Supplemental Material

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Hybrid Enzalutamide Derivatives with Histone Deacetylase Inhibitor Activity Decrease Hsp90 and the Androgen Receptor Levels and Inhibit Viability in Enzalutamide Resistant C4-2 Prostate Cancer Cells

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Synthesis of compounds 2-75, 1005, 3-52 and 1002.

General Methods for Chemistry. ^{1}H and ^{13}C NMR spectra were obtained with Varian Mercury 600MHz, Bruker Ultrashield 400 MHz, Advance 300 MHz spectrometers. Chemical shifts are reported as δ values in parts per million (ppm) relative to tetramethylsilane (TMS) for all recorded NMR spectra. All reagents and solvents were obtained commercially from Acros, Aldrich, and Fluka and were used without further purification. High resolution mass spectral data were collected using a LCT Premier XE KD128 instrument. All compounds submitted for biological testing were found to be >95% pure by analytical HPLC.

(E)-ethyl 3-(4-(3-(4-cyano-3-(trifluoromethyl)phenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl)-2-fluorophenyl)acrylate **2** (**1002**). Compound **1** (420 mg, 0.78 mmol), $Pd(OAc)_2$ (8.7 mg, 0.04 mmol) and P(*o*-tolyl)$_3$ (24 mg, 0.08 mmol) were mixed in a flask. DMF (6 ml) and DIPEA (2 ml) were added via syringe under argon followed by the addition of ethyl acrylate (0.1 mL, 1 mmol). The reaction mixture was heated at 80^{o}C for 5h. After cooled to ambient temperature, the reaction mixture was diluted with EtOAc and washed with water and brine, dried over Na_2SO_4.

Purification by silica gel column (Hexane/EtOAc=4/1) provided the title compound (297 mg, 75%) as slightly brown powder. ^{1}H NMR (400MHz, $CDCl_3$): δ 7.99-7.96 (m, 2H), 7.85-7.79 (m, 2H), 7.70 (t, *J*=8.2Hz, 1H), 7.17-7.11 (m, 2H), 6.61 (d, *J*=16.4Hz, 1H), 4.29 (q, *J*=7.2Hz, 2H), 1.62 (s, 6H), 1.36 (t, *J*=7.2Hz, 3H). ^{13}C NMR (100MHz, $CDCl_3$): δ 179.74, 174.48, 166.28, 161.14 (d, *J*=255.5Hz), 137.36 (d, *J*=10.2Hz), 136.86, 135.51 (d, *J*=1.0Hz), 135.24, 133.59 (q, *J*=33.3Hz), 132.12, 129.98 (d, *J*=4.0Hz), 127.03 (q, *J*=4.7Hz), 125.86 (d, *J*=3.7Hz), 123.16, 122.85 (d, *J*=6.5 Hz), 120.44, 117.91 (d, *J*=23.6Hz), 114.68, 110.31 (d, *J*=2.0Hz), 66.57, 60.88, 23.76, 14.21.

(E)-3-(4-(3-(4-cyano-3-(trifluoromethyl)phenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl)-2-fluorophenyl)acrylic acid (**3**). Compound **1002** (297 mg, 0.58 mmol) was dissolved in a mixture of acetonitrile (7 mL) and HCl (37%, 2 mL). The reaction mixture was refluxed until the completion shown by TLC. After the solvent was removed under reduced pressure, the residue was partitioned between EtOAc and water. The collected organic phase was washed with water and brine, dried over Na_2SO_4. Purification by silica gel column (Hexane/EtOAc/AcOH=3/2/0.5%) provided the title compound (257 mg, 91%) as white foam solid. ^{1}H NMR (400MHz, Acetone-d_6): δ 8.28 (d, *J*=8.0Hz,1H), 8.20 (d, *J*=1.6Hz,1H), 8.08 (dd, *J*=8.4Hz, 2.0Hz,1H), 8.01 (t, *J*=8.4Hz, 1H), 7.83 (d, *J*=16Hz, 1H,), 7.42-7.37 (m, 2H), 6.71 (d, *J*=16Hz, 1H), 1.68 (s, 6H). ^{13}C NMR (100MHz, Acetone-d_6): δ 181.38, 175.66, 167.21, 161.77 (d, *J*=251.8Hz), 139.59 (d, *J*=10.6Hz), 139.03, 136.66, 136.29 (d, *J*=3.1Hz), 134.28, 132.89 (q, *J*=32.7Hz), 130.69 (d, *J*=3.9Hz), 128.50 (q, *J*=4.9Hz), 127.52 (d, *J*=3.5Hz), 124.66, 124.23 (d, *J*=11.6Hz), 123.34 (d, *J*=5.8Hz), 118.96 (d, *J*=23.6Hz), 115.62, 110.29, 67.63, 23.65. LR-MS for $C_{22}H_{15}F_4N_3O_3S$ $[M-H]^-$ Calcd 476.1, found 476.2.

