Supplemental Material

A Novel Probe for Spliceosomal Proteins that Induces Autophagy and Death of Melanoma Cells Reveals New Targets for Melanoma Drug Discovery

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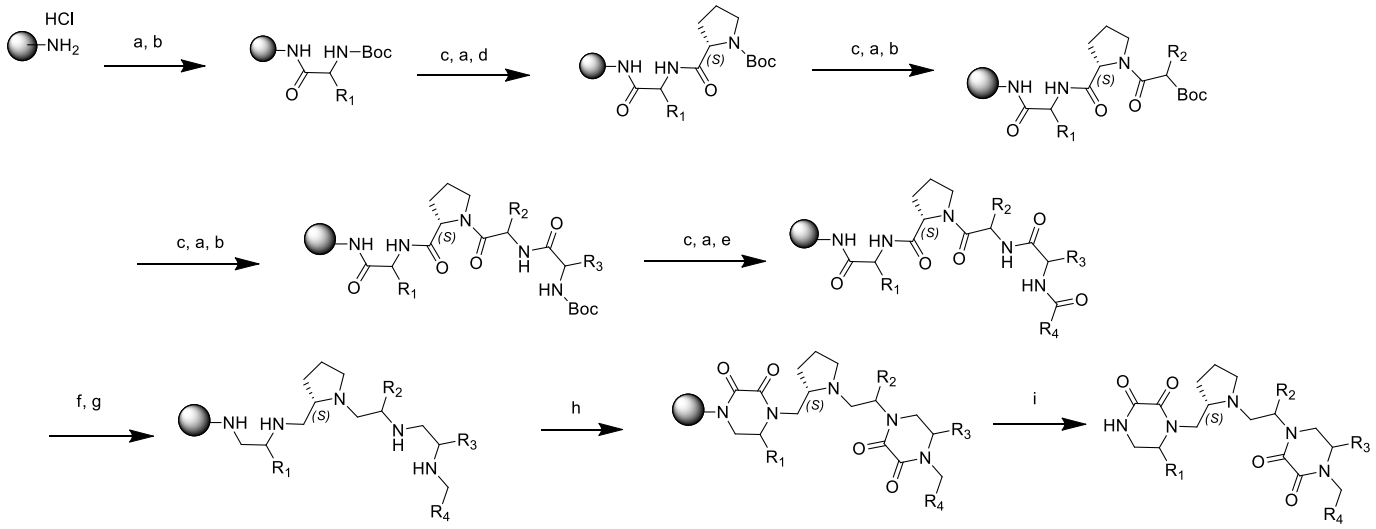
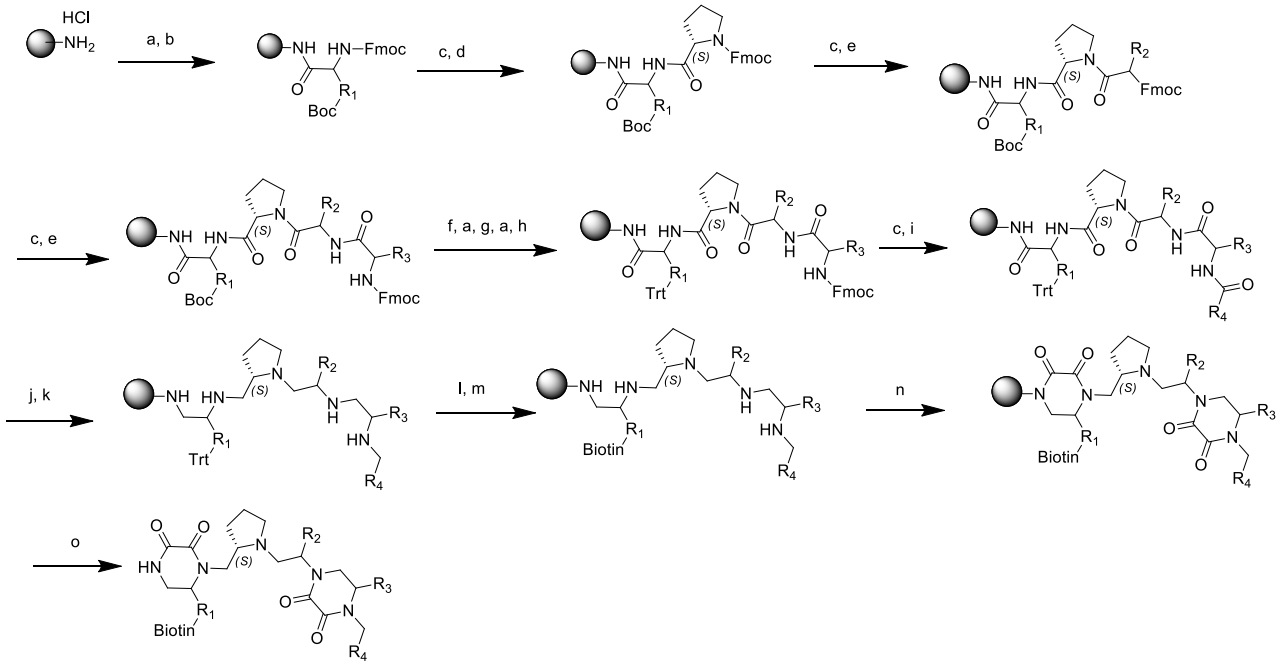
Compound purification and characterization.



