Supporting Information

Synthetic mRNA Splicing Modulator Compounds with *In Vivo* Anti-tumor Activity

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[†]C.L. performed the majority of the chemistry content of this work while A.P. performed the majority of the biology studies described herein; C.L. and A.P. should be considered co-equal contributors to this study.

Contents

(S,Z)-5-((1R,4R)-4-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dienyl)cyclohexylamino)-5-oxopent-3-en-2-yl 4-(3-methoxyphenyl)piperazine-1-carboxylate (8). The title compound was prepared from (S,Z)-5-((1R,4R)-4-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dienyl)cyclohexylamino)-5-oxopent-3-en-2-yl 4-nitrophenyl carbonate (6 mg, 10.3 μmol) and 1-(3-methoxyphenyl)piperazine (30 μL, 0.03 mmol, 1M stock solution) by following the procedure for compound **7**. Yield: 3 mg (46%) of **8** as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 7.9, 1H), 7.22 – 7.17 (m, 1H), 6.54 (dd, *J* = 7.9, 1.7 Hz, 1H), 6.50 – 6.43 (m, 2H), 6.27 (d, *J* = 15.7 Hz, 1H), 5.84 (d, *J* = 10.9 Hz, 1H), 5.70 – 5.61 (m, 2H), 5.58 – 5.48 (m, 2H), 4.53 – 4.40 (m, 1H), 4.18 – 4.10 (m, 1H), 3.80 (s, 3H), 3.63 (s, 4H), 3.20 – 3.12 (m, 4H), 2.56 (s, 2H), 2.12 – 2.05 (m, 2H), 2.04 – 1.87 (m, 2H), 1.72 (s, 3H), 1.63 – 1.54 (m, 5H), 1.40 (s, 3H), 1.38 – 1.35 (m, 4H), 1.28 (s, 3H), 1.26 – 1.13 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.05, 160.64, 155.17, 152.27, 136.64, 136.24, 133.74, 132.24, 129.96, 126.94, 126.23, 109.45, 105.22, 103.27, 73.05, 69.94, 69.63, 55.70, 55.25, 54.05, 51.06, 49.31, 45.35, 43.10, 42.47, 38.65, 36.61, 34.54, 31.55, 29.62, 27.48, 23.79, 20.64, 12.52; HRMS (ESI) *m/z* Calcd for $C_{37}H_{54}N_3O_6$ $(M+1)^+$ 636.4013, found 636.4005.

(S,Z)-5-((1R,4R)-4-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dienyl)cyclohexylamino)-5-oxopent-3-en-2-yl 4-methylpiperazine-1-carboxylate (9). The title compound was prepared from (S,Z)-5-((1R,4R)-4-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dienyl)cyclohexylamino)-5-oxopent-3-en-2-yl 4-nitrophenyl carbonate (6 mg, 10.3 μmol) and 1-methylpiperazine (31 μL, 0.03 mmol, 1M stock solution) by following the procedure for compound **7**. Yield: 2.5 mg (45%) of **9** as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, *J* = 7.9 Hz, 1H), 6.27 (d, *J* = 15.7 Hz, 1H), 5.83 (d, *J* = 10.9, 1H), 5.65 – 5.46 (m, 4H), 4.52 – 4.41 (m, 1H), 4.14 (s, 1H), 3.50 (s, 4H), 2.56 (s, 2H), 2.37 (t, *J* = 4.7 Hz, 4H), 2.31 (s, 3H), 2.10 – 2.04 (m, 2H), 2.01 – 1.86 (m, 2H), 1.71 (s, 3H), 1.64 – 1.55 (m, 6H), 1.41 (s, 3H), 1.38 – 1.32 (m, 4H), 1.28 (s, 3H), 1.27 – 1.12 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.09, 155.18, 136.46, 136.26, 133.63, 132.29, 126.93, 126.27, 73.05, 69.76, 69.63, 55.69, 54.66, 51.06, 46.20, 45.28, 42.47,

38.66, 36.64, 34.57, 31.55, 29.64, 29.49, 27.83, 27.48, 23.79, 20.64, 12.50; HRMS (ESI) *m/z* Calcd for $C_{31}H_{50}N_3O_5$ $(M+1)^+$ 544.3750, found 544.3737.