(E)-3-(4-(3-(4-cyano-3-(trifluoromethyl)phenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl)-2-fluorophenyl)-N-((tetrahydro-2H-pyran-2-yl)oxy)acrylamide (**4**). A flask was charged with compound **3** (90mg, 0.19mmol), NH_2OTHP (24mg, 0.21mmol) and BOP (93mg, 0.21mmol), then filled with argon, DMF (2mL) and DIPEA (65µL, 0.38mmol) were added using syringe. The reaction mixture was stirred overnight at ambient temperature and quenched by adding water. Product was extracted with EtOAc and washed with water and brine, dried over Na_2SO_4. Purification by silica gel column (DCM/EtOAc=4/1) provided the title compound (55mg, 51%) as white solid. ^{1}H NMR (400MHz, DMSO-d_6): δ 11.44 (s, 1H), 8.41 (d, *J*=8.4Hz, 1H), 8.30 (s, 1H), 8.10 (d, *J*=8.4Hz, 1H), 7.88 (t, 1H), 7.60 (d, *J*=16Hz, 1H), 7.44 (m, 1H), 7.34 (d, *J*=8.0Hz, 1H), 6.73 (d, *J*=16Hz, 1H), 4.95 (bs,1H), 4.01 (t, *J*=8.4Hz, 1H), 3.56 (m, 1H), 1.72 (bs, 3H), 1.56 (bs, 9H).

*(E)-3-(4-(3-(4-cyano-3-(trifluoromethyl)phenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl)-2-fluorophenyl)-N-hydroxyacrylamide (**1005**)*. Compound **4** (35mg, 0.06mmol) was dissolved in MeOH (2mL) and cooled in an ice bath, HCl (4N in dioxane, 0.3 mL) was added. The reaction was stirred at 0-4ºC for 1h and evaporated to dryness. The residue was washed with cold ether and the product was obtained as white solid (18 mg, 60%). ^{1}H NMR (400MHz, Methanol-d_4): δ 8.15-8.13 (m, 2H), 7.98 (d, *J*=8.0Hz, 1H), 8.01 (t, *J*=8.0Hz, 1H), 7.72 (d, *J*=16.0Hz, 1H), 7.33-7.27 (m, 2H), 6.67 (d, *J*=16.0Hz, 1H), 1.58 (s, 6H). ^{13}C NMR (100MHz, Methanol-d_4): δ 181.73, 176.52, 165.48, 162.34 (d, *J*=253.0Hz), 139.34, 136.83, 134.44, 133.89 (q, *J*=37.3Hz), 132.91, 130.90, 128.86 (q, *J*=4.8Hz), 127.70 (d, *J*=3.7Hz), 125.22 (d, *J*=11.7Hz), 125.01, 122.83, 122.30, 119.34 (d, *J*=23.8Hz), 115.96, 110.83, 68.04, 23.72. HR-ESI-MS *m/z* Calcd for $C_{22}H_{17}F_4N_4O_3S$ $[M+H]^+$ 493.0958, found 493.0960.

*4-(3-(4-cyano-3-(trifluoromethyl)phenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl)-2-fluorobenzoic acid (**5**)*. HCOOLi (161 mg, 3.09 mmol), Ac_2O (0.2 mL, 2.06 mmol) and DIPEA (0.36 ml, 2.06 mmol) were mixed in DMF (1 mL) and this mixture was stirred at ambient temperature for 1h. Then, LiCl (129 mg, 3.09 mmol), $Pd_2(dba)_3$ (24 mg, 0.025 mmol) and compound **11** (550 mg, 1.03 mmol, dissolved in 1 mL DMF) were added. The reaction mixture was stirred at 80^{o}C overnight. After cooled to ambient temperature, the reaction was diluted with EtOAc, washed with 2M HCl, water and brine, dried over Na_2SO_4. Purification by silica gel column (Hexane/EtOAc/AcOH=3/2/0.5%) provided the title compound (430 mg, 92%) as off-white solid.

