (t, J = 6.8 Hz, 2H), 3.14 – 3.05 (m, 4H), 2.92 (ddd, J = 17.4, 13.6, 5.5 Hz, 1H), 2.66 – 2.59 (m, 1H), 2.32 (qd, J = 13.2, 4.4 Hz, 1H), 2.04 – 2.01 (qd, J = 13.2, 4.4 Hz, 1H), 1.63 (p, J = 6.8 Hz, 2H), 1.57 (p, J = 7.2 Hz, 2H), 1.49 (p, J = 7.4 Hz, 2H), 1.38 – 1.22 (m, 8H).

^{13}C NMR (150 MHz, DMSO - d_6): δ 172.81, 172.14, 168.70, 162.85, 154.30, 133.64, 130.14, 124.37, 115.54, 114.96, 68.39, 52.69, 49.03, 45.56, 38.64, 36.20, 35.81, 31.89, 31.66, 31.19, 28.99, 28.87, 26.70, 25.65.

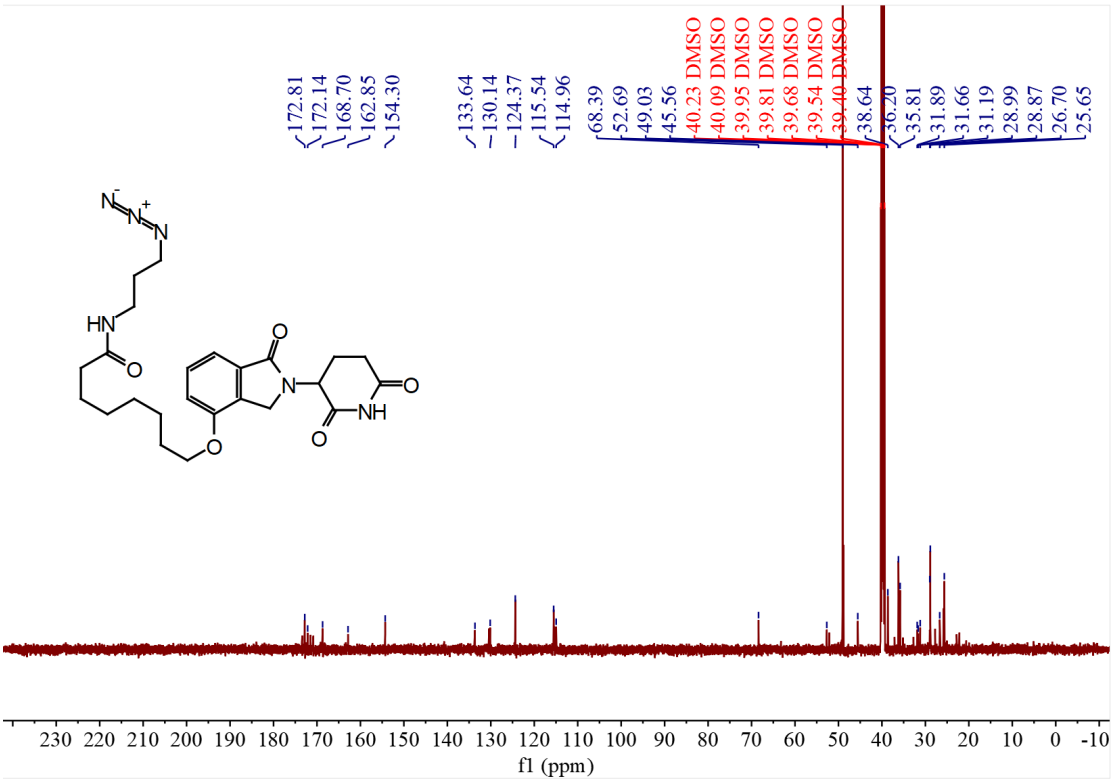
ESI-MS, $[C_{24}H_{32}N_6O_5]^+$. Theoretical value: 484.24. Measured value: 507.23 (M + Na^+).

HRMS, $[C_{24}H_{32}N_6O_5]^+$. Theoretical value: 484.2434. Measured value: 507.2328 (M + Na^+).

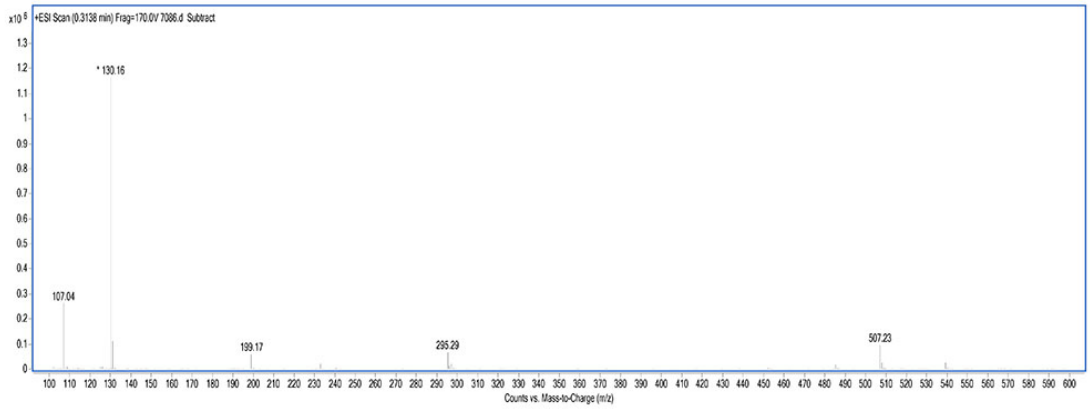
^{1}H NMR of compound **2c**



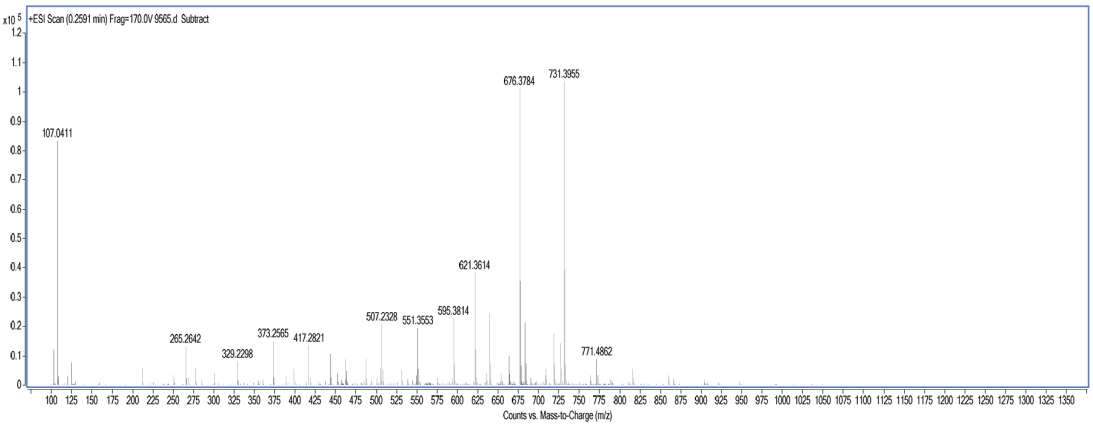
^{13}C NMR of compound **2c**



ESI-MS of compound **2c**



HRMS of compound **2c**

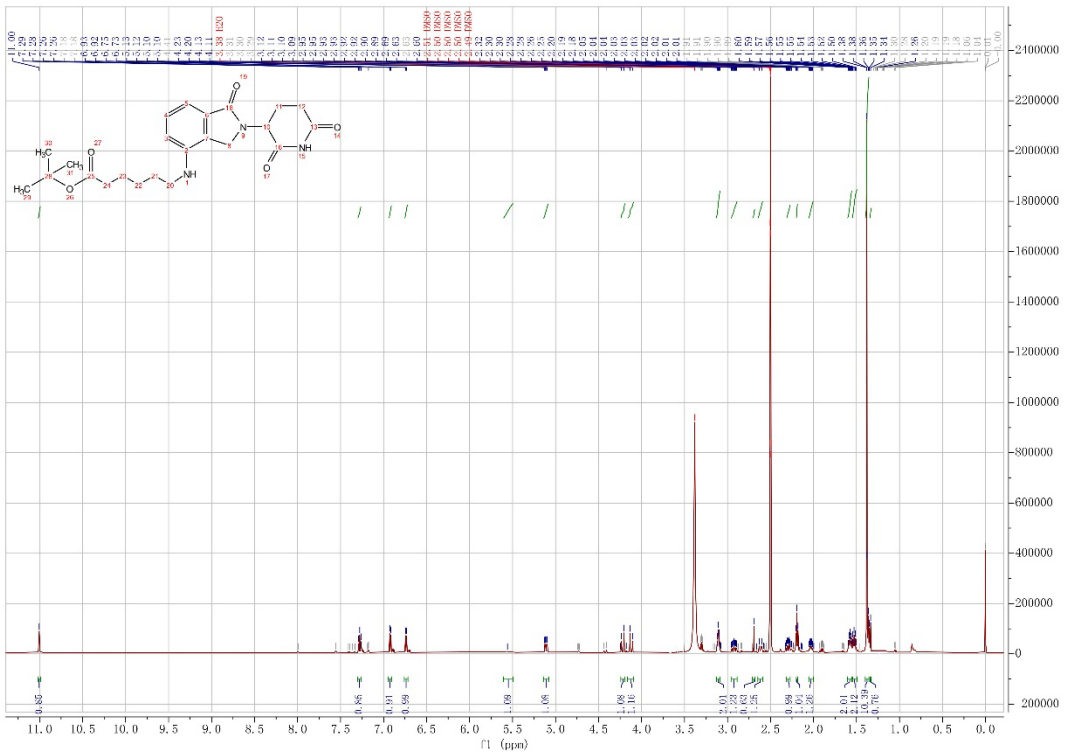


Compound 3a. 2 g lenalidomide (0.0077 mol, 1 eq) was dissolved in 30 mL NMP. Then 2.32 g *tert*-butyl 6-bromohexanoate (0.0090 mol, 1.2 eq) and 3 g DIPEA (0.0232 mol, 3 eq) were added. The mixture was stirred at 120 °C for 15 h. After reaction, the solution was diluted with 20 mL H_2O, extracted three times with 40 mL EtOAc. The combined organic layer was washed three times with 40 mL saturated NaCl, dried over anhydrous Na_2SO_4 overnight. After filtration and vacuum concentration, the crude product was purified by fast column chromatography (DCM : MeOH = 50 : 1) to obtain pale yellow powder product 3a (yield 40 %).

