Supporting Information

Utilizing PROTAC technology

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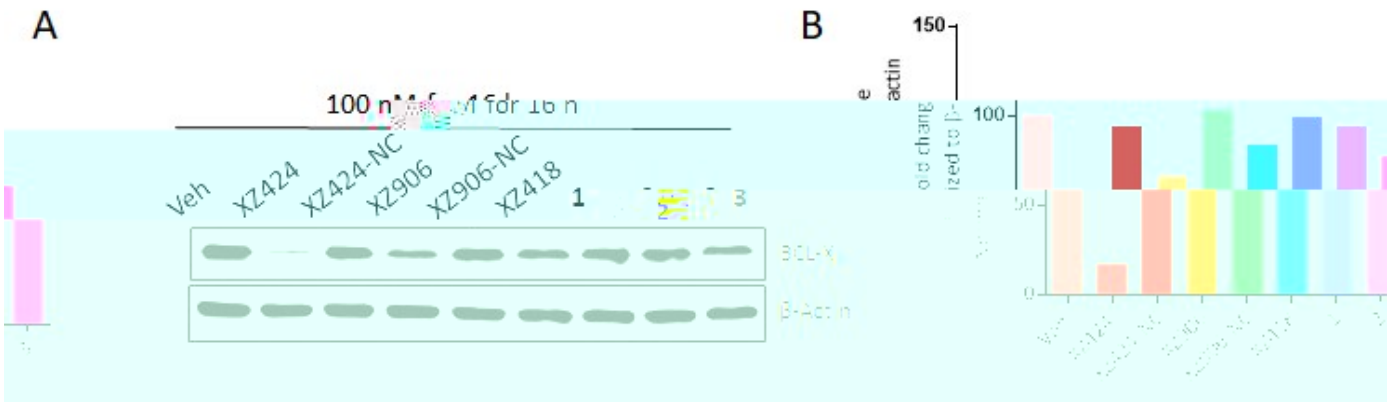


Figure S1 Western blot analysis of BCL-X_L degradation in MOLT-4 cells. (A) Effect of protein level change in MOLT-4 cells after 16 h treatment with 0.1% DMSO or XZ424, XZ424-NC, XZ906, XZ906-NC, XZ418, compounds **1**, **2**, and **3** at 100 nM. (B) BCL-X_L levels were normalized to β-actin.

Table S1 Cytotoxicity against MOLT-4 cells.

Compound	EC_{50} (nM)[a] (72h) MOLT-4
A-1155463	6.2
XZ906	123
XZ418	140
XZ424	51
XZ424-NC	684
XZ906-NC	832

[a]Each value was reproduced in three experiments.

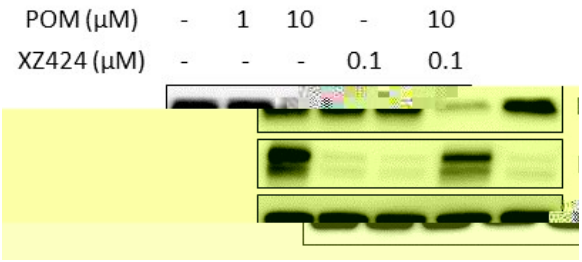


Figure S2 Western blot analysis of pomalidomide (POM), XZ-424, or combination induced changes of IZFK1 and BCL-X_L protein levels in MOLT-4 cells.

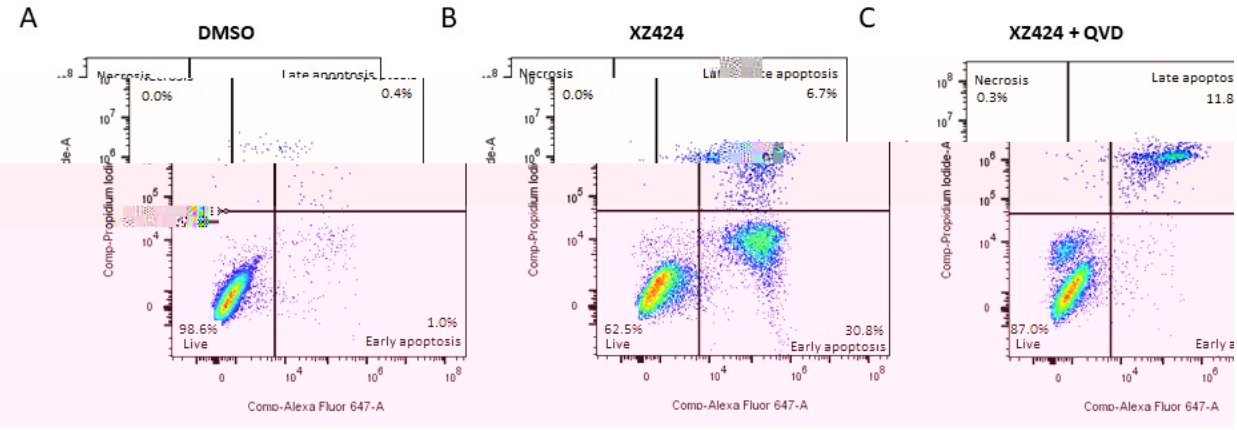
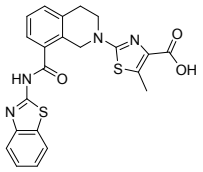


