

Epithelial-mesenchymal transition sensitizes breast cancer cells to cell death via the fungus-derived sesterterpenoid ophiobolin A

Keighley N. Reisenauer¹, Yongfeng Tao², Shuxuan Song¹, Saawan D. Patel¹, Alec Ingros¹, Peter Sheesley¹, Marco Masi³, Angela Boari⁴, Antonio Evidente³, Alexander V. Kornienko⁵, Daniel Romo², Joseph Taube^{1#}

Affiliations

¹Department of Biology, Baylor University, Waco, TX, USA

²Department of Chemistry and Biochemistry, Baylor University, Waco, TX, USA

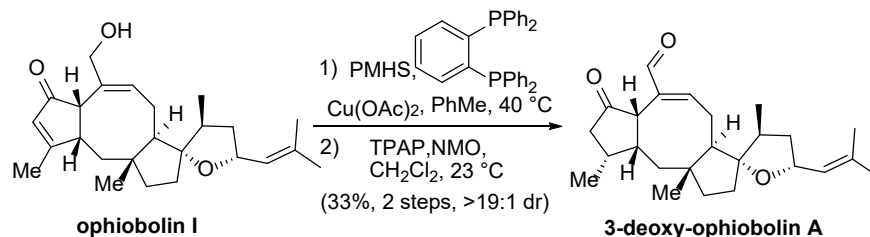
³Department of Chemical Sciences, University of Naples Federico II, Complesso Universitario Monte Sant'Angelo, Naples, Italy

⁴Institute of Sciences and Food Production, CNR, Bari, Italy

⁵Department of Chemistry and Biochemistry, Texas State University, San Marcos, TX, USA

#Corresponding author: Joseph Taube, [Joseph Taube@baylor.edu](mailto:Joseph.Taube@baylor.edu)