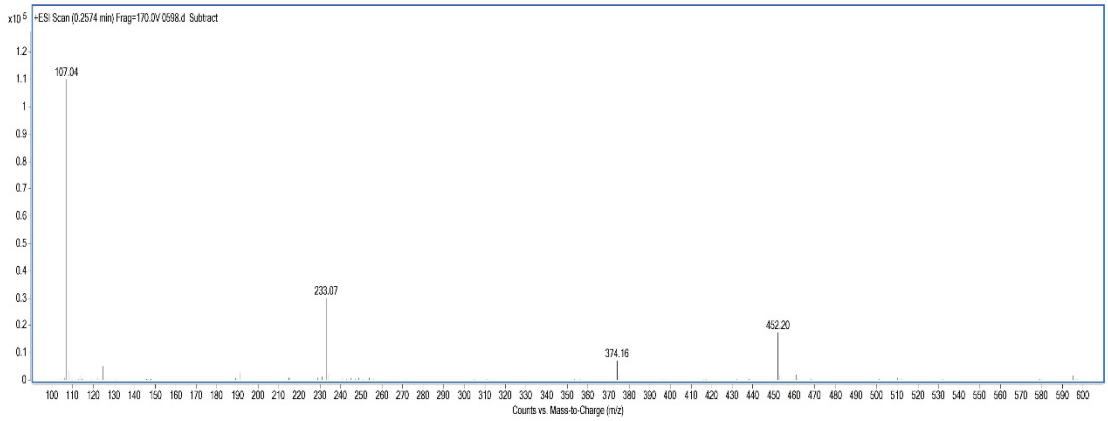
^{1}H NMR (600 MHz, DMSO - d_6): δ 11.00 (s, 1H), 7.28 (d, J = 7.7 Hz, 1H), 6.92 (d, J = 7.4 Hz, 1H), 6.74 (d, J = 8.0 Hz, 1H), 5.56 (s, 1H), 5.11 (dd, J = 13.3, 5.1 Hz, 1H), 4.22 (d, J = 17.0 Hz, 1H), 4.12 (d, J = 17.1 Hz, 1H), 3.10 (q, J = 6.4, 5.8 Hz, 2H), 2.92 (ddd, J = 17.3, 13.6, 5.4 Hz, 1H), 2.69 (s, 1H), 2.62 (d, J = 17.5 Hz, 1H), 2.29 (dd, J = 13.3, 4.5 Hz, 1H), 2.19 (s, 1H), 2.03 (ddq, J = 10.8, 5.6, 3.0, 2.5 Hz, 1H), 1.58 (q, J = 7.3 Hz, 2H), 1.52 (q, J = 7.5 Hz, 2H), 1.38 (s, 10H), 1.34 (s, 1H).

ESI-MS, $[C_{23}H_{31}N_3O_5]^+$. Theoretical value: 429.23. Measured value: 452.20 ($M + Na^+$).

^{1}H NMR of compound **3a**



ESI-MS of compound **3a**

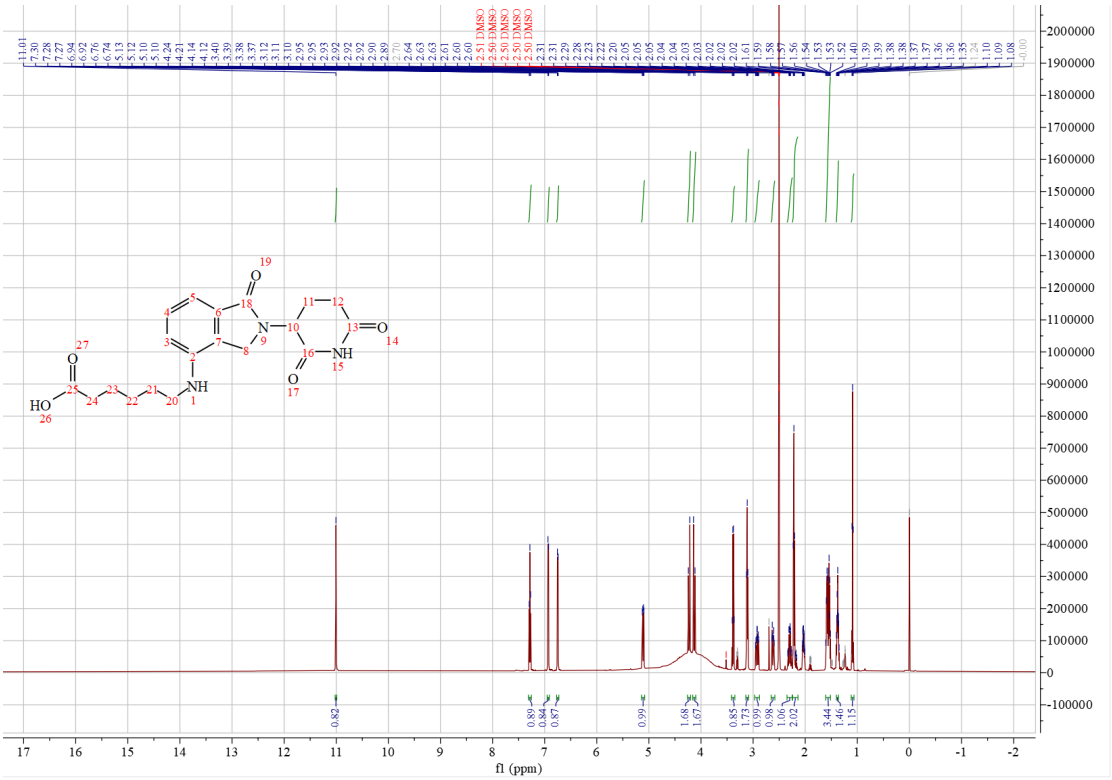


Compound 3b. 0.5 g 3a (0.0012 mol, 1 eq) was dissolved in 2 mL DCM. 6 g TFA (0.0524 mol, 45 eq) was added, and the mixture was stirred at room temperature for 2 h. After reaction, the solvent was evaporated, 5 mL Et_2O was added. The mixture was filtered, and the precipitate was collected to obtain pale yellow powder product 3b (yield 99 %).

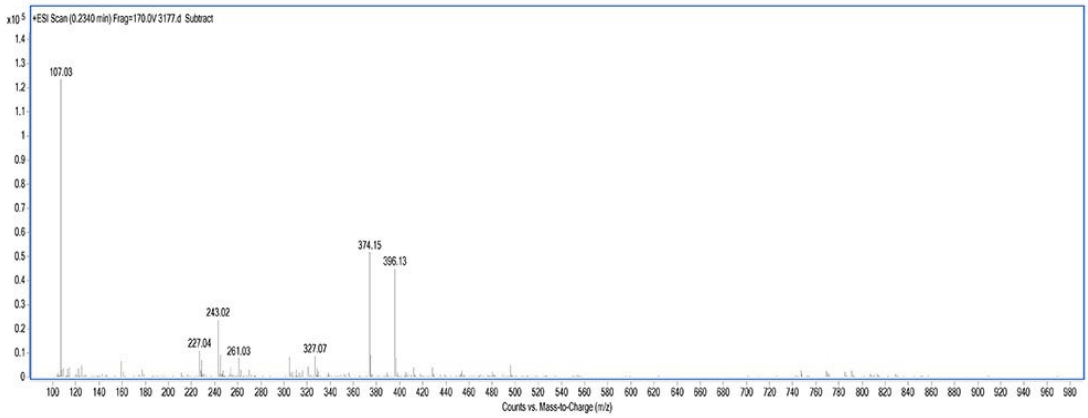
^{1}H NMR (600 MHz, DMSO - d_6) δ 11.01 (s, 1H), 7.28 (t, J = 7.7 Hz, 1H), 6.93 (d, J = 7.4 Hz, 1H), 6.75 (d, J = 8.0 Hz, 1H), 5.11 (dd, J = 13.3, 5.1 Hz, 1H), 4.23 (d, J = 17.1 Hz, 2H), 4.13 (d, J = 17.1 Hz, 2H), 3.38 (q, J = 7.0 Hz, 1H), 3.11 (t, J = 7.1 Hz, 2H), 2.92 (ddd, J = 17.3, 13.6, 5.4 Hz, 1H), 2.62 (dt, J = 17.2, 3.8 Hz, 1H), 2.30 (qd, J = 13.2, 4.5 Hz, 1H), 2.22 (t, J = 7.4 Hz, 2H), 1.56 (dp, J = 23.0, 7.4 Hz, 3H), 1.40 – 1.36 (m, 1H), 1.09 (t, J = 7.0 Hz, 1H).

ESI-MS, $[C_{19}H_{23}N_3O_5]^+$. Theoretical value: 373.16. Measured value: 374.15 & 396.13 ($M + H^+$ & $M + Na^+$).

^{1}H NMR of compound **3b**



ESI-MS of compound **3b**



Compound 3c. Solution A: 0.1 g 3b (0.00026 mol, 1 eq) was dissolved in 3 mL DMF. Then 0.102 g HBTU (0.00026 mol, 1 eq), 0.036 g HOBt (0.00026 mol, 1 eq), and 0.172 g DIPEA (0.0013 mol, 5 eq) were added. Solution B: 0.028 g 3 - azidopropylamine (0.00026 mol, 1 eq) was mixed with TFA ($V_{3-axi}d_{opropy}y_{lamine}$: VTFA = 1 : 1). Solution A was stirred for 10 min before adding Solution B. The reaction mixture was stirred at room temperature for 12 h. The crude product was purified by thin-layer chromatography (DCM : MeOH = 10 : 1) to obtain light yellow powder product 3c (yield 52 %).

