Supporting Information

Radiation Responsive PROTAC Nanoparticles for Specific Tumor Proteolysis Enhanced Radiotherapy

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Methods

Scheme S1 Structure and synthesis of MZ1.

Synthesis of tert-butyl ((S)-13-((2R,4S)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carbonyl)-14,14-dimethyl-11-oxo-3,6,9-trioxa-12-a zapentadecyl)carbamate (1)

Boc-NH-PEG₃-CH₂-COOH (85.6 mg, 0.279 mmol, 1.0 eq) and HATU (159.1 mg, 0.418 mmol, 1.5 eq) were dissolved in dichloromethane (DCM), then (S, R, S)-AHPC (120 mg, 0.279 mmol, 1.0 eq) and DIPEA (108.2 mg, 0.837 mmol, 3.0 eq) were added sequentially. After stirring for 4 h at room temperature, the mixture was washed with water and then dried with anhydrous sodium sulfate (Na₂SO₄). The crude production was purified via silica gel column chromatography to obtain compound 1 as light-yellow oily liquid (140.5 mg, 70% yield). ¹H-NMR (400 MHz, DMSO-d₆) δ: 8.99 (s, 1H), 8.60 (t, J = 6.0 Hz, 1H), 7.44 (s, 1H), 7.41 (s, 4H), 6.73 (t, J = 5.8 Hz, 1H), 5.17 (d, J = 3.6 Hz, 1H), 4.58 (d, J = 9.6 Hz, 1H), 4.44 (q, J = 7.2, 6.3 Hz, 2H), 4.39 (d, J = 6.4 Hz, 1H), 4.26 (dd, J = 15.8, 5.5 Hz, 1H), 3.98 (s, 2H), 3.64-3.49 (m, 12H), 3.05 (q, J = 5.9 Hz, 2H), 2.45 (s, 3H), 2.07 (dd, J = 12.9, 7.8 Hz, 1H), 1.97-1.86 (m, 1H), 1.37 (s, 9H), 0.95 (s, 9H).

Synthesis of (2R,4S)-1-((S)-2-(tert-butyl)-17-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-4,16-dioxo-6,9,12-trioxa-3,15-diazaheptadecanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (3, MZ1)

Compound 1 (140.5 mg, 0.195 mmol, 1.0 eq) was dissolved in DCM contained 30% TFA (V/V) and stirred at room temperature for 1 h, and the reaction was
monitored by thin layer chromatography (TLC). The crude compound 2 was used without further purification after the solvent was removed under reduced pressure. JQ1-COOH (64.8 mg, 0.162 mmol, 1.0 eq) and HATU (92.5 mg, 0.243 mmol, 1.5 eq) were dissolved in N,N-dimethylformamide (DMF), followed by compound 2 (100.4 mg, 0.162 mmol, 1.0 eq) and DIPEA (62.9 mg, 0.486 mmol, 3.0 eq). The reaction was stirred at room temperature for 4 h and then extracted with DCM. The organic phase was washed with water and saturated ammonium chloride (NH₄Cl) solution, dried over anhydrous Na₂SO₄ and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography to yield the compound 3 as white solid (112.0 mg, 69% yield).

\[ ^1H-NMR \quad (400 \text{ MHz, DMSO-d}_6) \delta: 8.99 (s, 1H), 8.64 (t, J = 5.9 \text{ Hz, 1H}), 8.32 (t, J = 5.1 \text{ Hz, 1H}), 7.49 (d, J = 1.5 \text{ Hz, 1H}), 7.41 (d, J = 13.1 \text{ Hz, 8H}), 5.19 (s, 1H), 4.57 (d, J = 9.5 \text{ Hz, 1H}), 4.50 (dd, J = 8.0, 6.2 \text{ Hz, 1H}), 4.46-4.40 (m, 1H), 4.25 (dd, J = 15.8, 5.6 \text{ Hz, 1H}), 3.98 (s, 2H), 3.59 (ddt, J = 16.7, 6.8, 4.1 \text{ Hz, 12H}), 3.44 (d, J = 5.8 \text{ Hz, 2H}), 3.25 (d, J = 7.5 \text{ Hz, 2H}), 2.59 (s, 3H), 2.44 (s, 4H), 2.41 (s, 4H), 2.06 (t, J = 10.4 \text{ Hz, 1H}), 1.90 (td, J = 8.7, 5.3 \text{ Hz, 1H}), 1.63-1.60 (m, 3H), 0.94 (s, 9H).\]