(S,Z)-5-((1R,4R)-4-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dienyl)cyclohexylamino)-5-oxopent-3-en-2-yl 4-(2-hydroxyethyl)piperazine-1-carboxylate (10). The title compound was prepared from (S,Z)-5-((1R,4R)-4-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dienyl)cyclohexylamino)-5-oxopent-3-en-2-yl 4-nitrophenyl carbonate (2 mg, 3.43 μmol) and 2-(piperazin-1-yl)ethanol (10.3 μL, 10.3 μmol, 1M stock solution) by following the procedure for compound **7**. Yield: 1 mg (51%) of **10** as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 8.0 Hz, 1H), 6.27 (d, *J* = 15.7 Hz, 1H), 5.82 (d, *J* = 10.7 Hz, 1H), 5.67 – 5.46 (m, 4H), 4.46 (ddd, *J* = 9.1, 6.7, 1.9 Hz, 1H), 4.13 (s, 1H), 3.68 – 3.61 (m, 2H), 3.53 – 3.47 (m, 4H), 2.61 – 2.54 (m, 4H), 2.48 (t, *J* = 4.9 Hz, 4H), 2.12 – 2.04 (m, 2H), 2.02 – 1.87 (m, 2H), 1.74-1.69 (m, 6H), 1.64 – 1.51 (m, 5H), 1.41 (s, 3H), 1.35 (d, *J* = 6.0 Hz, 3H), 1.34-1.32 (m, 1H), 1.28 (s, 3H), 1.25 – 1.13 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.04, 155.12, 136.57, 136.23, 133.64, 132.22, 126.97, 126.23, 73.05, 69.83, 69.62, 59.39, 57.80, 55.69, 52.56, 51.05, 45.30, 42.47, 38.66, 36.60, 34.55, 31.55, 29.63, 29.48, 27.82, 27.48, 23.79, 20.63, 12.51; HRMS (ESI) *m/z* Calcd for $C_{32}H_{52}N_3O_6$ $(M+1)^+$ 574.3856, found 574.3858.

(S,Z)-5-((1R,4R)-4-((2E,4E)-5-((2S,4R)-4-(chloromethyl)-4-hydroxy-6,6-dimethyltetrahydro-2H-pyran-2-yl)-3-methylpenta-2,4-dienyl)cyclohexylamino)-5-oxopent-3-en-2-yl 4-cycloheptylpiperazine-1-carboxylate (11). A solution of alcohol **4** (8 mg, 0.019 mmol) and pyridine (3.0 μL, 0.038 mmol) in THF/CH_3CN (2:1) (0.6 mL) was stirred at room temperature as 4-nitrophenylchloro formate (7.7 mg, 0.038 mmol) was added at once. The resulting solution was stirred at room temperature for overnight. The solvent was evaporated and the residue was purified by flash chromatography (20% ethyl acetate in hexane) to give 3.0 mg (27%) of chlorinated product as a liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 – 8.24 (m, 2H), 7.41 – 7.36 (m, 2H), 6.33 – 6.24 (m, 2H), 6.07 (br d, 1H), 5.97 (dd, *J* = 11.6, 8.1, 1H), 5.88 – 5.81 (m, 1H), 5.55 (dd, *J* = 15.6, 6.7, 1H), 5.46 (t, 1H), 4.53 – 4.44 (m,