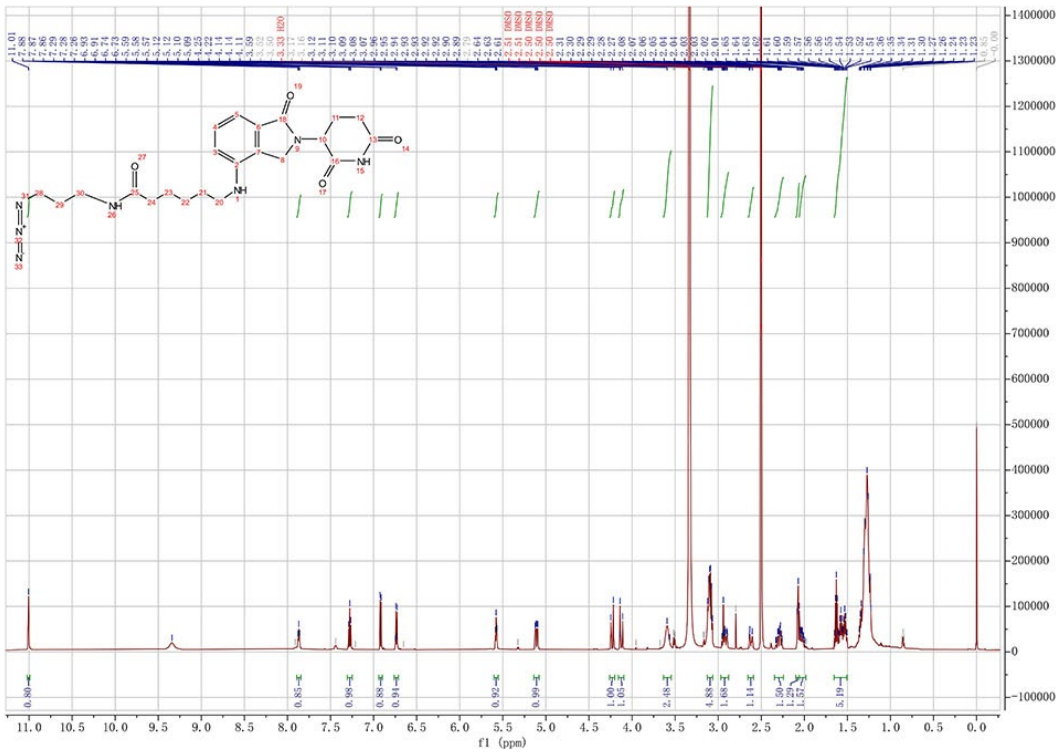
^{1}H NMR (600 MHz, DMSO - d_6): δ 11.01 (s, 1H), 7.87 (t, J = 5.6 Hz, 1H), 7.28 (t, J = 7.7 Hz, 1H), 6.92 (d, J = 7.4 Hz, 1H), 6.73 (d, J = 8.1 Hz, 1H), 5.58 (t, J = 5.5 Hz, 1H), 5.11 (dd, J = 13.3, 5.1 Hz, 1H), 4.23 (d, J = 17.1 Hz, 1H), 4.12 (d, J = 17.1 Hz, 1H), 3.58 (d, J = 18.2 Hz, 2H), 3.09 (dq, J = 12.8, 6.6 Hz, 5H), 2.97 – 2.88 (m, 2H), 2.65 – 2.59 (m, 1H), 2.29 (qd, J = 13.0, 4.6 Hz, 2H), 2.08 (d, J = 7.4 Hz, 1H), 2.06 – 1.98 (m, 2H), 1.65 – 1.51 (m, 5H).

^{13}C NMR (150 MHz, DMSO - d_6): δ 173.38, 172.57, 171.74, 169.37, 144.23, 132.51, 129.69, 126.92, 112.18, 110.38, 51.95, 48.90, 46.19, 43.07, 36.22, 35.86, 31.72, 28.93, 28.78, 26.78, 25.60, 23.31.

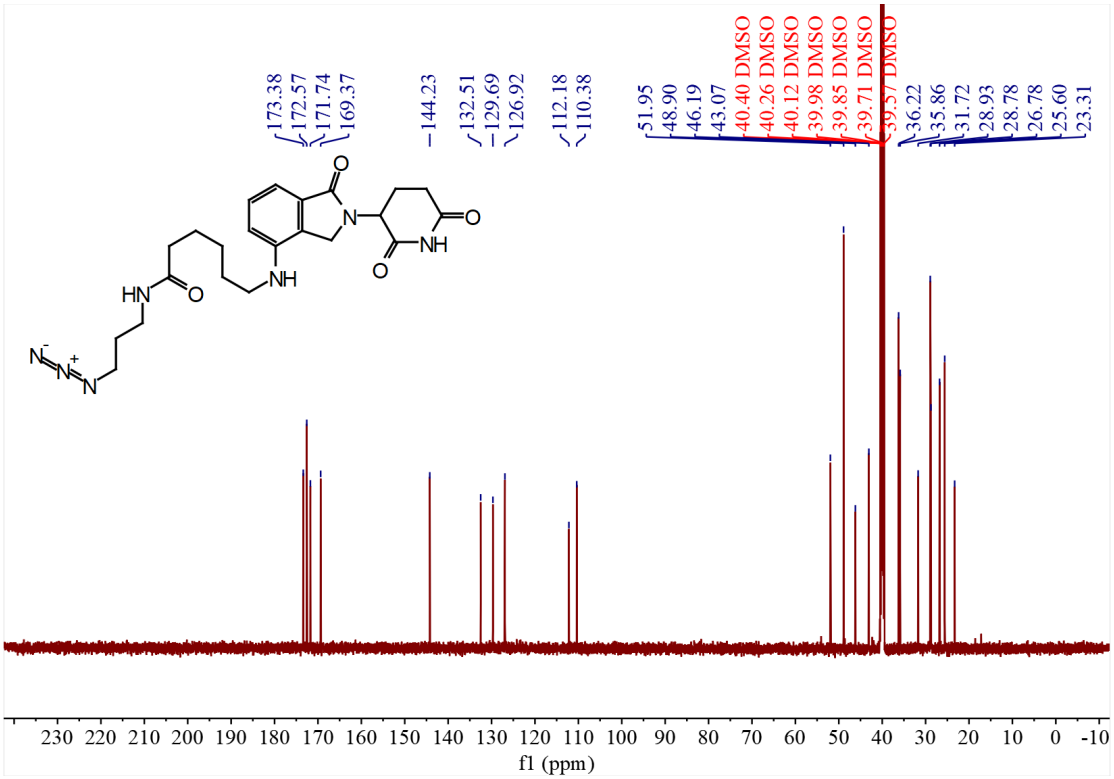
ESI-MS, $[C_{22}H_{29}N_7O_4]^+$. Theoretical value: 455.23. Measured value: 456.23 & 478.21 ($M + H^+$ & $M + Na^+$).

HRMS, $[C_{22}H_{29}N_7O_4]^+$. Theoretical value: 455.2281. Measured value: 478.2173 ($M + Na^+$).