Supplementary Figure 1A. Synthesis procedure for (S)-1((R)-1((S)-2-(((S)-6-benzyl-2,3-dioxopiperazin-1-yl)methyl)pyrrolidin-1-yl)-3-cyclohexylpropan-2-yl)-5-(4-hydroxybenzyl)-4-phenethylpiperazine-2,3-dione: (a)5%DIEA/95%DCM, 3x 2min; (b) Boc-AA (6eq), DIC(6eq) ,HOBt (6eq),DMF, 2hr; (c) 55%TFA/45%DCM, 30min; (d) Boc-L-Pro-OH (6eq),DIC (6eq), HOBt (6eq), DMF (6eq), 2hr; (e) RCOOH (10eq), DIC (10eq), HOBt (10eq), DMF, 2hr; (f) BH3/THF(40eq), 65C, 96hr; (g) Piperidine, 65C, 18hr; (h) (COIm)2 (10eq), Anhydrous Atmosphere, 18hr; (i)HF/Anisole, 0C, 1.5hr



Supplementary Figure 1B. Synthesis procedure for Biotin tagged pyrrollidine-bis-diketopiperazines used in present studies: (a)5%DIEA/95%DCM, 3x 2min; (b) Fmoc-AA(Boc) (6eq), DIC(6eq) ,HOBt (6eq),DMF, 2hr; (c) 20%Piperidine/80%DMF, 2x 30min; (d) Fmoc-L-Pro-OH (6eq),DIC (6eq), HOBt (6eq), DMF (6eq), 2hr; (e) Fmoc-AA (6eq), DIC(6eq) ,HOBt (6eq),DMF, 2hr; (f) 55%TFA/45%DCm, 30min; (g) Trt-Cl (5eq), DIEA (10eq), 10%DMF/90%DCM, 2hr; (h) Trt-Cl (5eq), DIEA (10eq), 10%DMF/90%DCM, 2hr; (i) RCOOH (10eq), DIC (10eq), HOBt (10eq), DMF, 2hr; (j) BH3/THF(40eq), 65C, 96hr; (k) Piperidine, 65C, 18hr; (l) 2%TFA/5%TRIS,95%DCM. 3x 2min; (m) Biotin (10eq), DIC (10eq), DMF, 2hr (n) (COIm)2 (10eq), Anhydrous Atmosphere, 18hr; (o)HF/Anisole, 0C, 1.5hr