1H), 4.10 – 4.04 (m, 1H), 3.47 (d, *J* = 1.4, 2H), 2.08 – 2.02 (m, 2H), 1.75 – 1.67 (m, 2H), 1.68 (s, 3H), 1.63 – 1.55 (m, 7H), 1.54 (d, *J* = 6.5, 3H), 1.45 (s, 3H), 1.38 – 1.30 (m, 2H), 1.26 (s, 3H), 1.24 – 1.15 (m, 2H); MS (ESI) m/z 641.1$(M+Na)^+$. A solution of above activated carbonate (3.0 mg, 4.85 μmol) in 1,2 dichloroethane (0.5 mL) was stirred at room temperature as 1-cycloheptyl piperizine (14.54 μL, 1.0M stock solution, 0.015 mmol) was added. The resulting solution was stirred for 3h and the solvent was removed under vacuum. The residue was purified by flash chromatography (1% - 2.5% MeOH in CH_2Cl_2) to give 1.5 mg (47%) of **11** as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 – 8.13 (br d, 1H), 6.29 (d, *J* = 15.7 Hz, 1H), 5.83 (d, *J* = 11.1 Hz, 1H), 5.66 – 5.46 (m, 4H), 4.54 – 4.42 (m, 1H), 4.20 – 4.05 (m, 1H), 3.50 – 3.42 (m, 4H), 2.62 – 2.53 (m, 1H), 2.51 – 2.45 (m, 4H), 2.12 – 2.05 (m, 2H), 1.82 – 1.75 (m, 2H), 1.74 – 1.67 (m, 5H), 1.60 – 1.51 (m, 14H), 1.51 – 1.44 (m, 5H), 1.45 (s, 3H), 1.42 – 1.37 (m, 2H), 1.35 (d, *J* = 6.0 Hz, 3H), 1.27 – 1.24 (m, 5H); MS (ESI) *m/z* 662.2 $(M)^+$.

(S,Z)-5-((1R,4R)-4-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dienyl)cyclohexylamino)-5-oxopent-3-en-2-yl furan-2-carboxylate (14). Yield: 8 mg (65.3%) of **14** as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (dd, *J* = 1.7, 0.9 Hz, 1H), 7.20 (dd, *J* = 3.5, 0.8 Hz, 1H), 6.98 (d, *J* = 7.6 Hz, 1H), 6.52 (dd, *J* = 3.5 Hz, 1.7, 1H), 6.26 (d, *J* = 15.7 Hz, 1H), 6.09 (dd, *J* = 8.2, 6.5 Hz, 1H), 5.82 (dt, *J* = 11.7, 10.0 Hz, 2H), 5.59 – 5.45 (m, 2H), 4.45 (ddd, *J* = 10.9, 6.7, 1.8 Hz, 1H), 4.18 – 4.07 (m, 1H), 2.57 (s, 2H), 2.15 – 2.06 (m, 2H), 2.03 – 1.87 (m, 2H), 1.71 (s, 4H), 1.67 – 1.56 (m, 6H), 1.50 (d, *J* = 6.5 Hz, 3H), 1.40 (s, 3H), 1.29-1.26 (m, 5H), 1.24 – 1.12 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.76, 158.57, 146.55, 144.53, 137.88, 136.25, 133.84, 132.01, 126.98, 125.62, 118.44, 111.96, 73.04, 69.99, 69.63, 55.69, 51.05, 45.58, 42.47, 38.64, 36.43, 31.55, 29.73, 29.50, 29.38, 27.88, 27.63, 23.79, 20.40, 12.48; IR (Neat Film) 3320, 2924, 2853, 1715, 1668**,** 1629, 1532, 1473, 1309, 1181, 1118, 1045 cm^{-1}; HRMS (ESI) *m/z* Calcd for $C_{30}H_{42}NO_6$ $(M+1)^+$ 512.3012, found 512.3007.