**Scheme S2** Structure and synthesis of RCNprotac (α) precursor.

**Synthesis of di-tert-butyl 2,2'-diselanediyldiacetate (4)**

The diselenide-bond containing carbon chain was synthesized according to the literature. Selenium powder (104.0 mg, 1.317 mmol, 1.0 eq) was suspended in aqueous solution, and the NaBH₄ (100.0 mg, 2.643 mmol, 2.0 eq) aqueous solution was dropped into the suspension under nitrogen (N₂) atmosphere. After the solution became colorless, another quantity of selenium powder (104.0 mg, 1.317 mmol, 1.0
eq) was added to obtain a brown-red aqueous solution of \( \text{Na}_2\text{Se}_2 \). After that, tert-butyl 2-bromoacetate (517.9 mg, 2.655 mmol, 2.0 eq) dissolved in tetrahydrofuran (THF) was dropped into the above solution and the mixture was stirred at 50 °C in \( \text{N}_2 \) atmosphere for 5 h. The crude product was then filtered and extracted with DCM, dried with anhydrous \( \text{Na}_2\text{SO}_4 \), and purified by silica gel column chromatography to yield the compound 4 as orange oily liquid (451.9 mg, 88.2% yield). \(^1\)H-NMR (400 MHz, DMSO-\( \text{d}_6 \)) \( \delta \): 3.33 (s, 4H), 1.42 (s, \( J = 3.4 \) Hz, 18H).

**Synthesis of 2-((2-(((3S,5R)-1-((R)-2-(tert-butyl)-17-((R)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-4,16-dioxo-6,9,12-trioxa-3,15-diazaheptadecanoyl)-5-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-3-yl)oxy)-2-oxoethyl)diselaneyl)acetic acid (6)**

Compound 4 was dissolved in DCM contained 30% TFA (V/V) and stirred at room temperature for 1 h. After removal of solvent under reduced pressure, the obtained crude compound 5 as red-orange solid was used without further purification. Compound 5 (16.5 mg, 0.060 mmol, 1.5 eq) and DMAP (1.0 mg, 0.008 mmol, 0.2 eq) were dissolved in anhydrous DCM solution containing DMF, stirred at 0 °C for 1 h. Then, Compound 3 (40.0 mg, 0.040 mmol, 1.0 eq) and EDCI (15.3 mg, 0.080 mmol, 2 eq) were sequentially added and the mixture was stirred at room temperature for 24 h. The crude product was then extracted with DCM, dried with anhydrous \( \text{Na}_2\text{SO}_4 \), and purified by silica gel column chromatography to yield the compound 6 as white solid (19.3 mg, 38.3%). \(^1\)H-NMR (400 MHz, DMSO-\( \text{d}_6 \)) \( \delta \): 12.42 (s, 1H), 8.98 (s, 1H), 8.63 (t, \( J = 6.1 \) Hz, 1H), 8.30 (t, \( J = 5.6 \) Hz, 1H), 7.50 (s, 1H), 7.42 (d, \( J = 13.6 \) Hz, 8H), 5.15 (d, \( J = 22.0 \) Hz, 1H), 4.57 (d, \( J = 9.6 \) Hz, 1H), 4.51 (dd, \( J = 8.0, 6.1 \) Hz, 1H), 4.44 (q, \( J = 7.7 \) Hz, 1H), 4.26 (dd, \( J = 15.8, 5.6 \) Hz, 1H), 3.98 (s, 1H), 3.64-3.54 (m, 10H), 3.44 (t, \( J = 5.9 \) Hz, 4H), 3.25 (dt, \( J = 11.8, 6.4 \) Hz, 4H), 3.06 (t, \( J = 7.0 \) Hz, 2H), 2.89-2.80 (m, 1H), 2.72 (t, \( J = 7.0 \) Hz, 2H), 2.59 (s, 3H), 2.44 (s, 4H), 2.41 (s, 3H), 1.62 (s, 3H), 0.95 (s, 9H).

**Synthesis of RCNprotac (α) precursor (7)**

Compound 6 (10.0 mg, 0.008 mmol, 1.0 eq) and HATU (4.5 mg, 0.012 mmol,
1.5 eq) were dissolved in DCM, then mPEG2000-NH$_2$ (15.9 mg, 0.008 mmol, 1.0 eq) and DIPEA (3.1 mg, 0.024 mmol, 3.0 eq) were added sequentially. After stirring for 6 h at room temperature, the product was dialyzed against DMSO and DI water to purify and lyophilized to obtain compound 7 as white solid (20.8 mg, 80.5%).