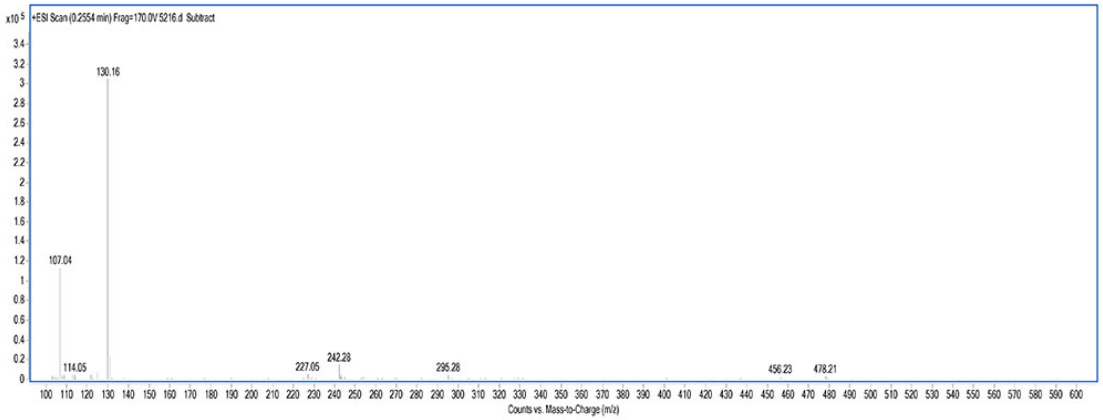
^{1}H NMR of compound **3c**



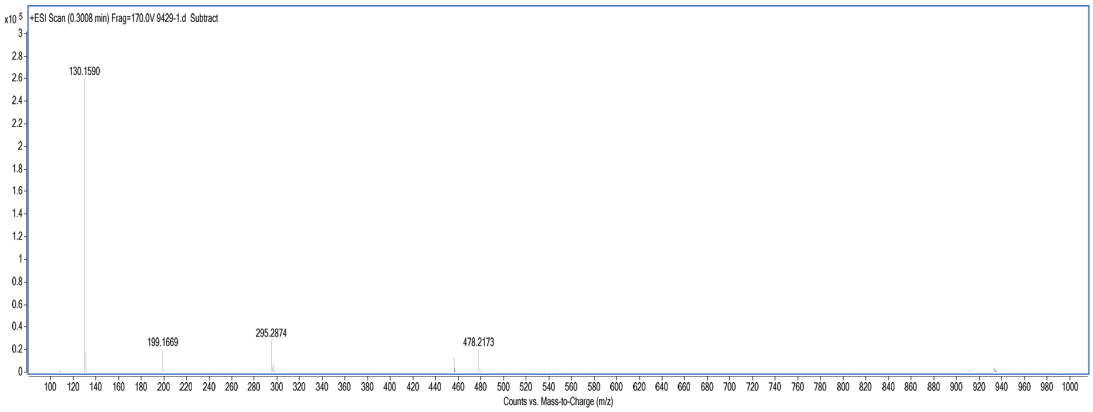
^{13}C NMR of compound **3c**



ESI-MS of compound **3c**



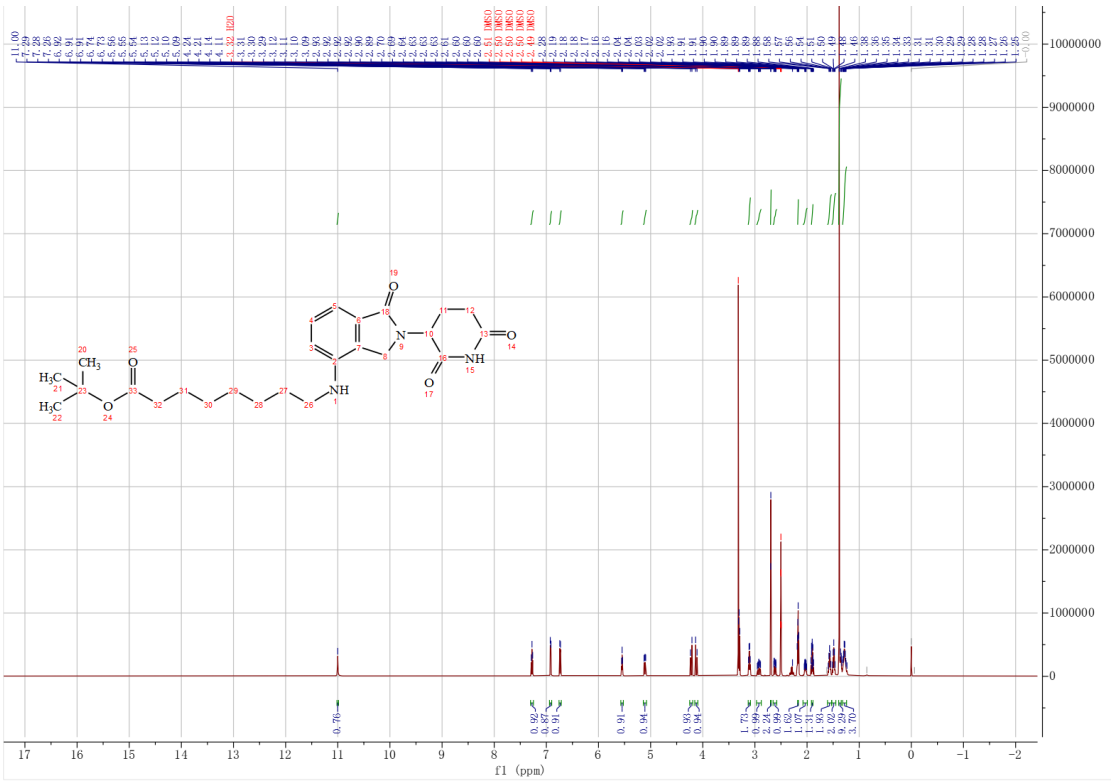
HRMS of compound **3c**



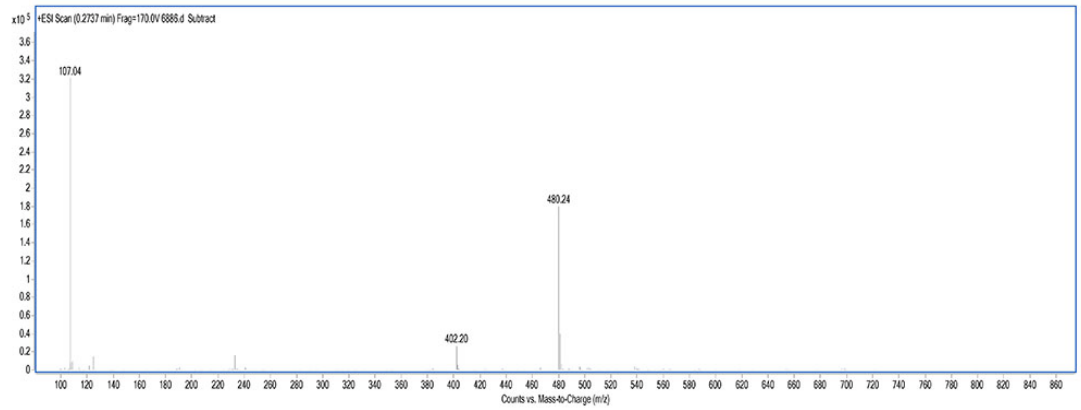
Compound 4a. 2 g lenalidomide (0.0077 mol, 1 eq) was dissolved in 30 mL NMP. Then 2.58 g *tert* - butyl 8 - bromooctanoate (0.0090 mol, 1.2 eq) and 3 g DIPEA (0.0232 mol, 3 eq) were added. The mixture was stirred at 120 °C for 15 h. Subsequent operations were consistent with the procedure for 3a. Pale yellow powder product 4a was obtained (yield 45 %). ^{1}H NMR (600 MHz, DMSO - d_6) δ 11.00 (s, 1H), 7.28 (t, J = 7.7 Hz, 1H), 6.94 – 6.90 (m, 1H), 6.74 (d, J = 8.0 Hz, 1H), 5.55 (t, J = 5.5 Hz, 1H), 5.11 (dd, J = 13.3, 5.2 Hz, 1H), 4.22 (d, J = 17.1 Hz, 1H), 4.12 (d, J = 17.1 Hz, 1H), 3.11 (q, J = 6.6 Hz, 2H), 2.92 (ddd, J = 17.3, 13.6, 5.4 Hz, 1H), 2.70 (d, J = 1.0 Hz, 2H), 2.62 (ddd, J = 17.3, 4.5, 2.4 Hz, 1H), 2.17 (d, J = 3.2 Hz, 2H), 2.03 (dtd, J = 12.6, 5.2, 2.2 Hz, 1H), 1.92 – 1.89 (m, 1H), 1.57 (p, J = 7.2 Hz, 2H), 1.49 (p, J = 7.3 Hz, 2H), 1.38 (s, 9H), 1.29 (ddt, J = 15.4, 11.7, 6.6 Hz, 4H).

ESI-MS, $[C_{25}H_{35}N_3O_5]^+$. Theoretical value: 457.26. Measured value: 480.24 ($M + Na^+$).

^{1}H NMR of compound **4a**



ESI-MS of compound **4a**

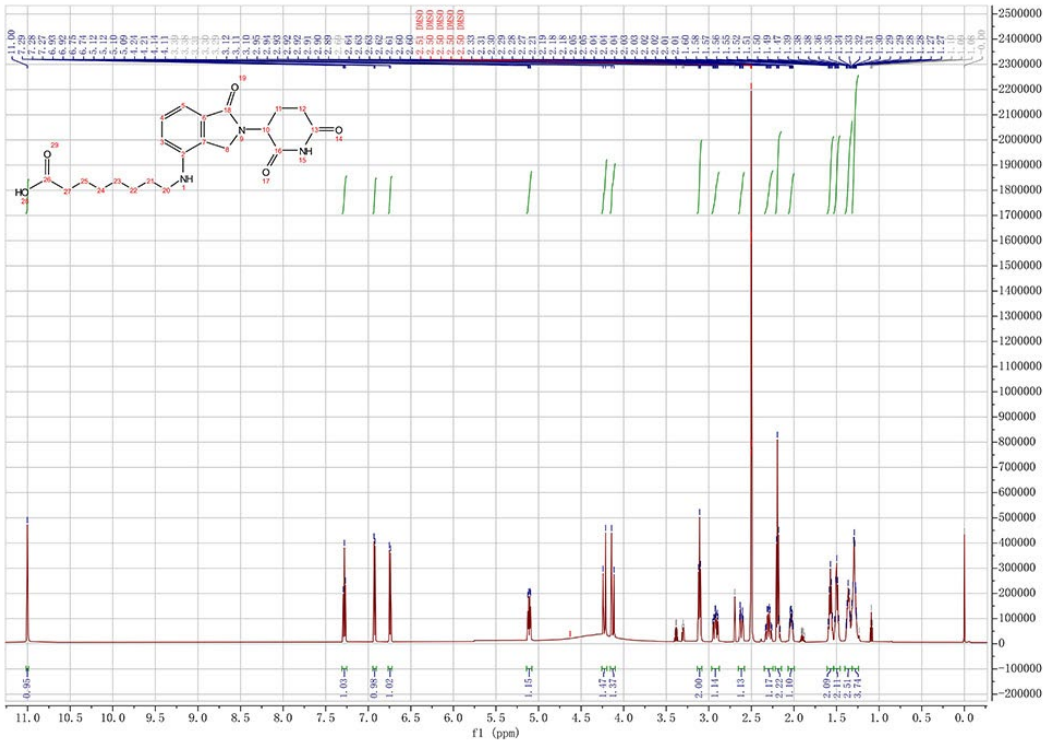


Compound 4b. 0.5 g 4a (0.0011 mol, 1 eq) was dissolved in 2 mL DCM. 5.61 g TFA (0.0492 mol, 45 eq) was added, and the mixture was stirred at room temperature for 2 h. Subsequent operations were consistent with the procedure for 3b. Pale yellow powder product 4b was obtained (yield 99 %).