(S,Z)-5-((1R,4R)-4-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dienyl)cyclohexylamino)-5-oxopent-3-en-2-yl 1-methyl-1H-pyrrole-2-carboxylate (15). Yield: 7 mg (56%) of **15** as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, *J* = 7.8 Hz, 1H), 6.98 (dd, *J* = 4.0, 1.8 Hz, 1H), 6.80

(t, *J* = 2.1 Hz, 1H), 6.25 (d, *J* = 15.7 Hz, 1H), 6.12 (dd, *J* = 4.0, 2.5 Hz, 1H), 5.95 – 5.87 (m, 1H), 5.85 – 5.71 (m, 2H), 5.56-5.46 (m, 2H), 4.52 – 4.38 (m, 1H), 4.16 (s, 1H), 3.91 (s, 3H), 2.57 (s, 2H), 2.13 – 1.83 (m, 4H), 1.80-1.75 (m, 1H), 1.69 (s, 3H), 1.65-1.55 (m, 6H), 1.45 (d, *J* = 6.5 Hz, 3H), 1.40 (s, 3H), 1.28 (s, 3H), 1.26-1.24 (m, 2H), 1.23-1.14 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.04, 161.38, 137.59, 136.19, 133.75, 131.98, 130.04, 127.03, 125.61, 122.13, 118.46, 108.07, 73.05, 69.61, 68.67, 55.68, 51.05, 45.20, 42.47, 38.64, 36.89, 36.80, 31.55, 29.73, 29.68, 29.56, 27.95, 27.64, 23.79, 20.53, 12.47; IR (Neat Film) 3318, 2923, 2852, 1703, 1667**,** 1628, 1530, 1412, 1245, 1047 cm^{-1}; HRMS (ESI) *m/z* Calcd for $C_{31}H_{45}N_2O_5$ $(M+1)^+$ 525.3328, found 525.3320.

(S,Z)-5-((1R,4R)-4-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dienyl)cyclohexylamino)-5-oxopent-3-en-2-yl benzoate (16). Yield: 8 mg (64%) of **16** as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 – 8.02 (m, 2H), 7.62 – 7.55 (m, 1H), 7.45 (dd, *J* = 10.6, 4.8 Hz, 2H), 7.32 (d, *J* = 7.8 Hz, 1H), 6.26 (d, *J* = 15.7 Hz, 1H), 6.05 (dd, *J* = 8.8, 6.5 Hz, 1H), 5.81 (dt, *J* = 11.7, 10.4 Hz, 2H), 5.59 – 5.46 (m, 2H), 4.44 (ddd, *J* = 11.0, 6.7, 1.9 Hz, 1H), 4.16 (s, 1H), 2.57 (s, 2H), 2.10 (dd, *J* = 11.2, 7.2 Hz, 2H), 2.03 – 1.86 (m, 2H), 1.82 – 1.68 (m, 2H), 1.68 – 1.58 (m, 6H), 1.52 (d, *J* = 6.5 Hz, 3H), 1.40 (s, 3H), 1.38 – 1.10 (m, 9H) ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.62, 164.90, 137.56, 136.23, 133.81, 133.27, 132.03, 129.98, 129.67, 128.44, 126.99, 125.80, 73.04, 69.86, 69.61, 55.69, 51.05, 45.39, 42.47, 38.63, 36.62, 31.55, 29.73, 29.62, 29.51, 27.86, 27.58, 23.79, 20.45, 12.49; IR (Neat Film) 3317, 2924, 2853, 1718, 1668**,** 1627, 1533, 1451, 1325, 1269, 1195, 1046 cm^{-1}; HRMS (ESI) *m/z* Calcd for $C_{32}H_{44}NO_5$ $(M+1)^+$ 522.3219, found 522.3209.