Fig. S3 (A, B, and C) Flow cytometry analysis of apoptosis using Annexin-V and PI staining. Cells were treated with DMSO and XZ424 (100 nM) for 24 h, XZ424 (100 nM) significantly increased the percentage of apoptotic cells and QVD (10 μM) pre-treatment for 4 h inhibited the apoptosis induced by XZ424. Data are presentative figures of two independent experiments.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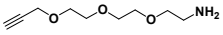
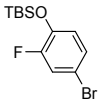
N
S
OH
HN
O
N
S


O
O
O
NH2

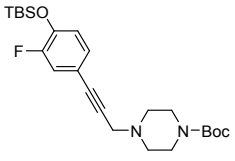
Experimental Section: Chemistry

General Methods. THF, DCM, and DMF were obtained via a solvent purification system by filtering through two columns packed with activated alumina and 4 Å molecular sieve, respectively. All other chemicals obtained from commercial sources were used without further purification. Flash chromatography was performed using silica gel (230–400 mesh) as the stationary phase. Reaction progress was monitored by thin layer chromatography (silica-coated glass plates) and visualized by UV light, and/or by LC-MS. ^{1}H NMR spectra were recorded in $CDCl_3$ or CD_3OD at 400 MHz or 600 MHz. Chemical shifts δ are given in ppm using tetramethylsilane as an internal standard. Multiplicities of NMR signals are designated as singlet (s), broad singlet (br s), doublet (d), doublet of doublets (dd), triplet (t), quartet (q), and multiplet (m). All final compounds for biological testing were of ≥95.0% purity as analyzed by LC–MS, performed on an Advion AVANT LC system with the expression CMS using a Thermo Accucore™ Vanquish™ C18+ UHPLC Column (1.5 µm, 50 x 2.1 mm) at 40 °C. Gradient elution was used for UHPLC with a mobile phase of acetonitrile and water containing 0.1% formic acid. High resolution mass spectra (HRMS) were recorded on a Bruker Impact II QTOF mass spectrometer.



S2

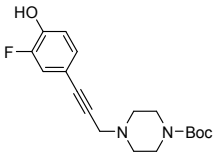
(4-Bromo-2-fluorophenoxy)(*tert*-butyl)dimethylsilane (S2): A mixture of 4-bromo-2-fluorophenol **S1** (1.0 g, 5.24 mmol), TBSCl (1.03 g, 6.83 mmol), and imidazole (713 mg, 10.48 mmol) in DMF (20 mL) was stirred at room temperature for 16 h. Then it was diluted with water (40 mL) and extracted with ethyl acetate. The organic phase was washed with water x1, brine x1, dried over Na_2SO_4, filtered and evaporated to dryness. The residue was further purified by column chromatography to afford the title compound as colorless oil (1.60 g, yield 100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (dd, J = 10.1, 2.4 Hz, 1H), 7.15–7.07 (m, 1H), 6.79 (t, J = 8.7 Hz, 1H), 1.00 (s, 9H), 0.19 (d, J = 0.9 Hz, 6H) ppm.



S4

***tert*-Butyl 4-(3-(4-(*tert*-butyldimethylsilyloxy)-3-fluorophenyl)prop-2-ynyl)piperazine-1-carboxylate (S4):** A mixture of compound **S2** (612 mg, 2.0 mmol), compound **S3** (448 mg, 2.0 mmol), $Pd(PPh_3)_4$ (68 mg, 0.06 mmol), CuI (12 mg, 0.06 mmol), and triethylamine (700 µL, 4.2 mmol) were stirred in DMF (15 mL) at 100 °C under an argon atmosphere for 20 h. The reaction mixture was poured into water (30 mL) and extracted with ethyl acetate. The

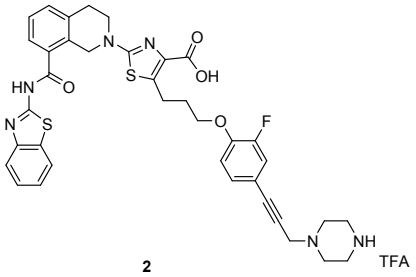
organic phase was washed with water x1, brine x1, dried over Na_2SO_4, filtered and evaporated to dryness. The residue was further purified by column chromatography to afford the title compound (220 mg, yield 24%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 (dd, *J* = 11.1, 2.0 Hz, 1H), 7.10–7.04 (m, 1H), 6.83 (t, *J* = 8.5 Hz, 1H), 3.58–3.41 (m, 6H), 2.68–2.50 (m, 4H), 1.47 (s, 9H), 1.00 (s, 9H), 0.19 (d, *J* = 0.9 Hz, 6H) ppm. LC-MS (ESI): m/z 449.3 $[M+H]^+$.



S5

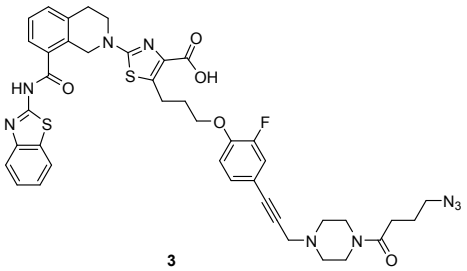
***tert*-Butyl 4-(3-(3-fluoro-4-hydroxyphenyl)prop-2-ynyl)piperazine-1-carboxylate (S5):** To a solution of compound **S4** (180 mg, 0.40 mmol) in THF (5 mL) was added TBAF solution (1.0 M in THF, 0.8 mL). The reaction mixture was stirred at room temperature for 30 min

2H), 4.00 (t, *J* = 6.2 Hz, 2H), 3.79–3.65 (m, 2H), 3.57–3.49 (m, 6H), 3.28 (t, *J* = 7.3 Hz, 2H), 3.09–2.88 (m, 2H), 2.74–2.46 (m, 4H), 2.30–2.06 (m, 2H), 1.46 (s, 9H) ppm. LC-MS (ESI): m/z 811.3 $[M+H]^+$.



