Supplementary Methods:

The DAP compounds used in this study, H-4073 ((3E,5E)-3,5-bis(4-fluorobenzylidene)piperidin-4-one), and HO-3867 (1- [(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)methyl]-(3E,5E)-3,5-bis(4-fluorobenzylidene)piperidin-4-one), were synthesized as described previously(1, 2). Antibodies against pSTAT3 Tyr705, pSTAT3 Ser727, pSTAT1, 2, 5 and 6, pAkt Ser473, Akt, Bcl-2, β-actin, caspase-3, and PARP were purchased from Cell Signaling Technology (Beverly, MA). Antibodies specific for STAT3, cyclin D1, cyclin D2, p53, STAT1, STAT3, STAT5 and 8-OHdG (15A3) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Enhanced chemiluminescence (ECL) reagents were obtained from Amersham Pharmacia Biotech (GE Healthcare, Piscataway, NJ). STATTIC, STAT3 inhibitor, Cucurbitacin Q, and all other reagents, of analytical grade or higher, were purchased from Sigma-Aldrich. Rodent chow containing HO-3867 was specially-prepared by Harlan-Teklad.

Cell lines and culture

Human ovarian cancer cell lines, A2780 and SKOV3, normal ovarian cell lines, hOSE (ATCC medium), Chinese hamster ovary (CHO), and the normal cardiac cell line H9C2 were used in this study. TR-127 and TR-182 primary ovarian cancer cell populations were generously provided by Dr. Gil Mor, of Yale University.

***In Silico* Docking Simulations for DAPs:** Previously published work by our group(1) has demonstrated that these compounds act upon the STAT3 pathway. This phenomenon was investigated in more detail through *in silico* molecular docking simulations using the freely-available program AutoDock (version 4.2) (3, 4). The target macromolecule used in these studies was a nontransformed murine STAT3 dimer downloaded from the RCSB Protein Data Bank (PDB code 3CWG). The monomeric structure was obtained by deletion of one of the chains from

the 3CWG structure. Energy-minimized 3D molecular topographies of the DAP compounds H-4073 and HO-3867 were obtained using the Dundee PRODRG2 server(5). Dockings were automatically ranked by AutoDock according to the lowest calculated binding energies (kcal/mol).

Evaluate the bio-absorption of DAPs in ovarian cancer cells using EPR: Our previous study showed that cellular uptake of HO-3867 was significantly greater than curcumin(6). We evaluated the bioabsorption of HO-3867 compounds in ovarian cancer cells and normal hOSE cells using EPR, as previously described (7).

Drug accumulation and metabolism using liquid chromatography mass spectrophotometry (LCMS): Cellular uptake of the curcumin analogues HO-3867 and H-4073 was analyzed by a liquid chromatography and tandem mass spectrometry (LC-MS/MS) assay. The assay utilizes a Thermo Finnigan TSQ Quantum Ultra triple quadruple mass spectrometer coupled with Dionex LC module. DAP compounds and internal standards were separated on a reverse phase column with isocratic flow of 50% acetonitrile containing 0.1% formic acid and sequentially monitored using selected reaction monitoring mode (SRM) by ion transitions as follows: HO-3867 *m/z* s 465.25>432.19; H-4073 *m/z* s 312.00>109.18; IS *m/z* s 445.94>428.80. The LC-MS/MS method was utilized to analyze both DAP compounds in a normal cell line (CHO), a cancer cell line (CS), and cell medium (control). For sample cleaning, cell medium samples were subjected to liquid-liquid extraction by ethyl acetate and cell samples were treated with 70% methanol to break down the cell membrane; dried extracts were reconstituted with 50% acetonitrile and 0.1% formic acid and injected for LC-MS analysis. Calibration curves of HO-3867 and H-4073 were tested to be linear over the concentration range of 1 to 1000 ng/mL in both normal and cancer cells, and cell medium. The linear regression coefficients (R^2) of calibration curves all exceeded

0.99. Quality control standards prepared in triplicates at concentrations of 5, 50, and 500 ng/mL were inserted throughout the run to ensure data quality.

Intracellular metabolite profiling: Cell samples (7.5 million cells) treated at 1 hour and 6 hours with the DAP compounds were extracted twice by MeOH, followed by ice water. The supernatants were combined, evaporated and resurrected in 50% acetonitrile and 0.1% formic acid to prepare the extract for intracellular metabolite profiling. A gradient LC elution program and full scan in the range of 100-700 Da under positive ionization were used in search for any intracellular metabolite formed in these cell treatment samples.

Ovarian tumor xenografts in mice: Cultured A2780 cancer cells (3 × 10^6 cells in 100μL of PBS) were subcutaneously injected into the flank of 6-week-old BALB/c nude mice from the National Cancer Institute. The groups were treated using the HO-3867 compound mixed with the animal feed (Harlan Teklad) at two different levels (50 and 100 ppm). The tumor volume was measured at the 4^{th} week, 28 days after the beginning of HO-3867 treatment, the mice were sacrificed and the tumors were resected. The tumor tissues were then subjected to immunoblot analysis, TUNEL assays, and histopathology experiments.

Histo-chemistry: Ovarian tumor and organ tissues were embedded in OCT medium (Tissue tek 4583) and stored at –80° C until sectioning. Consecutive 5 μm tissue sections were obtained and stained with hematoxylin and eosin (H&E), immunohistochemical (IHC) and TUNEL staining, following previously-described methods (8, 9) .

Primary ovarian carcinoma cell population isolation: Primary ovarian carcinoma cell populations used in this study were isolated from patient ascites at the time of primary surgery or collected at the time of tumor recurrence from patients with advanced stage ovarian carcinoma. All patients signed consent forms, and the use of patient samples was approved under the Ohio

State University Human Investigations Committee (IRB # 2004C0124). Cells were grown using MCDB/M199 media as previously described(10). Cells were treated with HO-3867 and viability, migration, invasion, and Annexin V assays were completed as previously described.

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