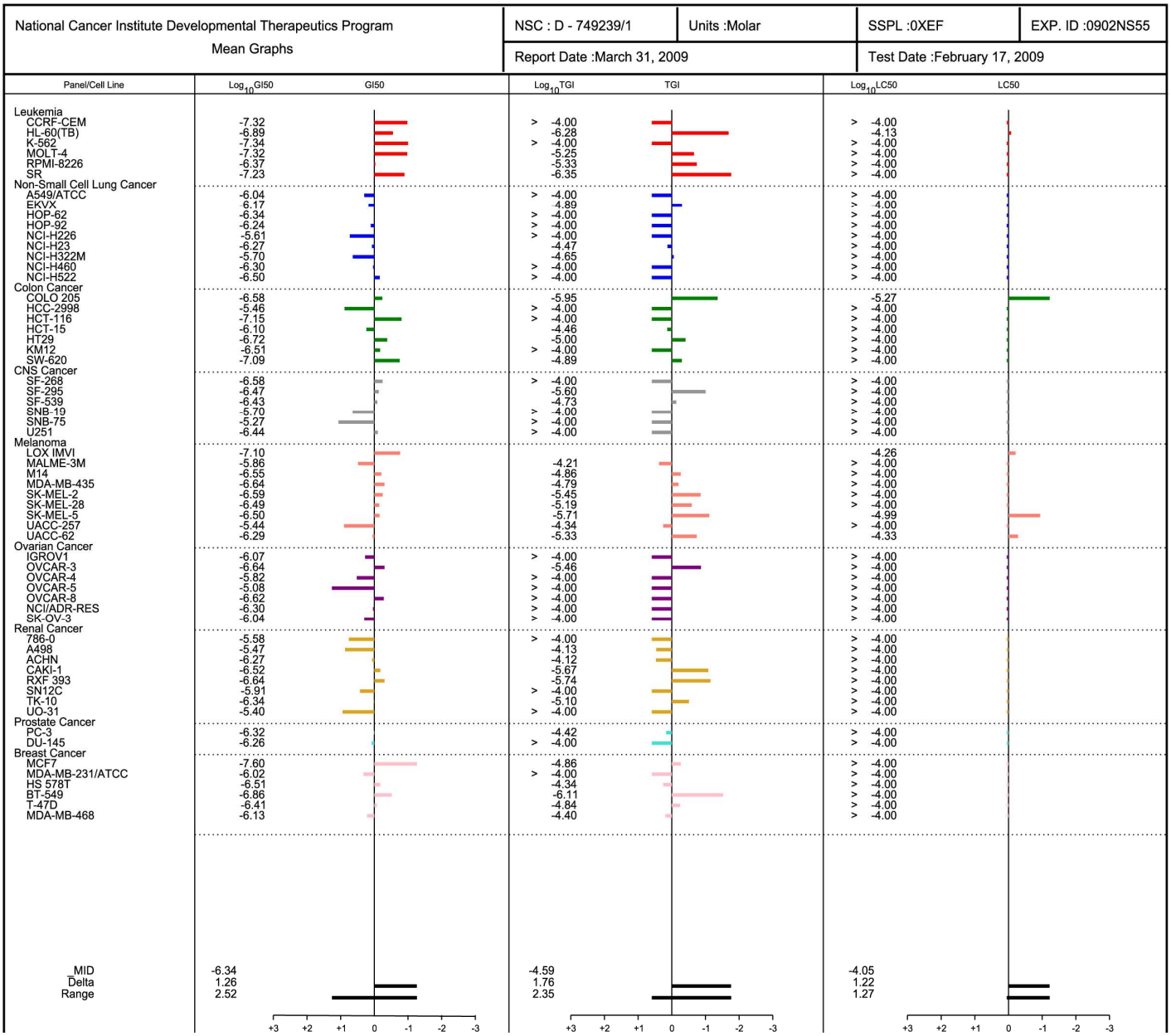
(S,Z)-5-((1R,4R)-4-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dienyl)cyclohexylamino)-5-oxopent-3-en-2-yl nicotinate (17). Yield: 3 mg (24%) of **17** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.23 (dd, *J* = 2.2, 0.8 Hz, 1H), 8.79 (dd, *J* = 4.9, 1.7 Hz, 1H), 8.34 – 8.25 (m, 1H), 7.40 (ddd, *J* = 8.0, 4.9, 0.9 Hz, 1H), 6.83 (d, *J* = 7.5 Hz, 1H), 6.30 -6.20 (m, 2H), 5.89 – 5.82 (m, 2H), 5.62 – 5.43 (m, 2H), 4.53 – 4.40 (m, 1H), 4.14 (s, 1H), 2.57 (s, 2H), 2.12-2.06 (m, 2H), 2.03 – 1.86 (m, 2H), 1.75-1.70 (m, 6H), 1.67-1.58 (m, 5H), 1.54 (d, *J* = 6.5 Hz, 3H), 1.51-1.45 (m, 1H), 1.40 (s, 3H), 1.28 (s, 3H), 1.24 – 1.12 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.16, 164.61, 153.64, 150.98, 138.62, 137.11, 136.16, 133.86, 131.87,