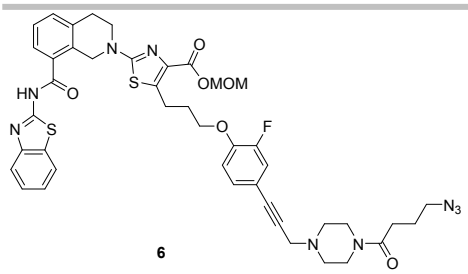
2

2-(8-(Benzo[*d*]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1*H*)-yl)-5-(3-(2-fluoro-4-(3-(piperazin-1-yl)prop-1-ynyl)phenoxy)propyl)thiazole-4-carboxylic acid trifluoroacetate (2): A mixture of compound **1** (130 mg, 0.16 mmol) and TFA (1.0 mL, 13.1 mmol) in DCM (3 mL) was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure and the crude product was crystallized in a mixture of Et_2O and MeOH to give the title compound as a pale yellow solid (110 mg, yield 83%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.93 (d, *J* = 7.7 Hz, 1H), 7.78 (d, *J* = 7.7 Hz, 1H), 7.63 (d, *J* = 7.2 Hz, 1H), 7.52–7.28 (m, 5H), 7.18–7.07 (m, 2H), 6.99 (t, *J* = 8.7 Hz, 1H), 4.91 (s, 2H), 4.07 (t, *J* = 6.1 Hz, 2H), 3.89–3.77 (m, 2H), 3.62 (s, 2H), 3.28–3.20 (m, 6H), 3.09–3.05 (m, 2H), 2.93–2.80 (m, 4H), 2.20–2.07 (m, 2H) ppm. LC-MS (ESI): m/z 711.2 $[M+H]^+$.



3

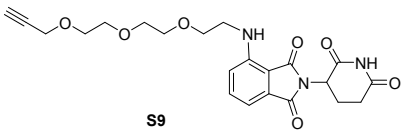
5-(3-(4-(3-(4-(4-Azidobutanoyl)piperazin-1-yl)prop-1-ynyl)-2-fluorophenoxy)propyl)-2-(8-(benzo[*d*]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)thiazole-4-carboxylic acid (3): 4-Azidobutanoyl chloride (17.7 mg, 0.12 mmol) was prepared according to the literature[2]. It was dissolved in DCM (660 µL) and added dropwise into a mixture of compound **2** (100 mg, 0.12 mmol) and triethylamine (157 µL, 1.13 mmol) in DCM (4 mL) at room temperature. The reaction mixture was stirred for 10 min and quenched with MeOH (1 mL), diluted with water, and extracted with DCM. The organic layer was washed with water x1, brine x1, dried over Na_2SO_4, filtered and evaporated to dryness. The crude product was crystallized in MeOH to give the title compound as pale yellow solid (85 mg, yield 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, *J* = 7.8 Hz, 1H), 7.69–7.59 (m, 2H), 7.44–7.29 (m, 4H), 7.15–7.05 (m, 2H), 6.82 (t, *J* = 8.7 Hz, 1H), 4.95 (s, 2H), 4.04 (t, *J* = 6.3 Hz, 2H), 3.81–3.64 (m, 6H), 3.44–3.24 (m, 6H), 3.06 (t, *J* = 5.9 Hz, 2H), 2.89–2.58 (m, 4H), 2.42 (t, *J* = 7.2 Hz, 2H), 2.22–2.11 (m, 2H), 1.99–1.87 (m, 2H) ppm. LC-MS (ESI): m/z 822.3 $[M+H]^+$.



6

Methoxymethyl 5-(3-(4-(3-(4-(4-azidobutanoyl)piperazin-1-yl)prop-1-yn-1-yl)-2-fluorophenoxy)propyl)-2-(8-(benzo[*d*]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1*H*)-yl)thiazole-4-carboxylate (6)

