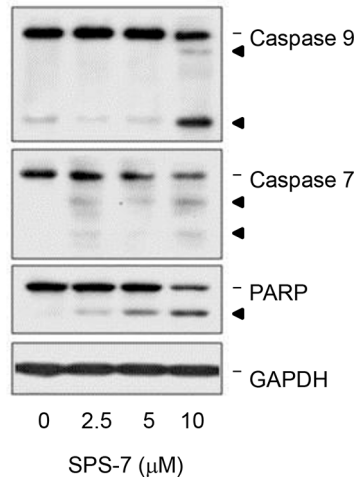


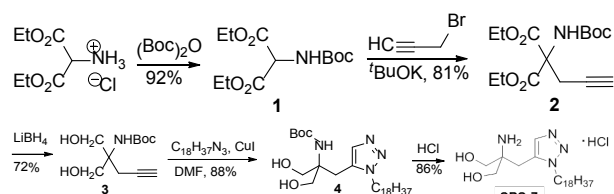
Non-immunosuppressive triazole-based small molecule induces anticancer activity against human hormone-refractory prostate cancers: the role in inhibition of PI3K/AKT/mTOR and c-Myc signaling pathways

Supplementary Materials



Supplementary Figure S1: : Effect of SPS-7 on the expression of caspases and PARP-1. PC-3 cells were incubated in the absence or presence of SPS-7 for 24 h. After the treatment, the cells were harvested and lysed for the detection of protein expressions by Western blot analysis. The data are representative of three independent experiments.

Scheme I. Synthesis of SPS-7



Diethyl 2-((*tert*-butoxycarbonyl)amino)malonate (1)

To a solution of diethylaminomalonate hydrochloride (211.9 g, 1.01 mmol, 1.0 equiv) and triethylamine (204.5 mg, 2.02 mmol, 2.0equiv) in dichloromethane (5.0 mL) was added di-*tert*-butyl dicarbonate (242.4 mg, 1.11 mmol, 1.1equiv). The reaction was stirred at rt for 12 h, then quenched with water, extracted with ethyl acetate, and excess solvent was evaporated under vacuum to give compound 1 (255.8 mg, 0.93mmol, 92%) as a colorless oil. ¹H NMR (300 MHz, CDCl₃) δ 5.54 (d, *J* = 8.0 Hz, 1H), 4.93 (d, *J* = 8.0 Hz, 1H), 4.36-4.12 (m, 4H), 1.42 (s, 9H), 1.23 (t, *J* = 7.0 Hz, 6H); ¹³C NMR (75 MHz, CDCl₃) 166.6, 154.7, 80.5, 62.4, 57.5, 28.1, 13.9; HRMS (ESI⁺) calcd for [M+Na]⁺(C₁₂H₂₁NO₆Na), 298.1267, found 298.1269.

Diethyl 2-(*tert*-butoxycarbonylamino)-2-(prop-2-ynyl)malonate(2)

To a solution of diethyl 2-(*tert*-butoxycarbonylamino)malonate (1, 278.1 mg, 1.01 mmol, 1.0 equiv) and propargyl bromide (144.1 mg, 1.21mmol, 1.2equiv) in 1,4-dioxane (5.0 mL) was added potassium-*tert*-butoxide (147.9 mg, 1.21mmol, 1.2equiv). The reaction was stirred heated at 90°C for 18 h, then quenched with water, extracted with ethyl acetate, and excess solvent was evaporated under vacuum. The crude product was purified by column chromatography (SiO₂: EtOAc/*n*-hexane, 35:65; *R*_f 0.56) to give compound 2 (256.3 mg, 0.81 mmol, 81%) as a colorless oil. ¹H NMR (300 MHz, CDCl₃) δ 6.09 (s, 1H), 4.26-4.22 (m, 4H), 3.22 (s, 2H), 1.96 (s, 1H), 1.41 (s, 9H), 1.24 (t, *J* = 7.0 Hz, 6H); ¹³C NMR (75 MHz, CDCl₃) 166.8, 153.9, 80.5, 78.4, 71.3, 65.5, 62.8, 28.1, 24.3, 13.9.

tert-Butyl 1-hydroxy-2-(hydroxymethyl)pent-4-yn-2-ylcarbamate(3)