127.08, 126.04, 125.37, 123.32, 73.05, 70.44, 69.59, 55.68, 51.05, 45.50, 42.47, 38.64, 36.52, 34.37, 31.55, 29.52, 29.43, 27.90, 27.68, 23.78, 20.32, 12.51; IR (Neat Film) 3333, 2921, 2851, 1723, 1667**,** 1628, 1534, 1327, 1275, 1119, 1045 cm^{-1}; HRMS (ESI) *m/z* Calcd for $C_{31}H_{43}N_2O_5$ $(M+1)^+$ 523.3172, found 523.3148.

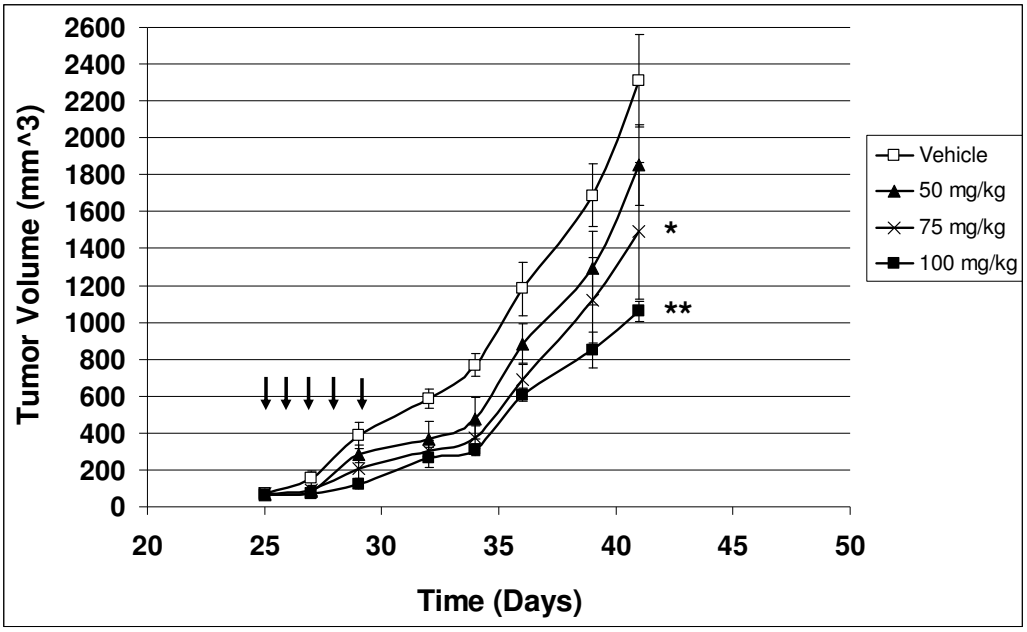
(S,Z)-5-((1R,4R)-4-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dienyl)cyclohexylamino)-5-oxopent-3-en-2-yl 1-(2-(trifluoromethyl)pyrimidin-4-yl)piperidine-4-carboxylate (18). Yield: 9 mg (56%) of **18** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, *J* = 4.8 Hz, 1H), 6.97 (d, *J* = 7.7 Hz, 1H), 6.73 (d, *J* = 4.8 Hz, 1H), 6.26 (d, *J* = 15.7 Hz, 1H), 5.88 (dt, *J* = 12.7, 6.3 Hz, 1H), 5.80 (dd, *J* = 11.7, 0.7 Hz, 1H), 5.66 (dd, *J* = 11.7, 8.9 Hz, 1H), 5.54 (dd, *J* = 15.7, 6.7 Hz, 1H), 5.47 (t, *J* = 7.5 Hz, 1H), 4.67 (dd, *J* = 9.5, 4.0 Hz, 2H), 4.46 (ddd, *J* = 11.1, 6.8, 2.1 Hz, 1H), 4.10 (s, 1H), 3.19 – 3.05 (m, 2H), 2.67 – 2.55 (m, 3H), 2.06 (td, *J* = 7.3, 2.9 Hz, 2H), 2.03 – 1.87 (m, 4H), 1.78 – 1.65 (m, 7H), 1.64 – 1.54 (m, 5H), 1.42 – 1.35 (m, 6H), 1.28 (s, 3H), 1.25 – 1.11 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.64, 164.67, 161.35, 160.10, 138.11, 136.16, 133.81, 131.95, 127.04, 125.46, 104.43, 73.04, 69.58, 69.37, 55.69, 51.06, 45.39, 43.09, 43.07, 42.47, 41.32, 38.64, 36.55, 34.40, 31.55, 29.53, 29.42, 27.89, 27.86, 27.79, 27.61, 23.78, 20.26, 12.48; IR (Neat Film) 3328, 2930, 2857, 1729, 1667**,** 1627, 1592, 1523, 1449, 1329, 1243, 1132, 1046 cm^{-1}; HRMS (ESI) *m/z* Calcd for $C_{36}H_{50}N_4O_5F_3$ $(M+1)^+$ 675.3733, found 675.3721.