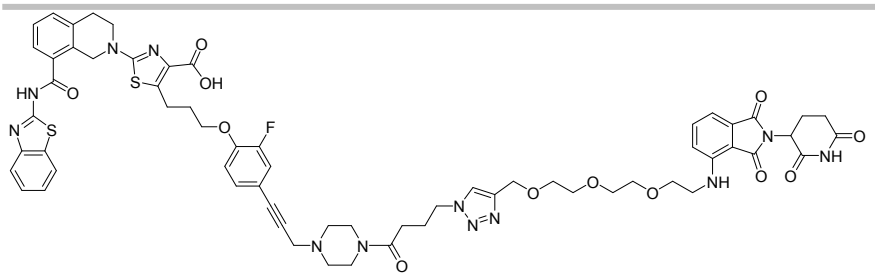
Compound **3** (26 mg, 0.032 mmol), Na_2CO_3 (4.1 mg, 0.039 mmol) and chloromethyl methyl ether (2.8 mg, 0.035 mmol) were stirred in DMF (2 mL) for 24 h. Then it was poured into water and extracted with ethyl acetate. The organic phase was washed with water x1, brine x1, dried over Na_2SO_4, filtered and evaporated to dryness. The resulting mixture was purified by silica gel flash column chromatography using DCM and MeOH as eluents to afford the title compound (16.0 mg, yield 84%, 8.0 mg **3** was recovered, yield was calculated based on recovered starting material). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90–7.77 (m, 1H), 7.54 (d, *J* = 7.6 Hz, 1H), 7.37–7.25 (m, 4H), 7.18 (t, *J* = 7.6 Hz, 1H), 7.12–7.04 (m, 2H), 6.81 (t, *J* = 8.4 Hz, 1H), 5.34 (s, 2H), 4.88 (s, 2H), 4.03 (t, *J* = 6.2 Hz, 2H), 3.81 (t, *J* = 6.0 Hz, 2H), 3.76–3.64 (m, 2H), 3.63–3.49 (m, 4H), 3.44 (s, 3H), 3.36 (t, *J* = 6.3 Hz, 2H), 3.25 (t, *J* = 7.4 Hz, 2H), 3.00 (t, *J* = 5.9 Hz, 2H), 2.71–2.53 (m, 4H), 2.40 (t, *J* = 7.2 Hz, 2H), 2.23–2.06 (m, 2H), 1.98–1.84 (m, 2H) ppm. LC-MS (ESI): m/z 866.3 $[M+H]^+$.



S9

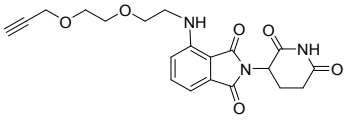
2-(2,6-Dioxopiperidin-3-yl)-4-(2-(2-(2-(prop-2-ynyloxy)ethoxy)ethoxy)ethylamino)isoindoline-1,3-dione (S9)

Compound **S7** (100 mg, 0.36 mmol), amine **S8** (68 mg, 0.36 mmol), and DIPEA (120 µL, 0.72 mmol) in DMF (4 mL) were stirred at 90 °C for 16 h. The reaction mixture was poured into water and extracted with ethyl acetate. The organic phase was washed with water x1, brine x1, dried over Na_2SO_4, filtered and evaporated to dryness. The residue was further purified by column chromatography to afford the title compound as a green solid (95 mg, yield 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.64–7.34 (m, 1H), 7.10 (d, *J* = 7.1 Hz, 1H), 6.93 (d, *J* = 8.6 Hz, 1H), 6.67–6.11 (m, 1H), 4.91 (dd, *J* = 12.1, 5.3 Hz, 1H), 4.20 (d, *J* = 2.2 Hz, 2H), 3.83–3.60 (m, 10H), 3.55–3.40 (m, 2H), 2.99–2.60 (m, 3H), 2.43 (t, *J* = 2.1 Hz, 1H), 2.21–2.03 (m, 1H) ppm. LC-MS (ESI): m/z 444.1 $[M+H]^+$.



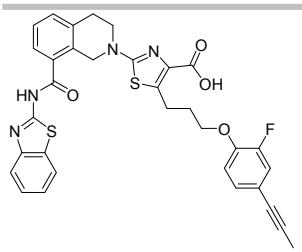
XZ906

2-(8-(Benzo[*d*]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1*H*)-yl)-5-(3-(4-(3-(4-(4-(4-((2-(2-(2-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-ylamino)ethoxy)ethoxy)ethoxy)methyl)-1*H*-1,2,3-triazol-1-yl)butanoyl)piperazin-1-yl)prop-1-ynyl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid (XZ906): To a mixture of compound **3** (18 mg, 0.022 mmol) and compound **S9** (10 mg, 0.023 mmol) in ^{t}BuOH (1 mL) under an Argon atmosphere was added $CuSO_4 \cdot 5H_2O$ (1.0 mg, 0.004 mmol) and sodium L-ascorbate (0.8 mg, 0.004 mmol) in water (0.2 mL). The mixture was stirred at 65 °C for 16 h and cooled to room temperature. Then it was poured into water and extracted with DCM. The organic phase was washed with brine x1, dried over Na_2SO_4, filtered and evaporated to dryness. The crude product was purified using reverse phase preparative HPLC to give the title compound as yellow solid (4.0 mg, yield 14%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.17 (s, 1H), 7.92–7.81 (m, 2H), 7.71 (d, *J* = 7.2 Hz, 1H), 7.54 (s, 1H), 7.50–7.42 (m, 2H), 7.37 (t, *J* = 7.6 Hz, 1H), 7.33–7.25 (m, 2H), 7.15–7.03 (m, 3H), 6.87 (d, *J* = 8.5 Hz, 1H), 6.79 (t, *J* = 8.4 Hz, 1H), 6.48 (s, 1H), 4.98–4.85 (m, 3H), 4.68–4.53 (m, 2H), 4.37 (t, *J* = 6.3 Hz, 2H), 4.05–3.94 (m, 4H), 3.84–3.54 (m, 16H), 3.42 (t, *J* = 5.2 Hz, 2H), 3.32–3.09 (m, 6H), 3.02 (t, *J* = 6.1 Hz, 2H), 2.89–2.67 (m, 3H), 2.33–2.08 (m, 7H) ppm. LC-MS (ESI): m/z 1265.3 $[M+H]^+$. HRMS *m/z* calcd for $C_{63}H_{66}FN_{12}O_{12}S_2$ 1265.4349, found 1265.4342 $[M+H]^+$.