To a solution of diethyl diethyl 2-(*tert*-butoxycarbonylamino)-2-(prop-2-ynyl)malonate (2, 316.4 mg, 1.01 mmol, 1.0 equiv) in dry THF (5.0 mL) was added

lithium borohydride (87.6 mg, 4.04 mmol, 4.0 equiv), and dry methanol (1.0 mL). The reaction was stirred heated at 0°C for 6 h, then quenched with water, dried over anhydrous magnesium sulfate, and excess solvent was evaporated under vacuum. The crude product was purified by column chromatography (SiO₂: EtOAc/*n*-hexane, 50:50; *R*_f 0.60) to give compound 3 (166.7 mg, 0.73 mmol, 72%) as a colorless oil. ¹H NMR (300 MHz, CDCl₃) δ 5.24 (s, 1H), 3.84 (d, *J* = 6.9 Hz, 2H), 3.62 (d, *J* = 6.9 Hz, 2H), 3.58 (s, 2H), 2.53 (d, *J* = 2.7 Hz, 2H), 2.06 (t, *J* = 2.7 Hz, 1H), 1.42 (s, 9H); ¹³C NMR (75 MHz, CDCl₃) 156.4, 80.5, 79.4, 71.6, 65.0, 58.5, 28.2, 22.9.

tert-Butyl 1,3-dihydroxy-2-((1-octadecyl-1H-1,2,3-triazol-4-yl)methyl)propan-2-ylcarbamate(4)

To a solution of alkyne 3 (59.6 mg, 0.26 mmol) and 1-azidooctadecane (92.2 mg, 0.31mmol) in DMF (2.0 mL) was added CuI (5.7 mg, 0.03mmol). The reaction was stirred at rt for 16 h, then quenched with water, extracted with ethyl acetate, and excess solvent was evaporated under vacuum. The crude product was purified by column chromatography (SiO₂: EtOAc/*n*-hexane, 55:45; *R*_f 0.64) to give compound 4 (120.7 mg, 0.23 mmol, 88%) as a solid (m.p. 82-83°C). ¹H NMR (300 MHz, CDCl₃) δ 7.47 (s, 1H), 5.59 (s, 1H), 4.77 (s, 2H), 4.27 (t, *J* = 7.2 Hz, 2H), 3.58 (d, *J* = 10.8 Hz, 2H), 3.46 (d, *J* = 12.0 Hz, 2H), 3.11 (s, 2H), 1.87-1.85 (m, 2H), 1.39 (s, 9H), 1.22 (s, 30H), 0.84 (t, *J* = 6.6 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) 156.9, 143.6, 123.4, 80.0, 65.1, 59.6, 50.4, 31.9, 30.2, 29.6, 29.5, 29.3, 28.9, 28.3, 27.7, 26.4, 22.6, 14.1; HRMS (ESI⁺) calcd for [M+Na]⁺(C₂₉H₅₆N₄O₄Na), 547.4199, found 547.4193.

1,3-Dihydroxy-2-((1-octadecyl-1H-1,2,3-triazol-4-yl)methyl)propan-2-aminium chloride (SPS-7)

A mixture of 4 (40.9mg, 0.078mmol), 10% methanolic HCl (0.28 mL, 0.78mmol), and methanol (1.0 mL) was stirred at rt for 6 h. The solvent was removed and the residue was washed with diethyl ether to give SPS-7 (30.8 mg, 0.067 mmol, 86%) as a white solid. ¹H NMR (300 MHz, (CD₃)₂SO) δ 7.99 (s, 1H), 7.94 (s, 3H), 4.29 (t, *J* = 6.6 Hz, 2H), 3.43 (s, 4H), 2.94 (s, 2H), 1.81-1.73 (m, 2H), 1.21 (s, 30H), 0.83 (t, *J* = 6.2 Hz, 3H); ¹³C NMR (75 MHz, (CD₃)₂SO) 140.5, 124.8, 61.2, 60.6, 49.8, 31.7, 30.1, 29.49, 29.48, 29.47, 29.46, 29.3, 29.1, 28.9, 27.2, 26.3, 14.1; HRMS (ESI⁺) calcd for [M-H]⁺(C₂₄H₄₈ClN₄O₂) 459.3466, found 459.3468.