$^1$H-NMR (400 MHz, DMSO-d$_6$) δ: 8.97 (s, 1H), 8.64 (s, 1H), 8.25 (d, $J$ = 6.0 Hz, 1H), 8.11 (d, $J$ = 4.1 Hz, 1H), 7.80 (d, $J$ = 5.7 Hz, 1H), 7.54-7.34 (m, 8H), 5.33 (d, $J$ = 4.8 Hz, 1H), 4.54-4.46 (m, 2H), 4.43-4.34 (m, 1H), 4.31-4.23 (m, 1H), 3.99-3.92 (m, 2H), 3.68 (dt, $J$ = 5.1, 2.8 Hz, 3H), 3.51 (s, 192H), 3.42 (d, $J$ = 2.4 Hz, 4H), 3.24 (s, 3H), 2.58 (d, $J$ = 2.3 Hz, 3H), 2.53 (d, $J$ = 6.6 Hz, 1H), 2.43 (d, $J$ = 4.2 Hz, 4H), 2.40 (d, $J$ = 3.4 Hz, 3H), 2.34-2.23 (m, 1H), 2.06-1.94 (m, 1H), 1.61 (s, 3H), 0.95 (s, 9H).

**Scheme S3** Structure and synthesis of RCNprotac (β) precursor.

**Synthesis of di-tert-butyl 3,3’-diselanediyldipropionate (8)**

Through the same synthetic pathway as before, obtained brown-red aqueous solution of Na$_2$Se$_2$. Tert-butyl 3-bromopropanoate (555.1 mg, 2.655 mmol, 2.0 eq) dissolved in THF was dropped into the Na$_2$Se$_2$ solution and stirred at 50 °C in N$_2$ atmosphere for 5 h. The mixture was then filtered and extracted with DCM, dried with anhydrous Na$_2$SO$_4$, and purified by silica gel column chromatography to yield the compound 8 as orange oily (471.4 mg, 85.3% yield). $^1$H-NMR (400 MHz, DMSO-d$_6$) δ: 3.04 (t, $J$ = 7.0 Hz, 4H), 2.70 (d, $J$ = 6.9 Hz, 4H), 1.41 (s, 18H).

**Synthesis of 3-((3-(((3R,5S)-1-((S)-2-(tert-butyl)-17-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-4,16-dioxo-6,9,12-trioxo-3,15-diazahexadecanoyl)-5-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-3-yl)oxy)-3-oxopropyl)diselanediyldipropionate**

10)
Compound 8 was dissolved in DCM contained 30% TFA (V/V) and stirred at room temperature for 1 h. After removal of solvent under reduced pressure, compound 9 was obtained as red-orange solid and used without further purification. Compound 9 (18.2 mg, 0.060 mmol, 1.5 eq) and DMAP (1.0 mg, 0.008 mmol, 0.2 eq) were dissolved in anhydrous DCM solution containing DMF and stirred at 0 °C for 1 h. After that, compound 3 (40.0 mg, 0.040 mmol, 1.0 eq) and EDCI (15.3 mg, 0.080 mmol, 2.0 eq) were added and stirred at room temperature for 24 h. Then, the mixture was extracted with DCM, dried with anhydrous Na₂SO₄, and purified by silica gel column chromatography to yield the compound 10 as white solid (21.1 mg, 40.9% yield). ¹H-NMR (400 MHz, DMSO-d₆) δ: 12.10 (s, 1H), 8.97 (s, 1H), 8.61 (s, 1H), 8.26 (s, 1H), 7.48 (d, J = 2.7 Hz, 1H), 7.46-7.35 (m, 8H), 5.34-5.28 (m, 1H), 4.49 (d, J = 7.4 Hz, 1H), 4.44-4.39 (m, 1H), 4.28 (d, J = 3.0 Hz, 1H), 4.03 (d, J = 7.1 Hz, 1H), 3.96 (s, 1H), 3.65-3.50 (m, 10H), 3.44 (dt, J = 7.1, 3.5 Hz, 4H), 3.25-3.20 (m, 2H), 2.97-2.84 (m, 4H), 2.59 (s, 3H), 2.43 (d, J = 2.8 Hz, 4H), 2.40 (s, 4H), 2.32 (td, J = 7.2, 2.6 Hz, 2H), 2.01-1.90 (m, 4H), 1.45 (s, 3H), 0.95 (s, 9H).