4

2-(2,6-Dioxopiperidin-3-yl)-4-((2-(2-(prop-2-yn-1-yloxy)ethoxy)ethyl)amino)isoindoline-1,3-dione (4): Compound **S7** (107 mg, 0.39 mmol), amine **S10** (84 mg, 0.58 mmol), and DIPEA (193 µL, 1.17 mmol) in DMF (5 mL) were stirred at 90 °C for 16 h. The reaction mixture was poured into water and extracted with ethyl acetate. The organic phase was washed with water x1, brine x1, dried over Na_2SO_4, filtered and evaporated to dryness. The residue was purified by column chromatography using ethyl acetate and hexanes as eluents to afford the title compound as a green solid (50 mg, yield 32%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.62–7.35 (m, 1H), 7.11 (d, *J* = 7.1 Hz, 1H), 6.93 (d, *J* = 8.5 Hz, 1H), 4.92 (dd, *J* = 11.9, 5.3 Hz, 1H), 4.21 (d, *J* = 2.3 Hz, 2H), 3.78–3.66 (m, 6H), 3.49 (t, *J* = 5.4 Hz, 2H), 2.93–2.68 (m, 3H), 2.48–2.41 (m, 1H), 2.18–2.09 (m, 1H) ppm. LC-MS (ESI): m/z 400.0 $[M+H]^+$.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S
N
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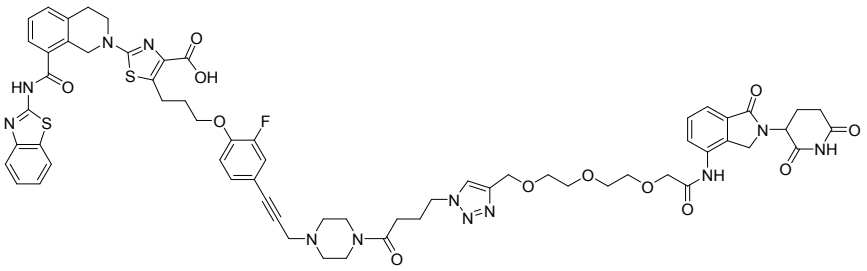
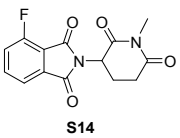
OH
O
F

1H), 7.49 (t, *J* = 7.7 Hz, 1H), 5.20 (dd, *J* = 13.3, 5.1 Hz, 1H), 4.45 (s, 2H), 4.14 (d, *J* = 3.4 Hz, 2H), 3.96 (s, 2H), 3.83–3.57 (m, 8H), 2.98–2.70 (m, 2H), 2.49–2.28 (m, 2H), 2.27–2.13 (m, 1H) ppm. LC-MS (ESI): m/z 444.2 $[M+H]^+$.



XZ418

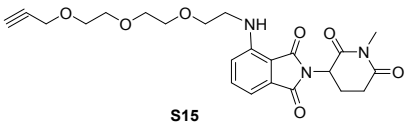
2-(8-(Benzo[*d*]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1*H*)-yl)-5-(3-(4-(3-(4-(4-(4-((2-(2-(2-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-ylamino)-2-oxoethoxy)ethoxy)ethoxy)methyl)-1*H*-1,2,3-triazol-1-yl)butanoyl)piperazin-1-yl)prop-1-ynyl)-2-fluorophenoxy)propyl)thiazole-4-carboxylic acid (XZ418): To a mixture of compound **6** (12.0 mg, 0.014 mmol) and compound **S13** (7.4 mg, 0.017 mmol) in ^{t}BuOH-THF (1:3, v/v, 4 mL) under an Argon atmosphere was added $CuSO_4 \cdot 5H_2O$ (0.70 mg, 0.0028 mmol) and sodium L-ascorbate (0.56 mg, 0.0028 mmol) in water (0.4 mL). The mixture was stirred at 55 °C for 16 h and cooled to room temperature. Then it was poured into water and extracted with DCM. The organic phase was washed with brine x1, dried over Na_2SO_4, filtered and evaporated to dryness. The crude product was purified by silica gel flash column chromatography using DCM and MeOH as eluents to afford an intermediate, which was dissolved in DCM-MeOH (3:1, v/v, 4 mL) and mixed with HCl solution (4.0 M in 1,4-dioxane, 0.1 mL). The mixture was stirred at room temperature for 10 min and the solvent was removed under reduced pressure. Then Et_2O was added into the residue and the formed solid was collected by filtration to afford the title compound (11.8 mg, yield 67%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.00–7.89 (m, 2H), 7.84–7.76 (m, 2H), 7.69 (d, *J* = 7.8 Hz, 1H), 7.64 (d, *J* = 7.1 Hz, 1H), 7.59–7.43 (m, 4H), 7.37 (t, *J* = 7.6 Hz, 1H), 7.31–7.19 (m, 2H), 7.06 (t, *J* = 8.5 Hz, 1H), 5.23–5.04 (m, 3H), 4.57–4.45 (m, 4H), 4.41 (t, *J* = 6.8 Hz, 2H), 4.33 (s, 2H), 4.23–4.12 (m, 4H), 3.93 (t, *J* = 5.7 Hz, 2H), 3.82–3.63 (m, 10H), 3.37–3.33 (m, 8H), 3.21 (t, *J* = 5.5 Hz, 2H), 2.96–2.67 (m, 2H), 2.57–2.36 (m, 3H), 2.28–2.11 (m, 5H) ppm. LC-MS (ESI): m/z 1265.3 $[M+H]^+$. HRMS *m/z* calcd for $C_{63}H_{66}FN_{12}O_{12}S_2$ 1265.4349, found 1265.4338 $[M+H]^+$.