(S)-1((R)-1((S)-2-(((S)-6-benzyl-2,3-dioxopiperazin-1-yl)methyl)pyrrolidin-1-yl)-3-cyclohexylpropan-2-yl)-5-(4-hydroxybenzyl)-4-phenethylpiperazine-2,3-dione. Using General Scheme (scheme 1) for the synthesis of bis-cyclic diketopiperazines compounds 2155-14 and 2529-1 were synthesized using the following reagents: (2 g) MBHA resin starting material, Boc-L-Phenylalanine -OH (R_1), Boc-D-Cyclohexylalanine-OH (R_2), Boc-L-Tyrosine(2-Br-Z)-OH (R_3), and Phenylacetic Acid (R_4). The final crude product was purified using HPLC as described above, with a gradient of (B) 0/5, 2/5, 4/20, 40/55. **Isolated Mass** 504.2mg, **%yield** 28.62%.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.80 - 1.05 (m, 2 H) 1.08 - 1.20 (m, 3 H) 1.24 (br. s., 2 H) 1.50 - 1.71 (m, 6 H) 1.78 - 1.96 (m, 2 H) 1.97 - 2.13 (m, 2 H) 2.55 - 2.65 (m, 2 H) 2.67 - 2.88 (m, 6 H) 2.90 - 3.04 (m, 3 H) 3.11 (d, *J*=12.96 Hz, 1 H) 3.19 - 3.32 (m, 2 H) 3.37 (br. s., 1 H) 3.52 (dd, *J*=13.08, 3.30 Hz, 2 H) 3.65 (d, *J*=10.03 Hz, 2 H) 3.70 - 3.94 (m, 2 H) 6.73 (m, *J*=8.07 Hz, 2 H) 7.01 (m, *J*=8.07 Hz, 2 H) 7.15 - 7.31 (m, 9 H) 8.50 (d, *J*=5.01 Hz, 1 H) **m/z** calcd $C_{44}H_{55}N_5O_5$ $[M+H]^+$ 734.42, found 734.15 (MALDI), 734.15 (MS ESI) **Purity** LC-MS: 99.0% (254 nm, peak height).

N-(4-((S)-1-(((S)-1-((R)-3-cyclohexyl-2-((S)-5-(4-hydroxybenzyl)-2,3-dioxo-4-phenethylpiperazin-1-yl)propyl)pyrrolidin-2-yl)methyl)-5,6-dioxopiperazin-2-yl)butyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide. Using Scheme 3 for the synthesis of biotin tagged pyrrollidine-bis-diketopiperazines compound 2529-3 was synthesized using the following reagents: (100 mg) MBHA resin starting material, Fmoc-L-Lysine(Boc) -OH (R_1), Fmoc-D-Cyclohexylalanine-OH (R_2), Fmoc-L-Tyrosine(2-Br-Z)-OH (R_3), and Phenylacetic Acid (R_4). The final crude product was purified using HPLC as described above, with a gradient of (B) 0/5, 2/5, 4/20, 40/55. **Isolated Mass** 4.9mg **%yield** 4.33%.

^{1}H NMR (400 MHz, DMSO-*d_6*) δ ppm 9.63 (br. s., 1H) 9.35 (br. s., 1H) 8.56 (br. s., 1 H) 7.81 (br. s., 1 H) 7.29 (br. s., 1 H) 7.24 (br. s., 2 H) 7.01 (br. s., 1 H) 6.72 (br. s., 1 H) 6.40 (d, J=15.89 Hz, 1 H) 4.90 (br. s., 1 H) 4.31 (br. s., 1 H) 3.93 - 4.17 (m, 1 H) 3.63 - 3.87 (m, 4 H) 3.60 (br. s., 1 H) 3.53 (br. s., 2 H) 3.44 (br. s., 14 H) 3.31 (br. s., 2 H) 3.20 (br. s., 2 H) 3.09 (br. s., 1 H) 3.04 (br. s., 1 H) 2.83 (d, J=11.49 Hz, 3 H) 2.74 (br. s., 1 H) 2.59 (d, J=12.72 Hz, 4 H) 2.28 (br. s., 1 H) 2.04 (br. s., 3 H) 1.89 (br. s., 1 H) 1.81 (d, J=9.41 Hz, 1 H) 1.69 (br. s., 2 H) 1.63 (br. s., 3 H) 1.48 (br. s., 2 H) 1.42 (br. s., 2 H) 1.29 (br. s., 4 H) 1.19 (br. s., 2 H) 0.96 (br. s., 2 H) **m/z** calcd $C_{51}H_{72}N_8O_7S$ $[M+H]^+$ 941.53, found 471.15 (MS ESI) **Purity** LC-MS: 99.0% (254 nm, peak height).