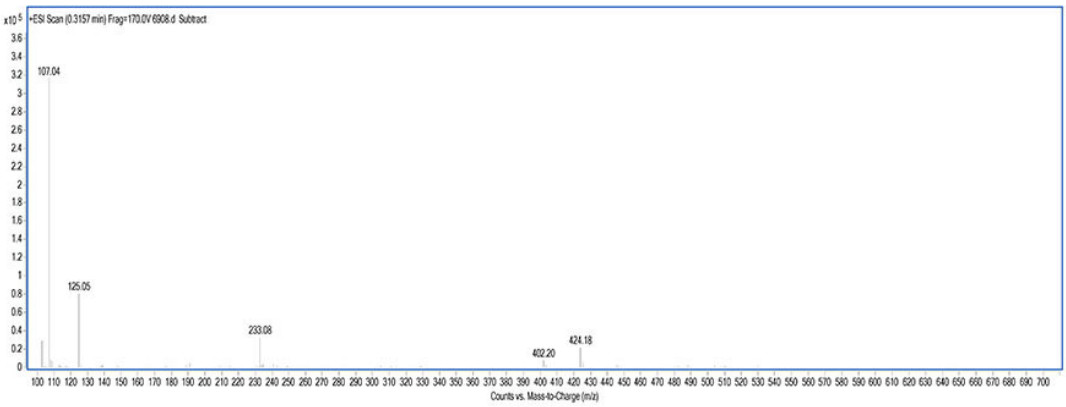
^{1}H NMR (600 MHz, DMSO - d_6): δ 11.00 (s, 1H), 7.28 (t, J = 7.7 Hz, 1H), 6.92 (d, J = 7.4 Hz, 1H), 6.74 (d, J = 8.0 Hz, 1H), 5.11 (dd, J = 13.3, 5.1 Hz, 1H), 4.23 (d, J = 17.1 Hz, 1H), 4.13 (d, J = 17.1 Hz, 1H), 3.11 (t, J = 7.1 Hz, 2H), 2.92 (ddd, J = 17.3, 13.6, 5.4 Hz, 1H), 2.65 – 2.58 (m, 1H), 2.30 (qd, J = 13.2, 4.4 Hz, 1H), 2.19 (t, J = 7.4 Hz, 2H), 2.03 (dtd, J = 12.8, 5.4, 2.4 Hz, 1H), 1.57 (p, J = 7.1 Hz, 2H), 1.50 (p, J = 7.3 Hz, 2H), 1.35 (dp, J = 11.9, 6.9, 6.0 Hz, 3H), 1.29 (td, J = 7.4, 6.2, 3.3 Hz, 4H).

ESI-MS, $[C_{21}H_{27}N_3O_5]^+$. Theoretical value: 401.20. Measured value: 402.20 & 424.18 (M + H^+ & M + Na^+).

^{1}H NMR of compound **4b**



ESI-MS of compound **4b**



Compound 4c. Solution A: 0.1 g 4b (0.00024 mol, 1 eq) was dissolved in 3 mL DMF. Then 0.094 g HBTU (0.00024 mol, 1 eq), 0.032 g HOBt (0.00024 mol, 1 eq), and 0.156 g DIPEA (0.00134 mol, 5 eq) were added. Solution B: 0.024 g 3 - azidopropylamine (0.00024 mol, 1 eq) was mixed with TFA ($V_{3-axi}d_{opropy}y_{lamine}$: VTFA = 1 : 1). Subsequent operations were consistent with the procedure for 3c. Pale yellow powder product 4c was obtained (yield 48 %).

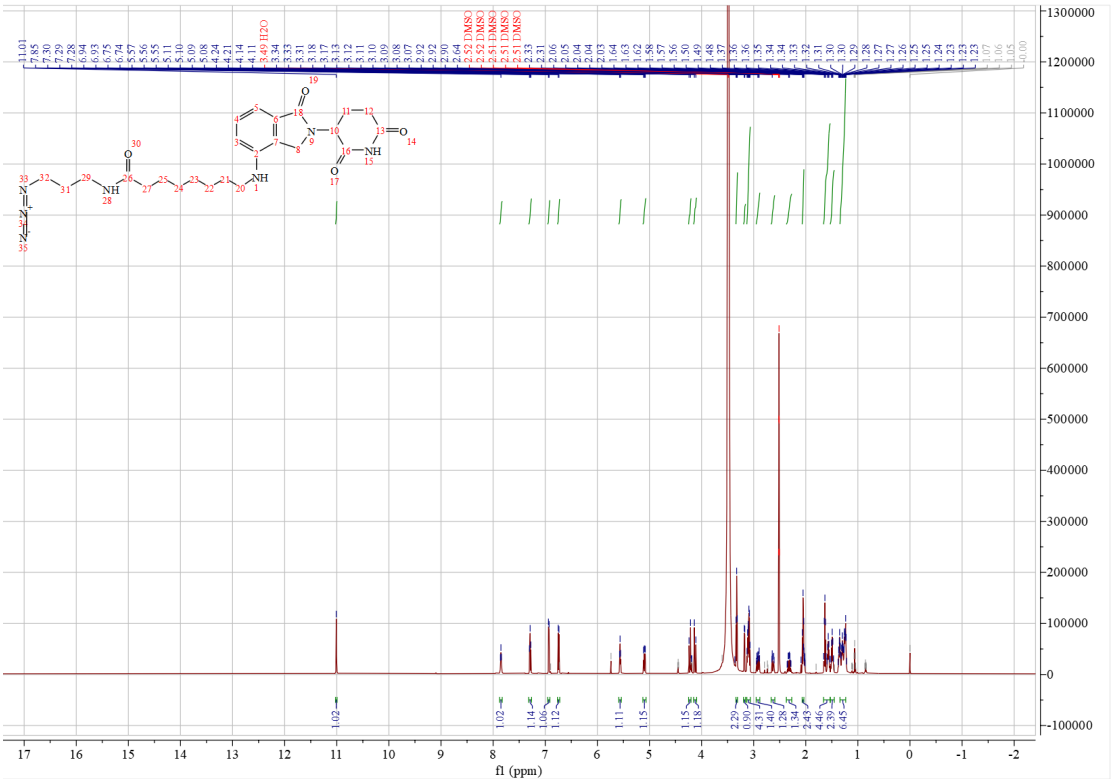
^{1}H NMR (600 MHz, DMSO - d_6) δ 11.01 (s, 1H), 7.85 (t, J = 5.8 Hz, 1H), 7.29 (t, J = 7.7 Hz, 1H), 6.93 (d, J = 7.4 Hz, 1H), 6.74 (d, J = 8.1 Hz, 1H), 5.56 (t, J = 5.5 Hz, 1H), 5.10 (dd, J = 13.3, 5.1 Hz, 1H), 4.23 (d, J = 17.1 Hz, 1H), 4.12 (d, J = 17.1 Hz, 1H), 3.33 (t, J = 6.8 Hz, 2H), 3.17 (d, J = 5.2 Hz, 1H), 3.13 – 3.06 (m, 4H), 2.92 (ddd, J = 17.4, 13.6, 5.5 Hz, 1H), 2.63 (dt, J = 17.3, 3.8 Hz, 1H), 2.32 (qd, J = 13.2, 4.4 Hz, 1H), 2.05 (t, J = 7.4 Hz, 2H), 1.60 (dp, J = 39.8, 7.0 Hz, 4H), 1.49 (p, J = 7.4 Hz, 2H), 1.34 – 1.23 (m, 6H).

^{13}C NMR (150 MHz, DMSO - d_6): δ 173.53, 172.77, 171.88, 169.52, 144.40, 132.66, 129.83, 127.07, 112.32, 110.51, 52.10, 49.05, 46.35, 43.34, 36.35, 36.00, 31.86, 29.29, 29.28, 29.14, 29.09, 27.18, 25.86, 23.45.

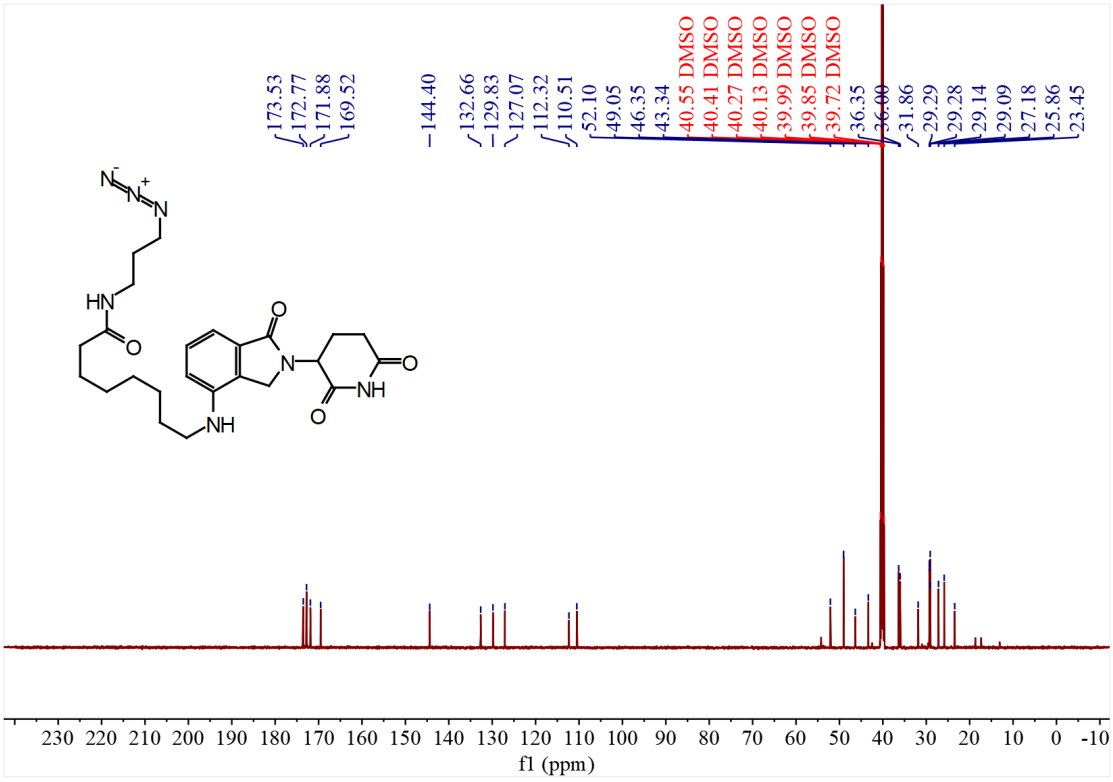
ESI-MS, $[C_{24}H_{33}N_7O_4]^+$. Theoretical value: 483.26. Measured value: 484.27 & 506.25 (M + H^+ & M + Na^+).

HRMS, $[C_{24}H_{33}N_7O_4]^+$. Theoretical value: 483.2594. Measured value: 506.2485 (M + Na^+).