Synthesis of RCNprotac (β) precursor (11)

Compound 10 (10.3 mg, 0.008 mmol, 1.0 eq), HATU (4.5 mg, 0.012 mmol, 1.5 eq), mPEG2000-NH₂ (15.9 mg, 0.008 mmol, 1.0 eq), and DIPEA (3.1 mg, 0.024 mmol, 3.0 eq) were dissolved in DCM and stirred for 6 h at room temperature. After the reaction was completed, the product was dialyzed against DMSO and DI water to purify and lyophilized to obtain compound 11 as white powder (21.8 mg, 85.6% yield). ¹H-NMR (400 MHz, DMSO-d₆) δ: 8.97 (s, 1H), 8.61 (d, J = 6.2 Hz, 1H), 8.26 (d, J = 5.7 Hz, 1H), 8.00 (t, J = 5.4 Hz, 1H), 7.80 (s, 1H), 7.50-7.38 (m, 8H), 5.34-5.30 (m, 1H), 4.51 (d, J = 8.3 Hz, 1H), 4.43 (d, J = 8.9 Hz, 1H), 4.39-4.34 (m, 1H), 4.29 (d, J = 5.8 Hz, 1H), 3.96 (s, 2H), 3.70-3.67 (m, 3H), 3.51 (s, 192H), 3.25 (s, 3H), 3.10-3.03 (m, 4H), 2.91 (s, 4H), 2.59 (s, 4H), 2.55 (s, 1H), 2.43 (s, 3H), 2.40 (s, 3H), 2.07-1.97 (m, 2H), 1.62 (s, 3H), 0.95 (s, 9H).

Scheme S4 Structure and synthesis of RCNprotac (γ) precursor.
Synthesis of di-tert-butyl 4,4’-diselanediyldibutyrate (12)

Na$_2$Se$_2$ brown-red solution was obtained by the same synthetic pathway as before. Tert-butyl 4-bromobutanoate (592.4 mg, 2.655 mmol, 2.0 eq) was dissolved in THF and dropped into the Na$_2$Se$_2$ solution. After stirred at 50 °C in N$_2$ atmosphere for 5 h, the reaction mixture was filtered, extracted by DCM, dried with anhydrous Na$_2$SO$_4$, and purified by silica gel column chromatography to yield the compound 12 as orange oily (451.1 mg, 81.6% yield). $^1$H-NMR (400 MHz, DMSO-d$_6$) δ: 2.90 (t, $J$ = 7.3 Hz, 4H), 2.34-2.28 (m, 4H), 1.89 (p, $J$ = 7.3 Hz, 4H), 1.40 (s, 18H).

Synthesis of 4-((4-(((3R,5S)-1-((S)-2-(tert-butyl)-17-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-4,16-dioxo-6,9, 12-trioxa-3,15-diazahexadecanoyl)-5-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-3-yl)oxy)-4-oxobutyl)diselaneyl)butanoic acid (14)

Compound 12 was dissolved in DCM contained 30% TFA (V/V) and stirred at room temperature for 1 h to obtain compound 13 as red-orange solid after removal of solvent. Compound 13 (19.9 mg, 0.060 mmol, 1.5 eq) and DMAP (1.0 mg, 0.008 mmol, 0.2 eq) were dissolved in anhydrous DCM solution containing DMF and react at 0 °C for 1 h. Then, compound 3 (40.0 mg, 0.040 mmol, 1.0 eq) and EDCI (15.3 mg, 0.080 mmol, 2.0 eq) were added and stirred at room temperature for 24 h. The mixture was extracted with DCM, dried with anhydrous Na$_2$SO$_4$, and purified by silica gel column chromatography to yield the compound 14 as white solid powder (15.3 mg, 29.1% yield). $^1$H-NMR (400 MHz, DMSO-d$_6$) δ: 12.09 (s, 1H), 8.98 (s, 1H), 8.63 (t, $J$ = 6.1 Hz, 1H), 8.28 (t, $J$ = 5.7 Hz, 1H), 7.49 (d, $J$ = 2.7 Hz, 1H), 7.47-7.37 (m, 8H), 5.31 (d, $J$ = 14.9 Hz, 1H), 4.52 (d, $J$ = 6.7 Hz, 1H), 4.47-4.41 (m, 1H), 4.28
(dd, \( J = 15.7, 5.7 \) Hz, 1H), 4.04 (q, \( J = 7.1 \) Hz, 1H), 3.97 (s, 1H), 3.58 (dd, \( J = 11.7, 4.6 \) Hz, 10H), 3.45 (t, \( J = 5.8 \) Hz, 4H), 3.25-3.21 (m, 2H), 2.98-2.84 (m, 4H), 2.59 (d, \( J = 2.2 \) Hz, 3H), 2.44 (s, 4H), 2.41 (d, \( J = 2.7 \) Hz, 4H), 2.36-2.22 (m, 4H), 2.00 (s, 2H), 1.93 (dd, \( J = 12.5, 7.3 \) Hz, 4H), 1.62 (s, 3H), 0.96 (s, 9H).