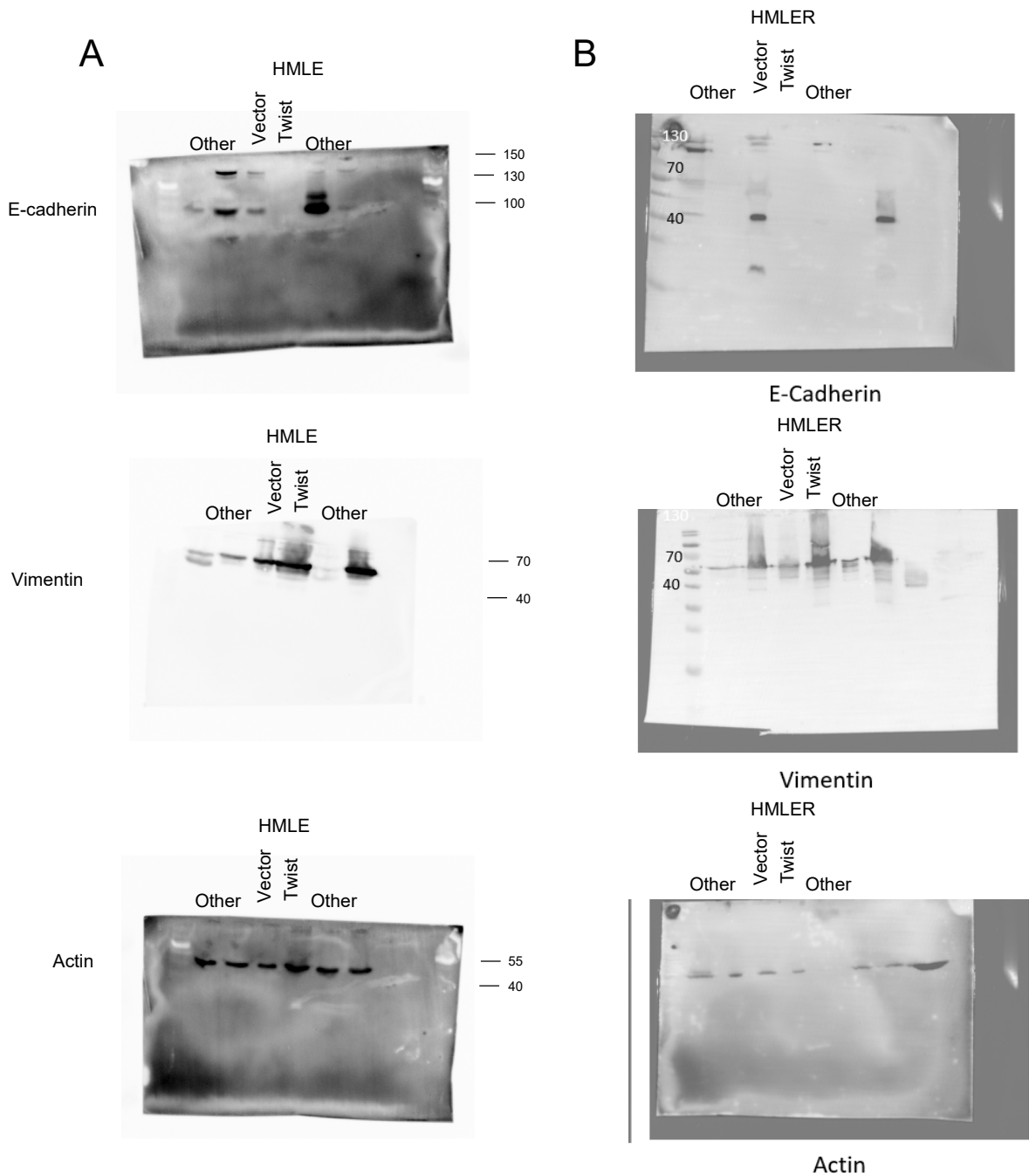
Supporting Information



Synthesis of 3-deoxy-ophiobolin A. To a dry 1-dram vial was added 3 mL dry PhMe, $\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$ (13 mg, 0.065 mmol, 5 equiv), 1,2-bis(diphenylphosphino)benzene (3.0 mg, 0.007 mmol, 0.5 equiv), *t*-BuOH (0.18 mL, 1.95 mmol, 150 equiv), polymethyl hydrosilane (PMHS) (0.12 mL, 1.95 mmol, 150 equiv). The mixture was stirred at 23 °C under argon for 30 min to generate a stock solution of the required reducing agent. In another dry 1-dram vial, ophiobolin I¹ (3.0 mg, 0.008 mmol, 1 equiv) was dissolved in 0.5 mL dry PhMe, and 0.3 mL of the above reagent stock solution was added. The reaction was heated to 40 °C under argon and stirred at this temperature for 2 h. The mixture was concentrated and filtered through a pad of silica gel with CH_2Cl_2 as eluent. The filtrate was then concentrated, dissolved in CH_2Cl_2 , and then *N*-methyl morpholine *N*-oxide (NMO, 1.2 mg, 0.01 mmol, 2 equiv) and *N*-tetra *n*-propyl ruthenium tetroxide (TPAP, 0.1 mg, 0.0003 mmol, 0.05 equiv) were added and stirred at 23 °C for 1 h. The mixture was concentrated and analysis of the crude ¹H NMR (600 MHz) suggested formation of a single diastereomer during the conjugate reduction (>19:1). The mixture was then purified by silica gel chromatography (0 → 10% EtOAc/hexanes) to afford 3-deoxy ophiobolin A (1.0 mg, 33%, >19:1 dr by ¹H NMR) as a colorless residue: TLC (EtOAc: hexanes, 1:2 v/v): R_f = 0.3; ¹H NMR (600 MHz, CDCl_3) δ 9.15 (s, 1H), 6.78 (dd, J = 7.0, 2.4 Hz, 1H), 5.06 (dq, J = 9.2, 1.5 Hz, 1H), 4.54 (dt, J = 8.6, 7.1 Hz, 1H), 3.08 (d, J = 9.4 Hz, 1H), 2.72 (dt, J = 19.8, 3.1 Hz, 1H), 2.53 (dd, J = 14.2, 4.2 Hz, 1H), 2.48 (dd, J = 17.3, 12.6 Hz, 1H), 2.36 (ddd, J = 17.3, 6.6, 1.6 Hz, 1H), 2.30 – 2.22 (m, 1H), 2.14 (q, J = 6.9 Hz, 1H), 1.91 (dd, J = 13.6, 3.5 Hz, 1H), 1.76 – 1.67 (m, 7H), 1.64 (d, J = 1.4 Hz, 3H), 1.60 (d, J = 1.3 Hz, 3H), 1.36 – 1.25 (m, 2H), 1.09 (d, J = 5.8 Hz, 3H), 0.97 (d, J = 6.9 Hz, 3H), 0.76 (s, 3H); ¹³C NMR (150 MHz, CDCl_3) δ 218.81, 193.72, 157.07, 142.60, 135.31, 126.74, 96.06, 71.99, 53.73, 50.81, 50.06, 47.88, 47.16, 42.27, 42.22, 42.02, 38.41, 35.33, 30.93, 29.57, 25.96, 22.73, 18.84, 18.22, 16.22; IR (thin film): 2357, 1738, 1664 cm^{-1} ; HRMS (ESI+) m/z calcd for $\text{C}_{25}\text{H}_{36}\text{O}_3\text{Na}$ $[\text{M}+\text{Na}]^+$: 407.2557, found: 407.2563.

¹Ophiobolin I was kindly provided by Prof. Antonio Evidente (University of Naples, Italy).

Supporting Information



Twist-induced protein expression. Western blotting for the indicated proteins was performed on whole cell lysates from vector or Twist-transduced HMLE (A) or HMLER (B) cells. Chemiluminescence was detected and imaged. Molecular weight of ladder proteins, indicated on the right, was used to trim the membranes prior to horizontal membrane cutting for (B).