^{1}H NMR (600MHz, $CDCl_3$): δ 8.21 (t, *J*=8.4 Hz, 1H), 7.99 (d, *J*=8.4 Hz, 1H), 7.94 (s, 1H), 7.82 (dd, *J*=8.4Hz, 1.8Hz, 1H), 7.23 (dd, *J*=9.0Hz, 1.8Hz, 1H), 7.20 (dd, *J*=10.2Hz, 1.8Hz, 1H), 1.63 (s, 6H). ^{13}C NMR (150MHz, $CDCl_3$): δ 179.63, 174.36, 167.43, 162.65 (d, *J*=264Hz), 141.19 (d, *J*=9.8Hz), 136.72, 135.33, 133.96, 133.71 (q, *J*=33Hz), 132.11, 127.03 (q, *J*=4.5Hz), 125.20 (d, *J*=3.4Hz), 121.78 (q, *J*=273Hz), 119.09, 118.93, 114.68, 110.46.

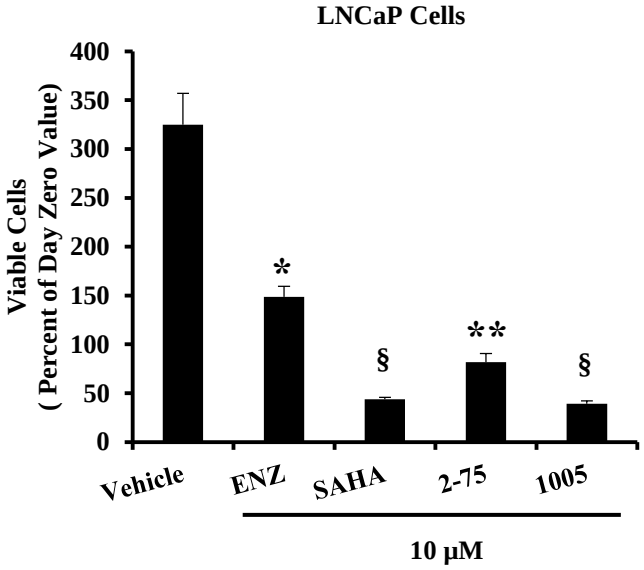
*4-(3-(4-cyano-3-(trifluoromethyl)phenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl)-2-fluoro-N-(7-oxo-7-(((tetrahydro-2H-pyran-2-yl)oxy)amino)heptyl)benzamide (**6**)*. A flask was charged with compound **5** (67 mg, 0.15 mmol), 7-amino-*N*-(tetrahydro-2*H*-pyran-2-yl)oxy heptanamide (74 mg, 0.3 mmol) and HBTU (86 mg, 0.225 mmol), then filled with argon, DMF (2 mL) and DIPEA (0.053 mL, 0.3 mmol)) were added via syringe. The reaction mixture was stirred overnight at room temperature and quenched by adding water. Product was extracted with EtOAc and washed with water and brine, dried over Na_2SO_4. Purification by silica gel column (DCM/MeOH=20/1) provided the title compound (30 mg, 30%) as yellow oil. ^{1}H NMR (600MHz, $CDCl_3$): δ 9.00 (bs, 1H), 8.18 (t, *J*=8.4Hz, 1H), 7.94 (s, 1H), 7.91 (s, 1H), 7.79 (dd, *J*=8.4Hz, 1.8Hz, 1H), 7.19 (dd, *J*=8.4Hz, 1.8Hz, 1H), 7.11 (dd, *J*=10.8Hz, 1.8Hz, 1H), 6.81-6.73 (m, 1H),

4.95-4.88 (m, 1H), 3.97-3.86 (m, 1H), 3.60-3.53 (m, 1H), 3.49-3.41 (m, 2H), 2.13-2.04 (m, 2H), 1.85-1.70 (m, 4H), 1.67-1.47 (m, 6H), 1.57 (s, 6H), 1.41-1.31 (m, 4H).