**Synthesis of RCNprotac (γ) precursor (15)**

Compound 14 (10.5 mg, 0.008 mmol, 1.0 eq), HATU (4.5 mg, 0.012 mmol, 1.5 eq), mPEG2000-NH₂ (15.9 mg, 0.008 mmol, 1.0 eq), and DIPEA (3.1 mg, 0.024 mmol, 3 eq) were dissolved in DCM and stirred for 6 h at room temperature. After that, the product was dialyzed against DMSO and DI water to purify and lyophilized to obtain compound 15 as white solid power (20.6 mg, 81.9% yield). ¹H-NMR (400 MHz, DMSO-d₆) δ: 8.98 (s, 1H), 8.63 (s, 1H), 8.29 (t, \( J = 5.6 \) Hz, 1H), 7.91 (s, 1H), 7.49 (d, \( J = 2.4 \) Hz, 1H), 7.44-7.39 (m, 8H), 5.31 (d, \( J = 16.1 \) Hz, 1H), 4.50 (s, 1H), 4.42 (d, \( J = 8.8 \) Hz, 1H), 4.38 (d, \( J = 5.8 \) Hz, 1H), 4.31-4.25 (m, 1H), 3.96 (d, \( J = 2.5 \) Hz, 2H), 3.68 (dd, \( J = 5.8, 3.9 \) Hz, 3H), 3.51 (s, 1H), 3.24 (s, 3H), 2.89 (t, \( J = 7.3 \) Hz, 4H), 2.59 (s, 3H), 2.44 (s, 4H), 2.40 (s, 4H), 2.18 (t, \( J = 7.4 \) Hz, 2H), 2.00 (q, \( J = 7.0 \) Hz, 2H), 1.94-1.88 (m, 4H), 1.76 (s, 1H), 1.61 (s, 3H), 0.95 (s, 9H).
Fig. S1 $^1$H-NMR spectrum (DMSO-d$_6$) (A) and MS spectrum (B) of compound 1.
Fig. S2 $^1$H-NMR spectrum (DMSO-d$_6$) (A) and MS spectrum (B) of compound 3 (MZ1).
Fig. S3 $^1$H-NMR spectrum of compound 4 (DMSO-d$_6$).
Fig. S4 $^1$H-NMR spectrum (DMSO-d$_6$) (A) and MS spectrum (B) of compound 6.
Fig. S5 $^1$H-NMR spectrum of compound 7 (DMSO-\textit{d}_6).

Fig. S6 $^1$H-NMR spectrum of compound 8 (DMSO-\textit{d}_6).
Fig. S7 $^1$H-NMR spectrum (DMSO-d$_6$) (A) and MS spectrum (B) of compound 10.
Fig. S8 $^1$H-NMR spectrum of compound 11 (DMSO-d$_6$).

Fig. S9 $^1$H-NMR spectrum of compound 12 (DMSO-d$_6$).
Fig. S10 $^1$H-NMR spectrum (DMSO-d$_6$) (A) and MS spectrum (B) of compound 14.
Fig. S11 $^1$H-NMR spectrum of compound 15 (DMSO-$d_6$).

Fig. S12 HPLC profiles of MZ1 in the presence of 0, 2, 4, 6, and 8 Gy radiation.
**Fig. S13** (A) Structure of seleninic acid produced by RCNprotac after exposure to X-ray radiation. (B) MS analysis of seleninic acid.

**Fig. S14** Radiation-responsive mechanism of RCNprotac.

**Fig. S15** Viabilities of MDA-MB-231 cells treated with different doses of X-ray radiation for 24 h ($n = 3$).
Fig. S16 Western blot analysis of BRD4 protein expression in MDA-MB-231 cells treated with different concentrations of MZ1 for 24 h.

Fig. S17 Normalized expression of BRD4 in MZ1 or RCNprotac + 4 Gy treated cells.

Fig. S18 Western blot analysis of γH2AX expression after different time points in cells exposure to 4 Gy radiation with or without 1 μM RCNprotac treated.

Fig. S19 Average weights of harvested tumors after treatments ($n = 5$). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. 

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**Fig. S20** Normalized expression of BRD4 in tumors of different treatment groups. *p < 0.05, **p < 0.01, ***p < 0.001.

**Fig. S21** H&E staining of major organs (including heart, liver, spleen, lung, and kidney) in mice 14 days after treating with PBS or RCNprotac + X-ray. (scale bar = 100 μm).

**Fig. S22** Biochemical indexes of mice treated with PBS or RCNprotac (n = 5).