S14

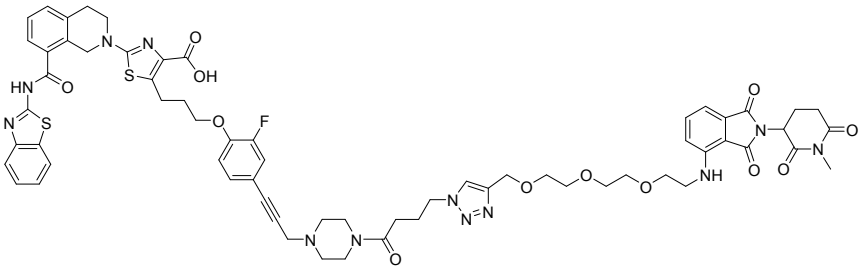
4-Fluoro-2-(1-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (S14): A mixture of **S7** (100 mg, 0.36 mmol), iodomethane (25 µL, 0.40 mmol), and ^{t}BuOK (81 mg, 0.72 mmol) in DMSO (1.5 mL) was stirred at room temperature for 2 h. The reaction mixture was diluted with water and extracted with ethyl acetate. The organic phase was washed with brine x1, dried over Na_2SO_4, filtered and evaporated to dryness. The crude product was

purified by column chromatography to afford the title compound (47 mg, yield 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82–7.68 (m, 2H), 7.46–7.39 (m, 1H), 5.03–4.93 (m, 1H), 3.21 (s, 3H), 3.04–2.94 (m, 1H), 2.93–2.71 (m, 2H), 2.18–2.06 (m, 1H) ppm. LC-MS (ESI): m/z 291.1 $[M+H]^+$.



S15

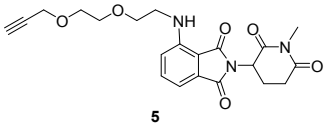
2-(1-Methyl-2,6-dioxopiperidin-3-yl)-4-((2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethyl)amino)isoindoline-1,3-dione (S15): A mixture of **S14** (80 mg, 0.28 mmol), **S8** (52 mg, 0.28 mmol), and DIPEA (230 µL, 1.39 mmol) in DMF (2.5 mL) was stirred at 90 °C overnight. The reaction mixture was diluted with water and extracted with ethyl acetate. The organic phase was washed with brine x1, dried over Na_2SO_4, filtered and evaporated to dryness. The crude product was purified by column chromatography to afford the title compound (49 mg, yield 38%). ^{1}H NMR (400 MHz, $CDCl_3$) 7.53–7.44 (m, 1H), 7.10 (dd, *J* = 7.1, 0.6 Hz, 1H), 6.93 (d, *J* = 8.5 Hz, 1H), 6.48 (t, *J* = 5.7 Hz, 1H), 4.97–4.86 (m, 1H), 4.20 (d, *J* = 2.4 Hz, 2H), 3.77–3.65 (m, 10H), 3.54–3.45 (m, 2H), 3.21 (s, 3H), 3.06–2.90 (m, 1H), 2.86–2.68 (m, 2H), 2.43 (t, *J* = 2.4 Hz, 1H), 2.17–2.04 (m, 1H) ppm. LC-MS (ESI): m/z 458.1 $[M+H]^+$.



XZ906-NC

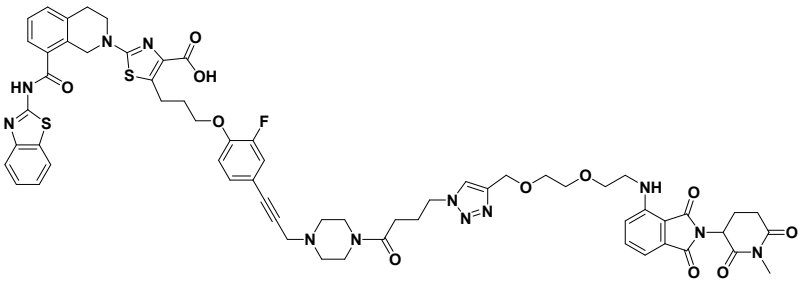
2-(8-(Benzo[*d*]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1*H*)-yl)-5-(3-(2-fluoro-4-(3-(4-(4-(4-((2-(2-(2-((2-(1-methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)methyl)-1*H*-1,2,3-triazol-1-yl)butanoyl)piperazin-1-yl)prop-1-yn-1-yl)phenoxy)propyl)thiazole-4-carboxylic acid (XZ906-NC): To a mixture of compound **6** (17.3 mg, 0.020 mmol) and compound **S15** (11.0 mg, 0.024 mmol) in ^{t}BuOH-THF (1:3, v/v, 4 mL) under an Argon atmosphere was added $CuSO_4 \cdot 5H_2O$ (1.0 mg, 0.004 mmol) and sodium L-ascorbate (0.8 mg, 0.004 mmol) in water (0.4 mL). The mixture was stirred at 55 °C for 16 h and cooled to room temperature. The reaction mixture was poured into water and extracted with DCM. The organic phase was washed with brine x1, dried over Na_2SO_4, filtered and evaporated to dryness. The crude product was purified by silica gel flash column chromatography using DCM and MeOH as eluents to afford an intermediate, which was dissolved in DCM-MeOH (3:1, v/v, 4 mL) and mixed with HCl solution (4.0 M in 1,4-dioxane, 0.1 mL). The mixture was stirred at room temperature for 2 h and the solvent was removed under reduced pressure. Then Et_2O was added into the residue and the formed solid was collected by filtration to afford the title compound (11.8 mg, yield 46%). ^{1}H NMR (600 MHz, CD_3OD) δ 7.99–7.91 (m,