(S,Z)-5-((1R,4R)-4-((2E,4E)-5-((3R,5S)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dienyl)cyclohexylamino)-5-oxopent-3-en-2-yl pivalate (19). Yield: 7 mg (58%) of **19** as viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (d, *J* = 7.9 Hz, 1H), 6.27 (d, *J* = 15.7 Hz, 1H), 5.81 (d, *J* = 11.7 Hz, 1H), 5.73 (tt, *J* = 12.7, 6.1 Hz, 1H), 5.61 (dd, *J* = 11.7, 9.2 Hz, 2H), 5.49 (t, *J* = 7.4, Hz, 1H), 4.46 (ddd, *J* = 11.1, 6.7, 1.8 Hz, 1H), 4.14 (d, *J* = 3.9 Hz, 1H), 2.57 (s, 2H), 2.11-2.04 (m, 2H), 2.02 – 1.88 (m, 3H), 1.75-1.68 (m, 5H), 1.65-1.55 (m, 5H), 1.40 (s, 3H), 1.36 (d, *J* = 6.4 Hz, 3H), 1.32 – 1.22 (m, 14H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.82, 164.89, 137.40, 136.24, 133.77, 132.09, 126.98, 125.73, 73.05, 69.60, 69.02, 55.69, 51.05, 45.23, 42.47, 38.69, 38.64, 36.67, 34.53, 31.55, 29.73, 29.64, 29.52, 27.84, 27.54, 27.11, 23.78, 20.24, 12.51; IR (Neat Film) 3320, 2924,