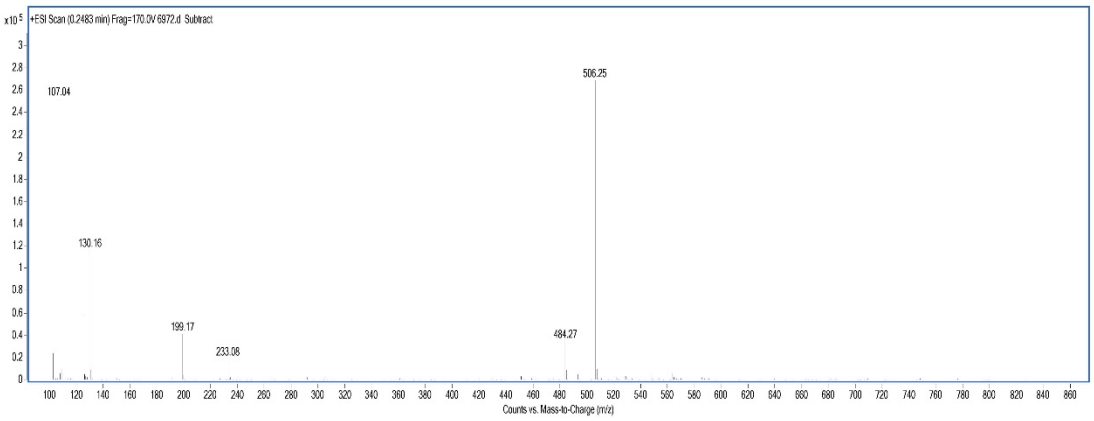
^{1}H NMR of compound **4c**



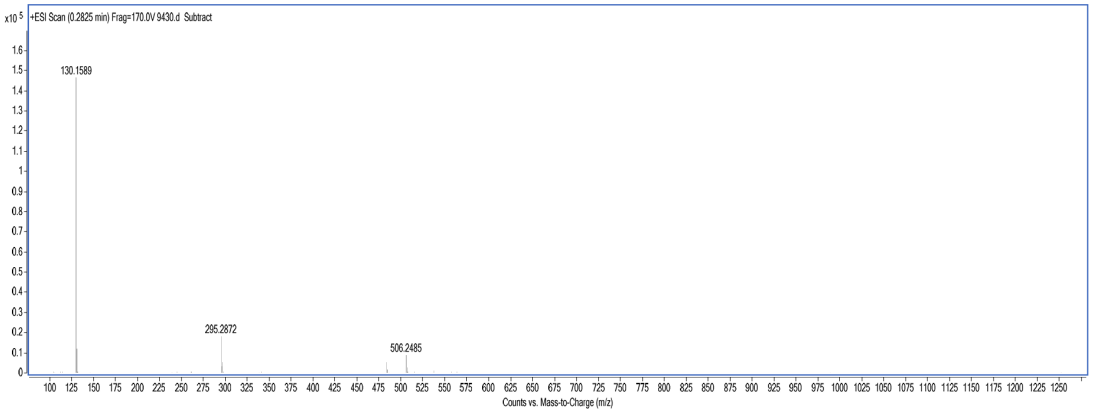
^{13}C NMR of compound 4c



ESI-MS of compound **4c**

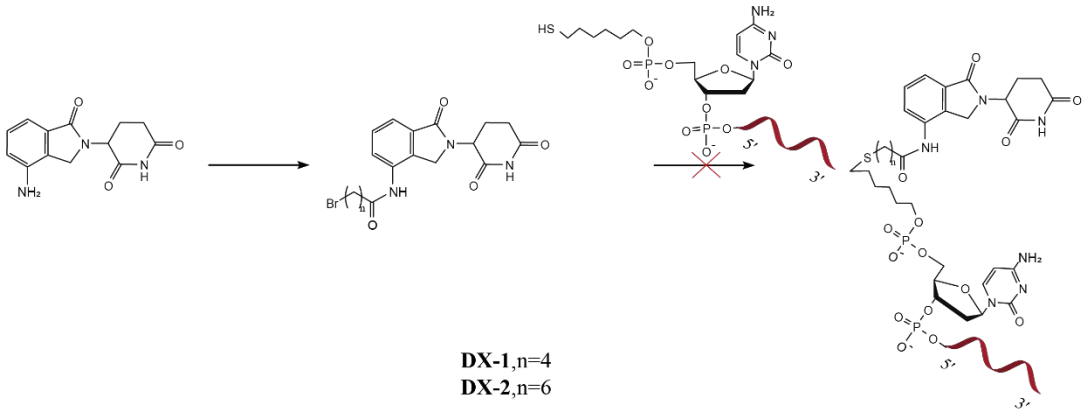


HRMS of compound **4c**

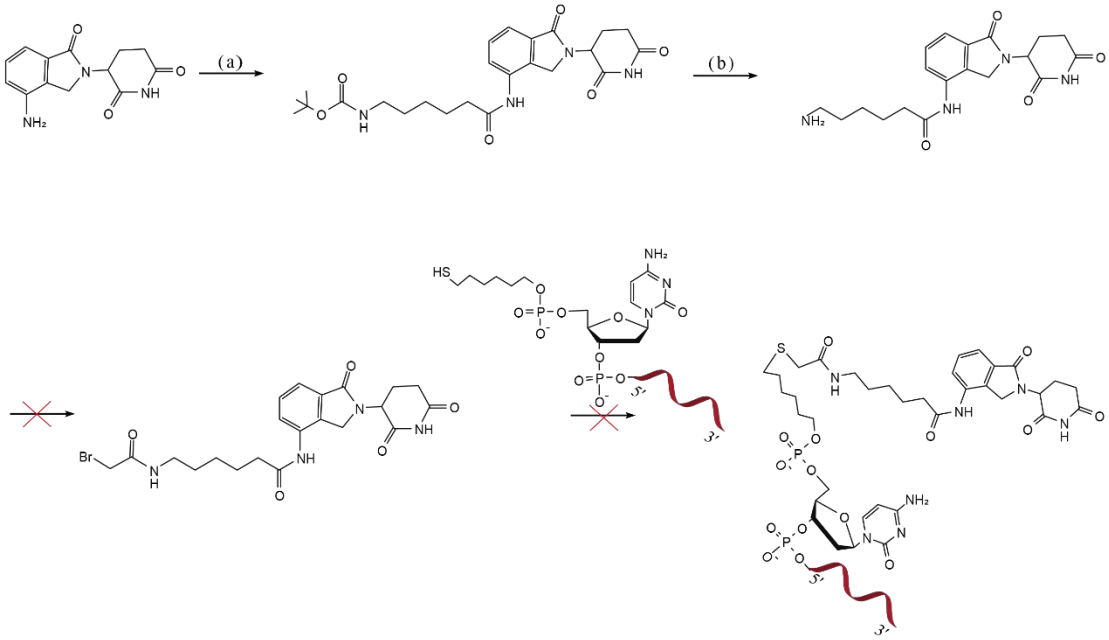


General procedure for the preparation of AS1411-PROTACs

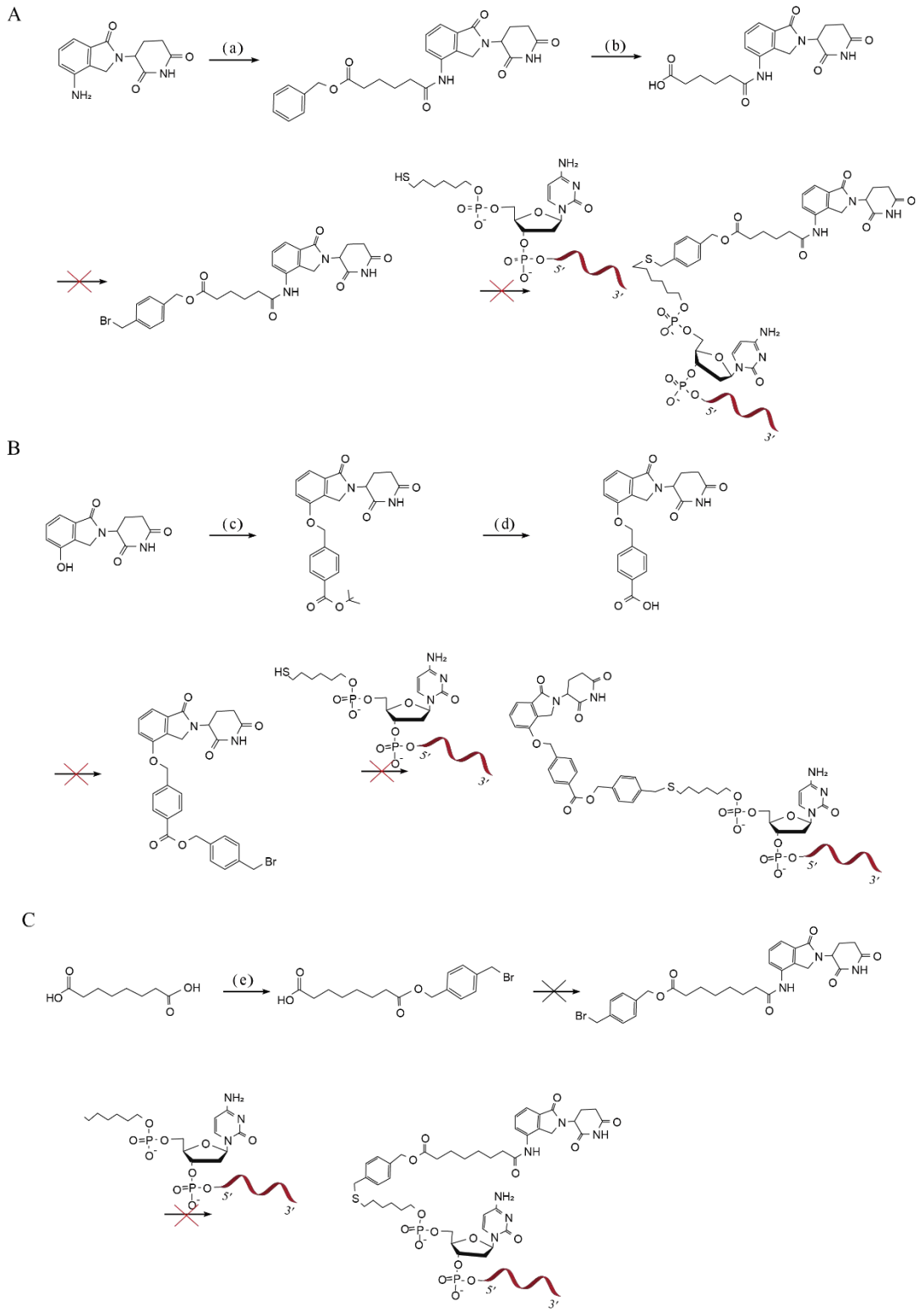
Preparation of 2.0 mol/L TEAA buffer: First, deionized water was used to configure 2.0 mol/L glacial acetic acid solution and 2.0 mol/L triethylamine solution. After mixing the two, the pH value was adjusted to 7.0, and an appropriate amount of deionized water was added to configure it to 2.0 mol/L. 200 μL TEAA, 250 μL DMSO, 50 μL Cu (II)-TBTA complex, 50 μL sodium ascorbate (10 mmol/L) were mixed at room temperature, and then 10 μL nucleic acid sequence (0.1 OD/μL, ddH_2O, which corresponds to the 5'-terminal alkynyl-modified AS1411) and 10 μL compound (1c, 2c, 3c, 4c) (n nucleic acid sequence : n compound = 1 : 20, DMSO) were added. After mixing, they were incubated at 37 °C for 2-12 h. The target chimeric molecule was purified by HPLC, followed by vacuum centrifugal drying to evaporate the solvent. Subsequently, it was enriched using a Sep-Pak column, desalted with approximately 20 mL of sterile water, and then eluted from the desalting column with about 3 mL of 70 % methanol solution. The chimeric molecule was quantified by an ultraviolet spectrophotometer, transferred to a 1.5 mL EP tube, and dried in a centrifugal dryer. After evaporation to dryness, it was stored at a low temperature (-20 ℃). 1c: yield 61 %; 2c: yield 59 %; 3c: yield 55 %; 4c: yield 62 %.