2H), 7.80 (d, *J* = 8.1 Hz, 1H), 7.74 (d, *J* = 7.3 Hz, 1H), 7.57–7.46 (m, 4H), 7.37 (t, *J* = 7.6 Hz, 1H), 7.31–7.22 (m, 2H), 7.10–7.04 (m, 2H), 7.02 (d, *J* = 7.1 Hz, 1H), 5.10–5.02 (m, 3H), 4.61 (s, 2H), 4.46 (t, *J* = 6.6 Hz, 2H), 4.36 (s, 2H), 4.15 (t, *J* = 5.9 Hz, 2H), 3.90 (t, *J* = 6.0 Hz, 2H), 3.74–3.63 (m, 12H), 3.49–3.46 (m, 2H), 3.33–3.30 (m, 8H), 3.17 (t, *J* = 5.9 Hz, 2H), 3.12 (s, 3H), 2.92–2.83 (m, 2H), 2.72–2.62 (m, 1H), 2.44 (t, *J* = 6.4 Hz, 2H), 2.24–2.16 (m, 4H), 2.12–2.05 (m, 1H) ppm. HRMS *m/z* calcd for $C_{64}H_{68}FN_{12}O_{12}S_2$ 1279.4505, found 1279.4508 $[M+H]^+$.



5

2-(1-Methyl-2,6-dioxopiperidin-3-yl)-4-((2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethyl)amino)isoindoline-1,3-dione (5): A mixture of **S14** (50 mg, 0.17 mmol), **S10** (25 mg, 0.17 mmol), and DIPEA (145 µL, 0.88 mmol) in DMF (1.5 mL) was stirred at 90 °C overnight. Water (10 mL) was added and the resulting mixture was extracted with ethyl acetate. The organic phase was washed brine, dried over Na_2SO_4, filtered, and evaporated to dryness. The crude product was purified by column chromatography to afford the title compound (34 mg, yield 48%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.10 (d, *J* = 7.1 Hz, 1H), 6.93 (d, *J* = 8.6 Hz, 1H), 6.48 (t, *J* = 5.8 Hz, 1H), 5.01–4.81 (m, 1H), 4.21 (d, *J* = 2.4 Hz, 2H), 3.79–3.62 (m, 6H), 3.57–3.42 (m, 2H), 3.21 (s, 3H), 3.06–2.65 (m, 3H), 2.44 (t, *J* = 2.4 Hz, 1H), 2.18–2.06 (m, 1H) ppm. LC-MS (ESI): *m/z* 414.1 $[M+H]^+$.



XZ424-NC

2-(8-(Benzo[*d*]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1*H*)-yl)-5-(3-(2-fluoro-4-(3-(4-(4-(4-((2-(2-((2-(1-methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)methyl)-1*H*-1,2,3-triazol-1-yl)butanoyl)piperazin-1-yl)prop-1-yn-1-yl)phenoxy)propyl)thiazole-4-carboxylic acid (XZ424-NC): compound **6** (15.0 mg, 0.017 mmol) and compound **5** (10.0 mg, 0.024 mmol) in ^{t}BuOH-THF (1:3, v/v, 4 mL) under an Argon atmosphere was added $CuSO_4 \cdot 5H_2O$ (0.84 mg, 0.003 mmol) and sodium L-ascorbate (0.67 mg, 0.003 mmol) in water (0.4 mL). The mixture was stirred at 55 °C for 16 h and cooled to room temperature. The reaction mixture was poured into water and extracted with DCM. The organic phase was washed with brine x1, dried over Na_2SO_4, filtered and evaporated to dryness. The crude product was purified by silica gel flash column chromatography using DCM and MeOH as eluents to afford an intermediate, which was dissolved in DCM-MeOH (3:1, v/v, 4 mL) and mixed with HCl solution (4.0 M in 1,4-dioxane, 0.1 mL). The mixture was stirred at room temperature for 2 h and

the solvent was removed under reduced pressure. Then Et_2O was added into the residue and the formed solid was collected by filtration to afford the title compound (8.6 mg, yield 41%). ^{1}H NMR (600 MHz, CD_3OD) δ 8.13 (s, 1H), 8.00 – 7.93 (m, 1H), 7.86 – 7.80 (m, 2H), 7.61 – 7.49 (m, 4H), 7.39 (t, *J* = 7.6 Hz, 1H), 7.29 (d, *J* = 8.4 Hz, 1H), 7.25 (dd, *J* = 11.5, 1.9 Hz, 1H), 7.11 – 7.06 (m, 2H), 7.03 (d, *J* = 7.0 Hz, 1H), 5.17 (s, 2H), 5.05 (dd, *J* = 12.9, 5.5 Hz, 1H), 4.68 (s, 2H), 4.51 (t, *J* = 6.7 Hz, 2H), 4.37 (s, 2H), 4.20 – 4.16 (m, 2H), 3.99 – 3.94 (m, 2H), 3.77 – 3.68 (m, 11H), 3.61 – 3.59 (m, 1H), 3.50 (t, *J* = 5.1 Hz, 2H), 3.39 – 3.34 (m, 4H), 3.27 – 3.23 (m, 2H), 3.11 (s, 3H), 2.93 – 2.81 (m, 2H), 2.71 – 2.61 (m, 1H), 2.56 – 2.41 (m, 2H), 2.27 – 2.18 (m, 4H), 2.14 – 2.04 (m, 1H) ppm. HRMS *m/z* calcd for $C_{62}H_{64}FN_{12}O_{11}S_2$ 1235.4243, found 1235.4234 $[M+H]^+$.