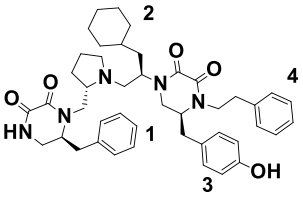
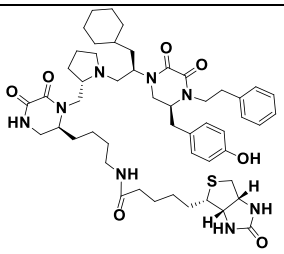
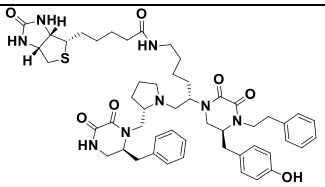
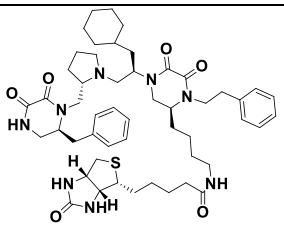
N-((S)-6-((S)-2-(((S)-6-benzyl-2,3-dioxopiperazin-1-yl)methyl)pyrrolidin-1-yl)-5-((S)-5-(4-hydroxybenzyl)-2,3-dioxo-4-phenethylpiperazin-1-yl)hexyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide. Using Scheme 3 for the synthesis of Biotin tagged pyrrollidine-bis-diketopiperazines compound 2529-5 was synthesized using the following reagents: (100mg) MBHA resin starting material, Fmoc-L-Phenylalanine -OH (R_1), Fmoc-L-Lysine(Boc) -OH (R_2), Fmoc-L-Tyrosine(2-Br-Z)-OH (R_3), and Phenylacetic Acid (R_4). The final crude product was purified using HPLC as described above, with a gradient of (B) 0/5, 2/5, 4/20, 40/55. **Isolated Mass** 6.4 mg **%yield** 5.70%.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.53 (br. s., 1 H) 9.35 (br. s., 1 H) 8.62 (br. s., 1 H) 7.82 (br. s., 1 H) 7.25 - 7.35 (m, 6 H) 7.22 (br. s., 1 H) 7.01 (br. s., 2 H) 6.72 (br. s., 1 H) 6.30 - 6.46 (m, 1 H) 4.76 (br. s., 1 H) 4.30 (br. s., 1 H) 4.12 (br. s., 1 H) 4.02 (d, J=12.47 Hz, 1 H) 3.78 (br. s., 2 H) 3.56 - 3.69 (m, 4 H) 3.51 (br. s., 10 H) 3.33 (br. s., 2 H) 3.16 (d, J=11.62 Hz, 2 H) 2.92 - 3.11 (m, 4 H) 2.76 - 2.92 (m, 4 H) 2.70 (br. s., 1 H) 2.57 (d, J=12.10 Hz, 1 H) 2.26 (br. s., 1 H) 2.02 (br. s., 3 H) 1.84 (br. s., 1 H) 1.59 (br. s., 1 H) 1.43 (br. s., 5 H) 1.29 (br. s., 2 H) **m/z** calcd $C_{51}H_{66}N_8O_7S$ $[M+H]^+$ 935.48, found 468.45 (MS ESI) **Purity** LC-MS: 99.0% (254 nm, peak height).