*4-(3-(4-cyano-3-(trifluoromethyl)phenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl)-2-fluoro-N-(7-(hydroxyamino)-7-oxoheptyl)benzamide (**2-75**)*. Compound **6** (26 mg, 0.038 mmol) was dissolved in MeOH (2mL) and cooled in an ice bath, HCl (4N in dioxane, 0.1 mL) was added. The reaction was stirred at 0-4ºC for 1h and evaporated to dryness. Purification by silica gel column (DCM/MeOH=20/1) provided the title compound (7 mg, 32%) as brown solid. ^{1}H NMR (600MHz, $CDCl_3$): δ 8.26-8.17 (m, 1H), 7.99 (d, *J*=8.4Hz, 1H), 7.95 (s, 1H), 7.82 (d, *J*=8.4Hz, 1H), 7.26-7.24 (m ,1H), 7.15 (d, *J*=11.4Hz, 1H), 6.80 (s, 1H), 3.52-3.41 (m, 2H), 2.23-2.09 (m, 2H), 1.75-1.62 (m, 4H), 1.61 (s, 6H), 1.44-1.32 (m, 4H). ^{13}C NMR (150MHz, $CDCl_3$): δ 179.77, 174.40, 171.20, 162.36, 160.31 (d, *J*=250Hz), 139.03 (d, *J*=10.2Hz), 136.76, 135.32, 133.67 (q, *J*=33.8Hz), 133.27, 132.12, 127.05 (q, *J*=4.5Hz), 126.22, 122.71, 121.84 (q, *J*=286Hz), 117.97 (d, *J*=25.6Hz), 114.68, 110.42, 66.64, 39.83, 32.50, 29.09, 28.08, 26.06, 24.92, 23.83. HR-ESI-MS *m/z* Calcd for $C_{27}H_{28}F_4N_5O_4S$ $[M+H]^+$ 594.1798, found 594.1777.

*Methyl 7-(4-(3-(4-cyano-3-(trifluoromethyl)phenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl)-2-fluorobenzamido)heptanoate **7(3-52**)*. A flask was charged with compound **17** (130 mg, 0.29 mmol), methyl 7-aminoheptanoate hydrochloride (86 mg, 0.44 mmol) and HBTU (167 mg, 0.44 mmol), then filled with argon, DMF (3 mL) and DIPEA (0.15 mL, 0.87 mmol)) were added via syringe. The reaction mixture was stirred overnight at room temperature and quenched by adding water. Product was extracted with EtOAc and washed with water and brine, dried over Na_2SO_4. Purification by silica gel column (Hexane/EtOAc=2/1) provided the title compound (80 mg, 47%) as yellow oil. ^{1}H NMR (600MHz, $CDCl_3$): δ 8.24 (t, *J*=8.4Hz, 1H), 7.97 (d, *J*=8.4Hz, 1H), 7.94-7.92 (m, 1H), 7.81 (dd, *J*=8.4Hz, 1.8Hz, 1H), 7.22 (dd, *J*=8.4Hz, 1.8Hz, 1H), 7.13 (dd,

J=11.4Hz, 1.8Hz, 1H), 6.71-6.66 (m, 1H), 3.65 (s, 3H), 3.51-3.45 (m, 2H), 2.30 (t, *J*=7.8Hz, 2H), 1.67-1.61 (m, 4H), 1.60 (s, 6H), 1.43-1.33 (m, 4H). ^{13}C NMR (150MHz, $CDCl_3$): δ 179.72, 174.42, 174.14, 161.95, 160.34 (d, *J*=248Hz), 138.90 (d, *J*=10.8Hz), 136.78, 135.29, 133.66 (q, *J*=33.3Hz), 133.38 (d, *J*=3.4Hz), 132.11, 127.04 (q, *J*=4.5Hz), 126.15 (d, *J*=3.0Hz), 122.90 (d, *J*=12.1Hz), 121.79 (q, *J*=273Hz), 117.89 (d, *J*=26.1Hz), 114.69, 110.39, 66.60, 51.50, 40.09, 33.91, 29.23, 28.70, 26.54, 24.75, 23.82. HR-ESI-MS *m/z* Calcd for $C_{28}H_{29}F_4N_4O_4S$ $[M+H]^+$ 593.1846, found 593.1829.



Supplemental Figure 1: Effects on cell viability in androgen-sensitive cells.

LNCaP cells were seeded in 96-well plates and 24h later, they were treated with the indicated compounds (10 uM) or with vehicle (DMSO). Cell density was measured by the MTT assay on Days 0 and 3 of treatment. The error bars represent standard deviation of experimental sextuplicate samples. Where indicated, $P < 0.05$