Biological Methods.

Competitive binding assay. To determine the binding affinities of compounds to BCL-X_L protein, AlphaScreen competitive binding assay was performed at room temperature and all reagents were diluted in a buffer containing 25 mM HEPES pH 7.5, 100 mM NaCl, 0.1% BSA, and 0.005% Tween-20. Purified recombinant His-tagged BCL-X_L (Cat. No. SRP0187 for BCL-X_L and Cat. No. SRP0186 for BCL-2, Sigma-Aldrich, St. Louis, MO, USA) were incubated with 4-fold serially diluted compounds and a fixed concentration of biotin-tagged BAD peptides (Cat. No., AnaSpec, Fremont, CA) to a final volume of 40 µL in 96-well PCR plate. After 2 h incubation, 5 µL 6X His-Acceptor beads (final concentration 20 µg/mL) (Cat. No. AL128M, PerkinElmer, Houston, TX) were added to each well and incubated for 1 h. Thereafter, 5 µL streptavidin-donor beads were added (final concentration 20 µg/mL) (Cat. No. 6760002 Perkin Elmer) to each well and incubated for 30 min. 17 µL of each sample was transferred into adjacent wells of 384-well proxy plate (Cat. No. 6008280, Perkin Elmer) prior to luminescence detection on Biotek's Synergy Neo2 multi-mode plate reader equipped with AlphaScreen filter cube. The inhibition constant (K_i) was calculated using non-linear regression, one site, competitive binding with peptide concentration value and experimentally determined K_d value between peptide and BCL-X_L inputted.

Cell lines and culture. MOLT-4 (Cat# CRL-1582) cell line was recently purchased from American Type Culture Collection (ATCC, Manassas, VA). MOLT-4 cells were cultured in RPMI 1640 media (Life Technologies, Carlsbad, CA, USA) supplemented with 10% FBS (Atlanta Biologicals, Flowery Branch, GA, USA) and 1% penicillin-streptomycin solution (Thermo Fisher Scientific, Waltham, MA, USA).

Cell viability assay. Cell viability was measured by Tetrazolium-based MTS assay (Promega, Madison, WI, USA). 5×10^4 to 1×10^5 MOLT-4 cells were seeded and treated in 96-well plates for 72 h. The EC_{50} values of individual agents were calculated with GraphPad Prism 7 software (GraphPad Software, La Jolla, CA, USA).

Immunoblotting. Cells were collected and lysed in Lysis buffer (Boston Bio Products, Ashland, MA, USA) supplied with protease and phosphatase inhibitor cocktails (Sigma-Aldrich, St. Louis, MO, USA). The equal amount of protein lysates

was separated on pre-casted 4-20% Tris-glycine gels (Bio-Rad, Hercules, CA, USA). Thereafter, the proteins were transferred to PVDF membranes (MilliporeSigma, Billerica, MA, USA). The membranes were blocked with 5% w/v non-fat dry milk in TBS + Tween-20 (0.1% v/v), and then probed with primary antibodies for overnight at 4° C. Next day, the membranes were washed and incubated with appropriate HRP-conjugated secondary antibodies. The signal was detected using ECL substrate (MilliporeSigma) and captured on X-ray films or ChemiDoc MP Imaging System (Bio-Rad). The band intensities were calculated on ImageJ software and normalized to equal loading control β-actin.

Human platelet isolation and viability assays. Platelet rich plasma (PRP) was purchased from Zenbio (Cat. No. SER-PRP-SDS, Research Triangle Park, NC, USA). PRP was transferred into a 50 mL tube containing 5 mL acid citrate buffer (Cat. No. sc-214744, Santa Cruz Biotechnology). To prevent clotting, prostaglandin E1 (PGE1, Cat. No. sc-201223A, Santa Cruz Biotechnology) and apyrase (Cat. No. A6237, Sigma-Aldrich) were added to final concentrations of 1 µM and 0.2 units/mL, respectively. After gently mixing the solution, platelets were pelleted by centrifugation at 1200 g for 10 min without break. Pelleted platelets were gently washed without disrupting platelets in 2 mL HEPES Tyrode's buffer (Cat. No. PY-921WB, Boston BioProducts, Ashland, MA, USA) containing 1 µM PGE1 and 0.2 units/mL apyrase. After washing, pellets were slowly suspended in 10 mL HEPES Tyrode's buffer containing 1 µM PGE1 and 0.2 units/mL apyrase. Then platelets number was counted using a HEMAVET 950FS hematology analyzer (Drew Scientific, Inc., Oxford, CT, USA). For viability assays, platelet number was adjusted