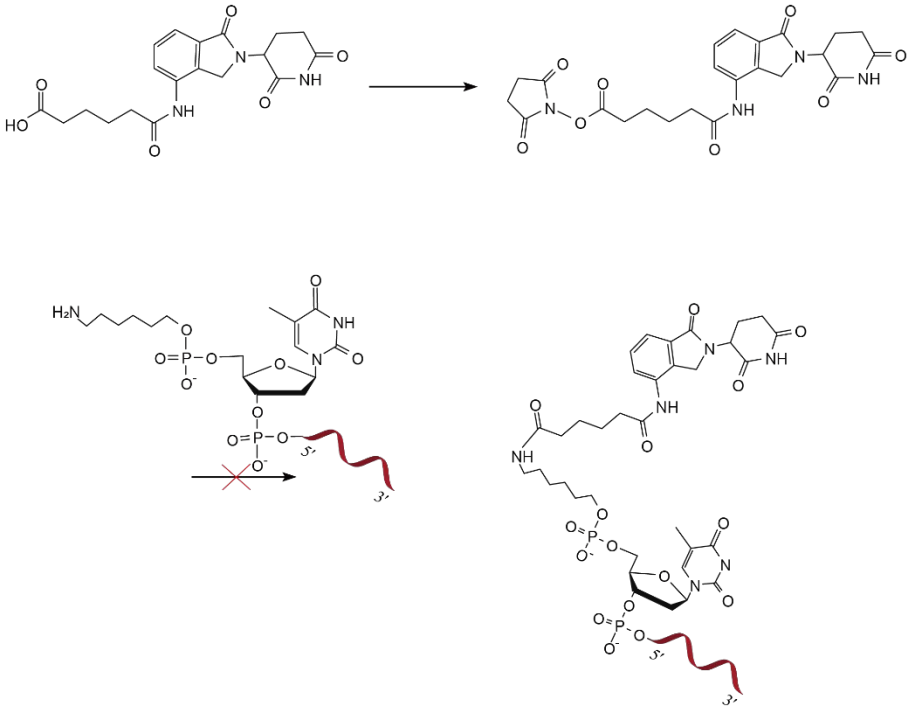
Scheme S1. A halogen-thiol-based synthetic strategy. Reagents and conditions: 5-Bromovaleric acid/7-Bromoheptanoic acid, $SOCl_2$, Anhydrous tetrahydrofuran, 80 °C, 7 h.



Scheme S2. A synthetic strategy based on halogenated acetyl bromide-thiol. Reagents and conditions: **(a)** 6-(boc-amino)caproic acid, pyridine, $POCl_3$, Acetonitrile, rt, 17 h; **(b)** TFA, DCM, rt, 2 h.

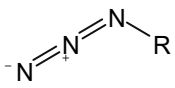
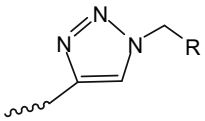
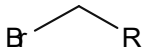
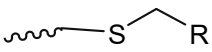
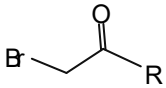
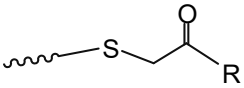
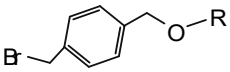
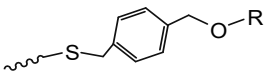
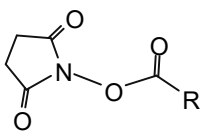
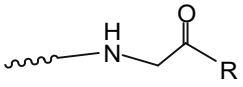


Scheme S3. A synthetic strategy based on bromobenzene-thiol. Reagents and conditions: **(a)** 6-oxo-6-phenylmethoxy-hexanoic acid, HATU, DIPEA, DMF, rt, overnight; **(b)** 10%Pd/C, MeOH, N_2, H_2, rt, 5 h; **(c)** (4-(Bromomethyl) phenyl) methanol, K_2CO_3, DMF, 50 °C, 17 h; **(d)** TFA, DCM, rt, 2 h; **(e)** (4-(Bromomethyl)phenyl) methanol, DMAP, EDAC, DCM, THF, rt, 3 h.



Scheme S4. A synthetic strategy based on N-hydroxy-succinimidyl esters (NHS-Esters) and amines. Reagents and conditions: NHS, EDAC, DCM, DMF, rt, overnight.

Table S1. Schematic diagram of the synthesis strategy of AS1411-lenalidomide targeting degraded chimeras.

Synthesis strategy	5′-AS1411 modification	Compound modifications	Predict product structure
Azides - alkynes			
Halogens - sulfhydryl groups			
Halogenated acetyl bromide - sulfhydryl groups			
Bromine - sulfhydryl			
N-hydroxysuccinimide - amino			

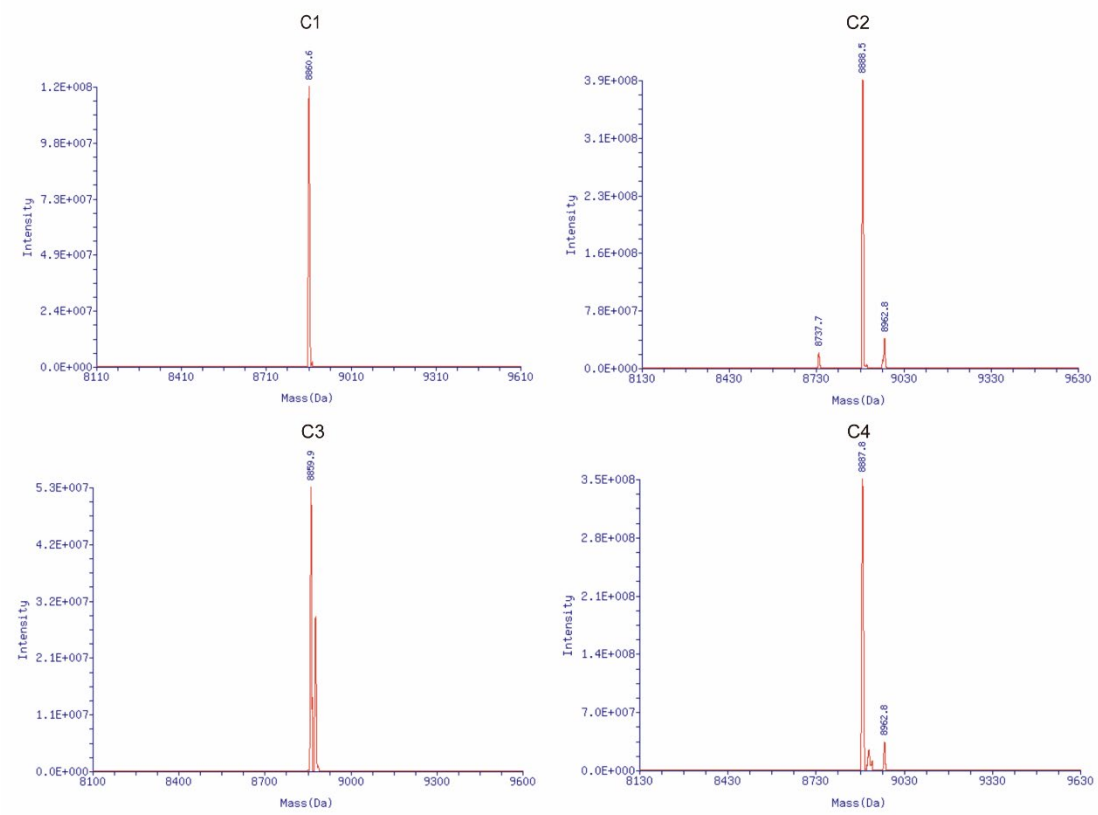


Figure S1. MOLDI-TOF-MS spectrum of C1, C2, C3 and C4.