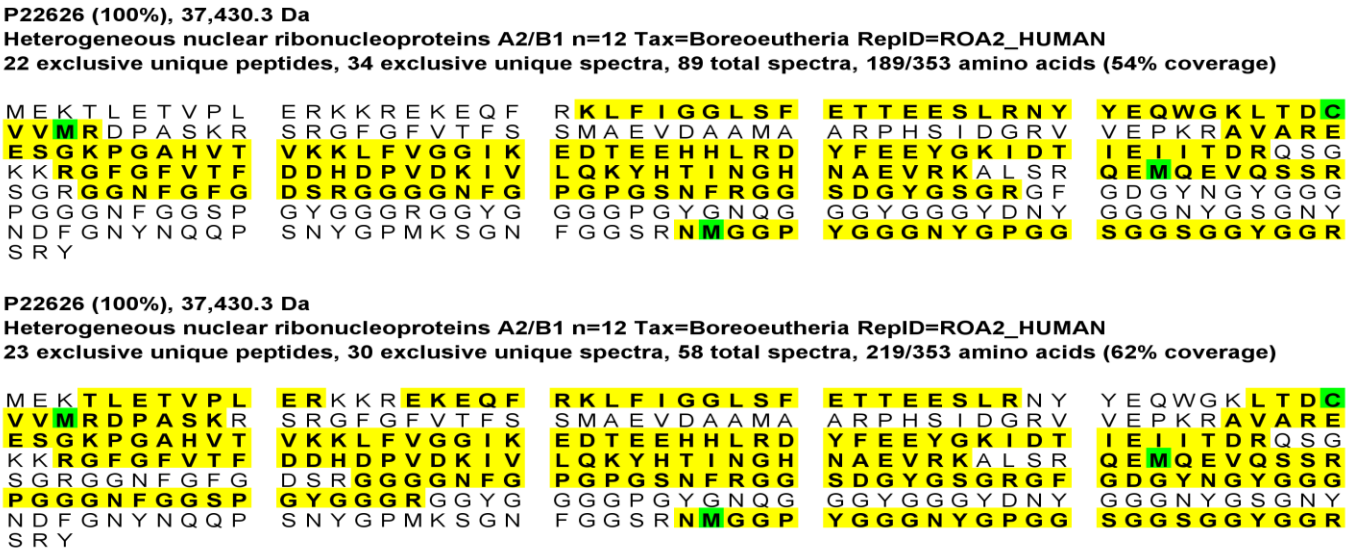
N-(4-((S)-4-((R)-1-((S)-2-(((S)-6-benzyl-2,3-dioxopiperazin-1-yl)methyl)pyrrolidin-1-yl)-3-cyclohexylpropan-2-yl)-5,6-dioxo-1-phenethylpiperazin-2-yl)butyl)-5-((3aR,4R,6aS)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide. Using Scheme 3 for the synthesis of Biotin tagged pyrrolidine-bis-diketopiperazines compound 2529-7 was synthesized using the following reagents: (100mg) MBHA resin starting material, Fmoc-L-Phenylalanine -OH (R_1), Fmoc-D-Cyclohexylalanine-OH (R_2), Fmoc-L-Lysine(Boc) -OH (R_3), and Phenylacetic Acid (R_4). The final crude product was purified using HPLC as described above, with a gradient of (B) 0/5, 2/5, 4/20, 40/55. **Isolated Mass** 16.1mg **%yield** 14.50%.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.39 (br. s., 1 H) 8.65 (br. s., 1 H) 7.78 (br. s., 1 H) 7.33 (br. s., 5 H) 7.28 (br. s., 3 H) 6.29 - 6.47 (m, 1 H) 4.86 (br. s., 1 H) 4.21 - 4.36 (m, 1 H) 4.12 (br. s., 1 H) 4.03 (d, J=12.72 Hz, 2 H) 3.79 (br. s., 4 H) 3.59 - 3.75 (m, 7 H) 3.52 (d, J=18.58 Hz, 3 H)3.25 - 3.43 (m, 2 H) 3.18 (br. s., 2 H) 2.97 - 3.10 (m, 4 H) 2.92 (br. s., 2 H) 2.70 - 2.87 (m, 1 H) 2.57 (d, J=11.62 Hz, 1 H) 2.29 (br. s., 1 H) 2.04 (br. s., 2 H) 1.99 (br. s., 1 H) 1.85 (br. s., 2 H) 1.65 (br. s., 2 H) 1.60 (br. s., 3 H) 1.48 (br. s., 2 H) 1.33 - 1.44 (m, 3 H) 1.29 (br. s., 4 H) 1.13 (br. s., 3 H) 0.72 - 0.96 (m, 2 H) **m/z** calcd $C_{51}H_{72}N_8O_6S$ $[M+H]^+$ 925.53, found 463.20 (MS ESI) **Purity** LC-MS: 95.0% (254 nm, peak height).

Supplemental Table 1. Viability testing of biotinylated analogs of compound 2155-14 with WM266-4 cells. All units are IC_{50}, µM (n=3). Numbers in 2155-14 structure indicate positions of substitutions of basic scaffold.

ID	Structure	R1	R2	R3	R4	IC_{50}, µM
2155-14/2529-1						4.0/1.6
2529-3		Biotin				>100
2529-5			Biotin			>100
2529-7				Biotin		3.3

Supplementary Figure 2. Comparison of sequence coverage of bands 3 and 4 from pulldown experiment. Yellow = identified peptides, green = modified amino acids. Top = coverage of band 4, bottom = coverage of band 3. Please note that there is a difference in coverage of amino acids 3-14 between band 3 and 4 suggesting that band 3 is hnRNP B1 and band 4 is hnRNP A2.



Supplementary Figure 3. Optimization of DDX1, hnRNP H2, and hnRNP A2/B1 siRNA knock-down conditions. Encircled concentrations of respective siRNAs were chosen for downstream experiments. C1 = scrambled siRNA control at 100nM.