Supporting Information

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SI Materials and Methods

Chemicals. Exemestane and letrozole were purchased from LKT Laboratories. *R,S*-sulforaphane was from Santa Cruz Biotechnology. Tamoxifen was obtained from Cayman Chemical. 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT), *tert*-butyl hydroperoxide, sodium hydrosulfite ($Na_2S_2O_4$), 2',7'-dichlorodihydrofluorescein diacetate (DCFDA), and other chemicals were from Sigma-Aldrich. All cell culture media and FBS were obtained from Invitrogen (Life Technologies).

Cell Cultures. All cell lines were maintained in 5% CO_2 at 37 °C in the following media: α-MEM supplemented with 10% heat- and charcoal-treated FBS (murine hepatoma Hepa1c1c7 cells), DMED (high glucose) supplemented with 10% heat-inactivated FBS (murine macrophage-like RAW264.7 cells and rat H9c2 myocardiocytes), a mixture of equal volumes of DMEM and Hanks' F-12 medium supplemented with 10% heat- and charcoal-treated FBS (human adult retinal pigment epithelial ARPE-19 cells), or MEM (2 mM L-glutamine) supplemented with 10% heat-treated FBS and 1% nonessential amino acids (murine 308 keratinocytes). Mouse embryonic fibroblasts (MEFs) derived from day 13.5 embryos of wild-type or Nrf2-knockout *(nrf2$^{-/-}$)* C57BL/6 mice (1) were grown in Iscove's modified Dulbecco's medium plus 10% heat-inactivated FBS. The stably transfected human monocytic U937 cells with an NF-κB reporter construct, which contains the luciferase reporter gene regulated by a promoter containing three NF-κB sites (U937-3xκB-LUC cells) (2), were maintained in RPMI 1640 (2 mM L-glutamine) supplemented with 10% heat-inactivated FBS, 50 U/mL penicillin, 50 μg/mL streptomycin and 75 μg/mL hygromycin B to ensure selection of cells that retained the construct.

NAD(P)H:quinone oxidoreductase 1 (NQO1) Assay. Cells (10,000 per well) were grown in 96-well plates for 24 h, and then exposed to serial dilutions of inducers for 48 h and lysed in 0.08% digitonin. Enzymatic activity of NQO1 in cell lysates was determined with menadione as substrate (3, 4). Concentrations required to double the specific activity of NQO1 (CD values) were used to quantify inducer potency.

mRNA Isolation and Real-Time PCR Analysis. H9c2 cells (100,000 per well) or RAW264.7 cells (500,000 per well) were grown in six-well plates for 24 h, and then exposed to vehicle or 10 μM exemestane for 6 h (H9c2), or incubated with exemestane, sulforaphane, combination of both compounds, or vehicle control for 30 min, and exposed to LPS (10 ng/mL) for an additional 6 h (RAW264.7). Total mRNA from cells was extracted with TRIzol reagent (Invitrogen). cDNA was synthesized by the iScript cDNA Synthesis Kit (Bio-Rad Laboratories). Quantitative real-time PCR analysis was performed using the 7000 Sequence Detection System (Applied Biosystems). All primers were optimized, and a final primer concentration of 300 nM was used for all reactions. Primer sequences for gene amplification were as follows: NAD(P)H:quinone oxidoreductase 1 (NQO1), fwd 5′-tccagaaacgacatcacagg-3′, rev 5′-ttcagctacaatatccgggc-3′; heme oxygenase-1 (HO-1), fwd 5′-cagggtgacagaagaggctaagac-3′, rev 5′-tgaggacccatcgcaggag; cyclooxygenase 2 (COX-2), fwd 5′-gaagtctttggtctggtgcctg-3′, rev 5′-gtctgctggtttggaatagttgc-3′; and β-actin (endogenous control), fwd 5′-ccccattgaacacggcatt-3′, rev 5′-catcttttcacggttggcctta-3′. The reactions were assembled using 5 ng of cDNA, 1×Power SYBR Green PCR Master Mix (Applied Biosystems), forward and reverse primers, and nuclease-free water. Relative mRNA expression was normalized to β-actin. Gene expression was calculated using the comparative $2^{-\Delta\Delta CT}$ method (5).