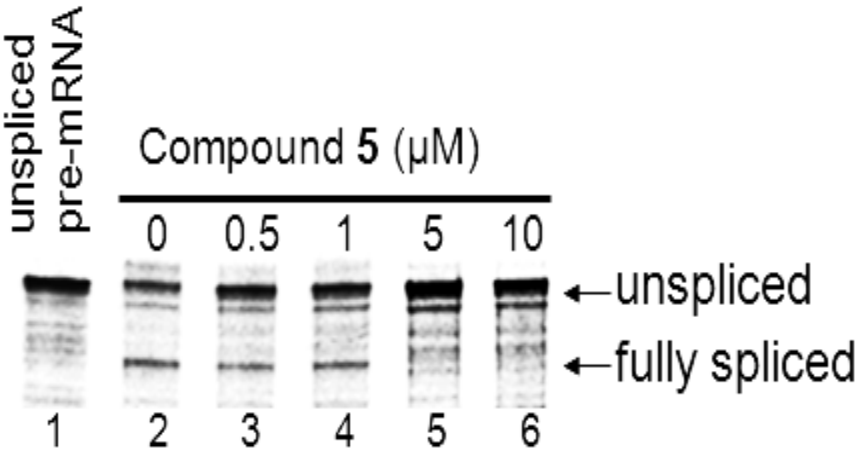
2931, 2853, 1728, 1668**,** 1627, 1533, 1479, 1396, 1278, 1163, 1048 cm^{-1}; HRMS (ESI) *m/z* Calcd for $C_{30}H_{50}NO_5$ $(M+1)^+$ 502.3532, found 502.3518.



Supplemental Figure S1. Growth inhibition and cytotoxic activity of compound **5** in the NCI-60 cell line panel. Compound **5** was screened against the NCI-60 cell line panel by the National Cancer Institute Developmental Therapeutics Program. Anti-cancer activity was measured using the Sulforhodamine B (SRB) protein dye. Three dose response parameters are presented for compound **5**: 50% growth inhibition compared to control (GI50), total growth inhibition (TGI), and 50% lethal concentration compared to control (LC50). Data are presented as a mean graph with bars depicting the variation of each cell line from the overall mean value of all the cancer cell lines. A bar projecting to the right represents a cell line more sensitive than the mean of all the cell lines and a bar projecting to the left represents a cell line more resistant than the mean of all the cell lines.



Supplemental Figure S2. *In vivo* JeKo-1 tumor treatment study with compound **26**. JeKo-1 tumors were transplanted to *Es-1*/SCID mice (these mice carry a heritable mutation of an allele of the esterase locus *Es-1*, designated *Es1*^{e}, resulting in reduced plasma esterase enzyme activity)[1,2] on day 1 and, beginning on day 25, the mice (4 per group) received IV injections of vehicle, 50, 75, or 100 mg/kg of compound **26** daily for five consecutive days. The average tumor volume in all cohorts of mice at the commencement of treatment on day 25 was 75 mm^3. Arrows indicate the dosing schedule. Vehicle alone did not inhibit tumor growth as compared to saline-treated mice (data not shown). The data are represented as mean ± SEM. Tumor volumes were modeled with treatment, linear and quadratic trends over time, and interaction of treatment with linear and quadratic trends over time in a repeated measure framework using autoregressive 1 AR(1) variance-covariance structure and empirical sandwich error estimates for fixed effect. Significance is reported as the comparison of tumor volumes in vehicle-treated mice to compound-treated animals. All analyses were performed using SAS software (SAS Institute, Cary, NC), Windows version 9.1. *, $p < 0.05$; **, $p < 0.0001$.



Supplemental Figure S3. Inhibition of splicing activity *in vitro* by compound **5**. Standard mRNA splicing reactions (see Experimental Section) containing HeLa nuclear extract were incubated in the presence or absence of compound **5** at 30°C for 1 hour, then terminated and the RNA products analyzed by autoradiography following electrophoresis on a 6% polyacrylamide TBE-urea gel. Lane 1 contains the unspliced target pre-mRNA and lanes 2 to 6 contain all reaction components including the nuclear extract. The data shown are representative of repeated analyses.

References

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