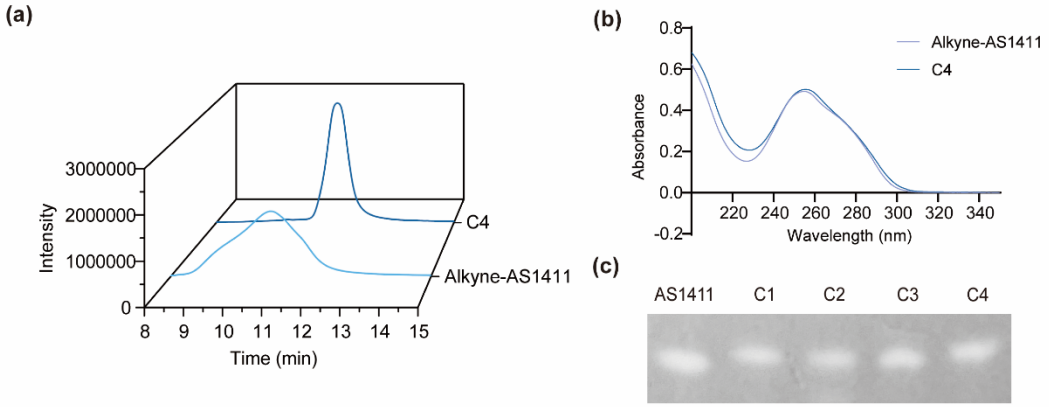


Figure S2. The characterizations of AS1411-PROTACs. **(a)** Synthesis monitoring of AS1411-PROTACs by HPLC. **(b)** The absorption spectrum of modified AS1411 and AS1411-PROTACs by UV-Vis spectrum. **(c)** Analysis of AS1411-PROTACs structure by N-PAGE electrophoresis.

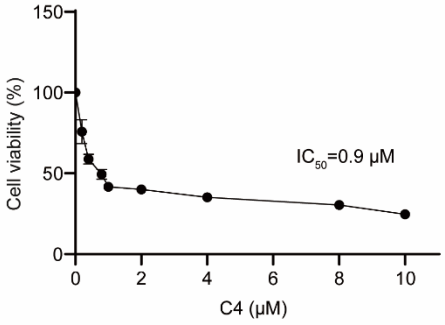


Figure S3. CCK-8 assay to detect the inhibitory effect of C4 on MCF-7 cells.

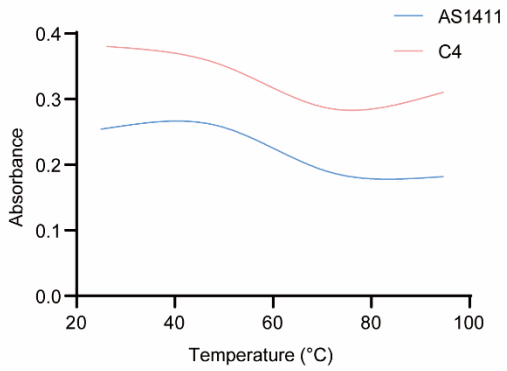


Figure S4. The sigmoidal curves responding to the Tm values of AS1411 and C4. (AS1411: Tm = 62.75 °C; C4: Tm = 66.62 °C)

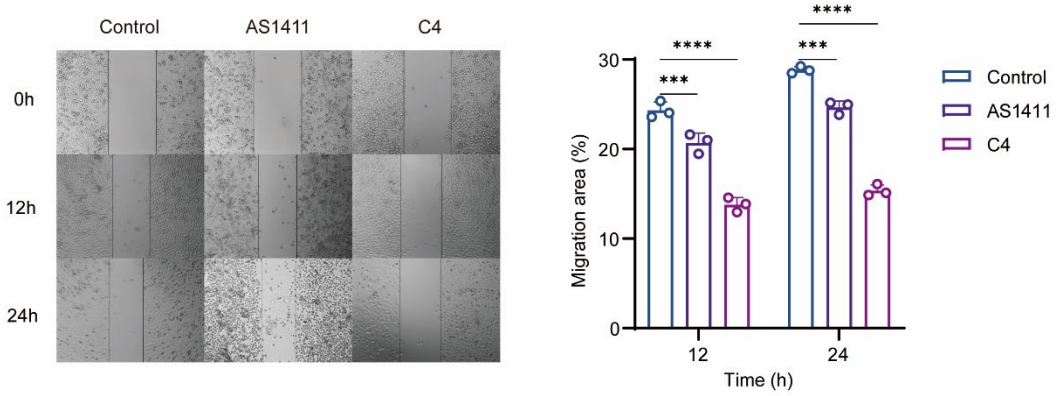


Figure S5. Cell scratch assay was used to detect the effect of AS1411 and C4 on the migration of MCF-7 cells. The colored circles (blue, purple, pink) correspond to the biological replicates of each experimental group (Control, AS1411, C4). ***P <0.001, ****P < 0.0001.

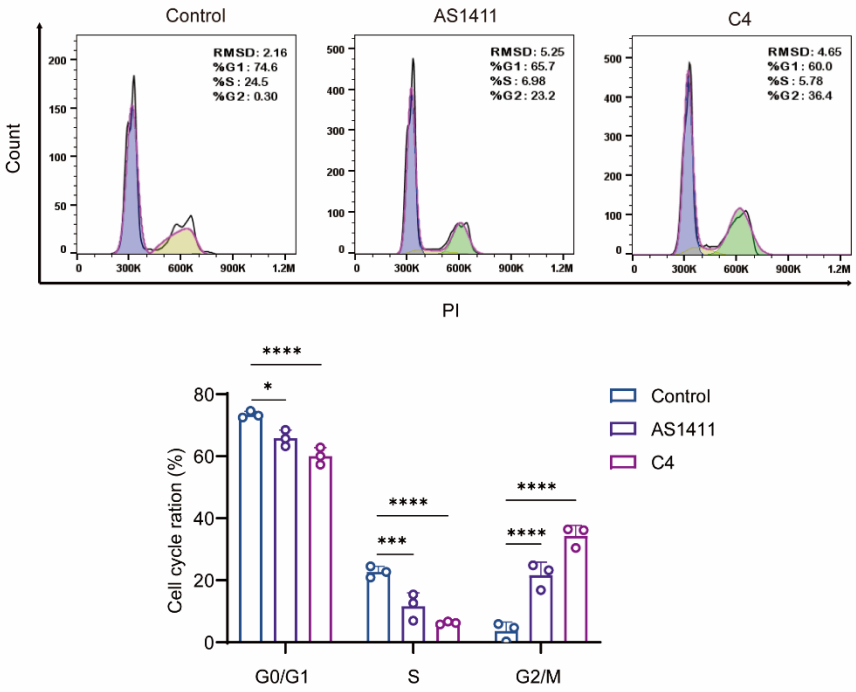


Figure S6. The cell cycle of MCF-7 cells treated with AS1411 and C4 detected by flow cytometry. The colored circles (blue, purple, pink) correspond to the biological replicates of each experimental group (Control, AS1411, C4). *P < 0.05, ***P <0.001, ****P < 0.0001.

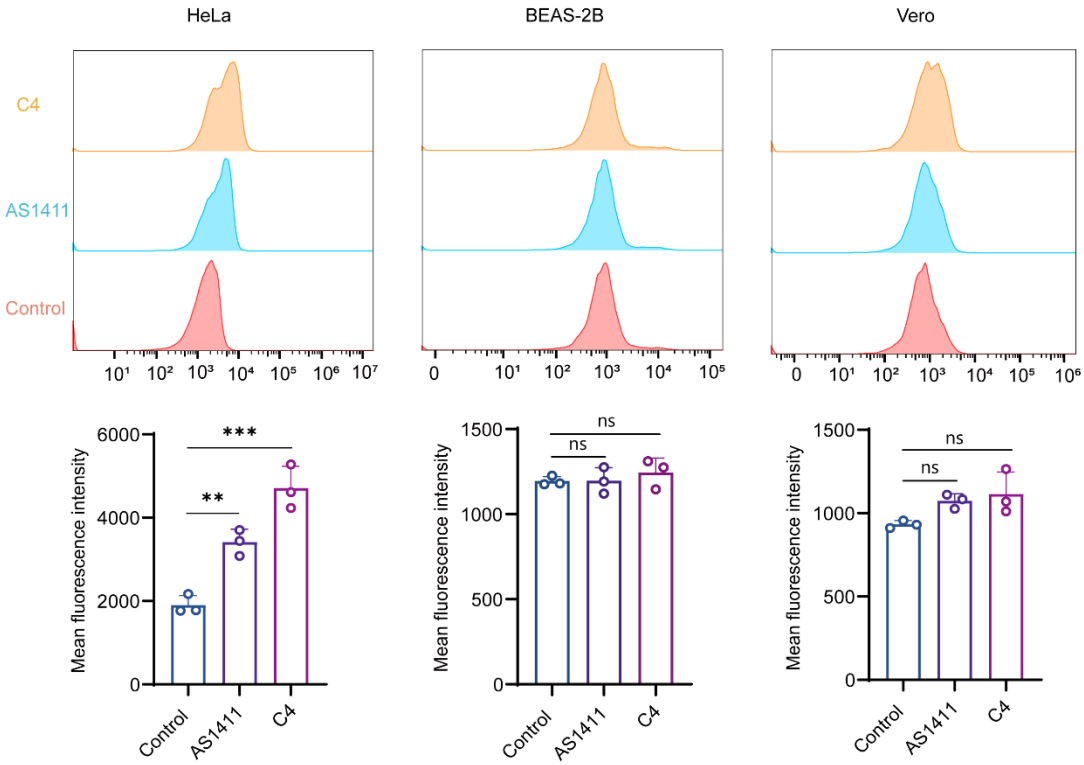


Figure S7. Representative fluorescence histograms of the AS1411 and C4 uptake pathway in HeLa, BEAS-2B and Vero cells. The colored circles (blue, purple, pink) correspond to the biological replicates of each experimental group (Control, AS1411, C4). ns: no significant difference. $^{**}P < 0.01$, $^{***}P < 0.001$.

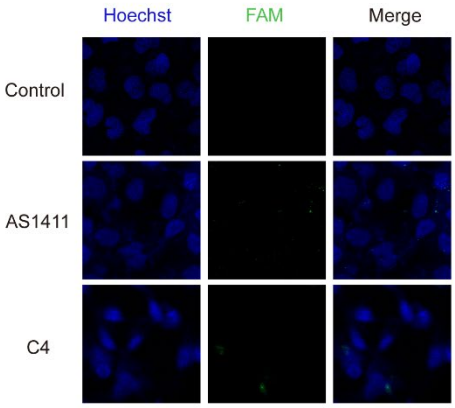


Figure S8. Confocal laser scanning microscope was used to observe the fluorescence of AS1411 and C4 uptake by BEAS-2B cells. "Hoechst" (blue) labels cell nuclei, and "FAM" (green) labels AS1411/C4.