NQO1 Induction in Mouse Skin. Eleven-month-old female SKH-1 hairless mice *(n = 5)* purchased from Charles River Laboratories were treated three times at 24-h intervals on their backs with 0, 1, and 2 μmol of exemestane dissolved in 80% aqueous acetone (40 μL) over about a 2.0 cm^2 area. Mice were euthanized 24 h after the final dose. Each treated segment of the dorsal skin was harvested, pulverized in liquid N_2, and homogenized in 10 volumes of 0.25 M sucrose in 10 mM Tris buffer (pH 7.4). After three freeze-thaw cycles, the homogenates were centrifuged at 12,000 × *g* and 4 °C for 30 min, and the supernatant fractions were analyzed for protein concentration (6) and NQO1 activity (3, 4). All animal experiments were in compliance with National Institutes of Health Guidelines and were approved by The Johns Hopkins University Animal Care and Use Committee.

Detection of Intracellular Reactive Oxygen Species. ARPE-19 or MEF cells (10,000 per well) in 96-well plates (black-walled wells with clear bottom) were treated with serial dilutions of exemestane for 24 h and then incubated with 20 μM DCFDA for 30 min (7). After washing with Dulbecco's PBS (DPBS) twice, the cells were challenged with 500 μM *tert*-butyl hydroperoxide for 30 min, and fluorescence intensity was measured in a SpectraMax GeminiEM plate reader (Molecular Devices) with excitation at 485 nm and emission at 530 nm. Murine 308 keratinocytes (40,000 per well) were plated in 24-well plates and grown for 24 h, and then exposed to different concentrations of exemestane (0, 2, 4, 8 μM). After further incubation for 24 h, cells were incubated with 20 μM DCFDA for 30 min, washed twice with DPBS, and exposed to 5 or 10 J/cm^2 UVA (340-400 nm). Immediately after UVA irradiation, fluorescence intensity was measured with a microplate reader.

Measurement of Cytotoxicity of Oxidative Stress. ARPE-19 cells (10,000 cells per well) in 96-well plates were treated with serial dilutions of exemestane for 24 h and exposed to *tert*-butyl hydroperoxide or 4-hydroxynonenal in serum-free medium for 4 h. Cells were then incubated in serum-free medium for an additional 20 h and washed with DPBS, and cell viability was determined using the colorimetric procedure based on the reduction of a water-soluble tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) (8). H9c2 cells (10,000 cells per well) in 96-well plates were treated with a range of concentrations of exemestane. After 24 h, the cells were washed with DPBS and exposed to 2 mM $Na_2S_2O_4$ in serum-free medium for 1 h. The cells were then washed with DPBS and incubated in complete medium containing 10% FBS for 24 h, and cell viability was measured by the MTT assay (8).

Preparation of Peritoneal Macrophages from *nrf2*$^{-/-}$ Mice. *Nrf2*$^{-/-}$ mice on C57BL/6 background were initially established by Itoh et al. (9). Wild-type C57BL/6 mice were purchased from The Jackson Laboratory. Macrophages were harvested by lavage from the peritoneal cavity with DPBS 4 d after an i.p. injection of thioglycolate broth (Brewer, 4%). Isolated cells were plated into 96-well plates at a density of 10^5 cells per well in RPMI1640 medium containing 2 mM L-glutamine, 10% heat-inactivated FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. After 3-h incubation at 37 °C in 5% CO_2, nonmacrophage cells were removed by washing with DPBS. The remaining macrophages were exposed immediately to serial dilutions of exemestane, sulforaphane, or a combination of both compounds in the presence

of 100 ng/mL LPS and incubated for 24 h before measurement of inducible nitric oxide synthase (iNOS).

Measurement of iNOS Induction. RAW264.7 cells (20,000 per well) in 96-well plates were grown for 24 h and exposed to serial dilutions of exemestane in the presence of 10 ng/mL LPS. After 48-h incubation, nitrite concentration in culture supernatant fractions of RAW264.7 cells or mouse peritoneal macrophages, measured as an indicator of iNOS induction, was determined by the Griess reaction (10, 11). Nitrite values of cells treated with LPS but without test compounds were used as controls.

Measurement of Luciferase Activity. Human monocyte U937-3xκB-LUC cells (2) were transferred to medium with 2% heat-inactivated FBS in 24-well plates (1×10^6 cells per well) and incubated overnight. Then cells were incubated with exemestane, sulforaphane, combination of both compounds, or vehicle control for 30 min and exposed to LPS (10 ng/mL). After an additional 6-h incubation, cells were harvested, and NF-κB-driven luciferase activity was assayed according to the manufacturer's instructions (Promega). Luciferase activity was normalized to total protein concentration (6) to compensate for possible cell toxicity of the compounds.

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