Supporting Information

Wu et al. 10.1073/pnas.1002890107

S1 Materials and Methods

Reagents. All reagents were purchased from Sigma-Aldrich unless otherwise stated. All compounds were prepared as 10 mM stocks in DMSO and stored at −20 °C. The compounds were thawed to room temperature before use. The structures and previously reported biological activities are described in Table S1.

Primers for qPCR. GAPDH-5′-TGCTGTAAGCACAAATTGG-3′, GAPDH-5′-CTGACCTCAACAGGACCAC-3′, HO-1-5′-CTGCCCTTCTCCTGTGC-3′, HO-1-5′-CTGCCGTCCTTGTTCG-3′, HO-1-5′-CTGCCGTCCTTGTTCG-3′, Nrf-2-for-5′-TGTCAATCAAATCTCATG-3′, Nrf-2-for-5′-ACACGGTCCACAGTCT-3′.

Antibodies for Immunoblotting. HO-1 (OSA-110) antibody was purchased from Assay Designs. Nrf2 (MAB3925) and Keap1 (MAB3024) antibodies were purchased from R&D Systems. β-Actin antibody (A5491) was purchased from Sigma-Aldrich.

Measurement of Cytotoxicity by Using MTT Uptake Assay. Cells (2.5 × 10^4) were plated in each well of a round-bottom 96-well plate and incubated with various concentrations of drugs. After 48 h incubation in the 5% CO_2 humidified incubator at 37 °C, 100 μL of medium was removed. MTT (Sigma-Aldrich) was added to each well at the final concentration of 0.83 mg/mL and incubated overnight. Cells were lysed by MTT lysis buffer (15% SDS, 0.01 M HCl) and uptake of MTT was measured at 570 nm absorbance using a multi-well reading UV-Vis spectrometer.

Measurement of ARE Activities by GeneBlazer Reporter Assay. ARE-blkB HepG2 cells (Invitrogen) were cultured in Dulbecco modified eagle medium (DMEM) supplemented with Glutamax, 10% dialyzed FBS, 0.1 mM nonessential amino acid, 25 mM Hepes, 100 U/mL penicillin, 100 μg/mL streptomycin, and 5 μg/mL basicidin. ARE-blkB HepG2 cells (3.12 × 10^4) were seeded in the clear-bottom, black 96-well plate and cultured in 5% CO_2 humidified 37 °C incubator overnight. After the addition of various concentrations of compounds, the cells were incubated in the incubator for 16 h. Fluorescent measurements were made using GENios (Tecan). The β-lactamase activities were measured by the ratio of blue product (465 nm) and the green substrate (535 nm) fluorescence.

Streptavidin Pull-Down Assay. Biotin was conjugated to EA using EZ-Link Biotin-LC-Hydrazide (Thermo Scientific) in our laboratory and isolated by HPLC. The cells were treated with biotinylated EA at 15 and 50 μM for 4 h. At the end of the treatments, the cells were washed with PBS solution briefly. The cells were then lysed in Phosphosafe Extraction Buffer with protease inhibitor by sonicating for 1 min, passing repeatedly through a 28-g needle insulin, and incubating on ice for 10 min. The insoluble fraction was removed by centrifugation. Then 100 μL of streptavidin beads (Thermo Scientific) were added to a final volume of 500 μL in RIPA buffer (50 mM NaCl, 50 mM Tris HCl, 0.5% Nonidet P-40) containing 800 μg of total protein lysates. The mixture was incubated overnight at 4 °C. The flow-throughs were saved and the beads were washed three times. The beads were boiled for 4× NuPAGE Sample Buffer (Invitrogen) supplemented with 1 mM DTT.

FACS Analysis. At least 1 × 10^7 freshly isolated PBMCs or B cells were resuspended in 300 μL of Dulbecco PBS solution containing 0.2% BSA (BD Pharamingen). The cells were blocked overnight with FcR blocking reagent (Miltenyi Biotec), and then stained with CD19-FITC antibody (BD Pharmingen) for 1 h. After washing, the cells were enumerated in a FACSCalibur instrument (BD Biosciences). At least 10,000 events were collected for analysis. The FACS spectra were analyzed with FlowJo software (FlowJo).

Table S1. Structures and pharmacological properties of electrophilic and antioxidant compounds

<table>
<thead>
<tr>
<th>Compound class/name</th>
<th>Structure</th>
<th>Source</th>
<th>Pharmacological properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-β unsaturated carbonyl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parthenolide</td>
<td></td>
<td>Feverfew (1)</td>
<td>Anti-migraine (1) Anti-cancer (2) Anti-inflammatory (3) Inhibits IκB kinase (3, 4)</td>
</tr>
<tr>
<td>Hypoestoxide</td>
<td>Shrub Hypoestes rosea (5)</td>
<td></td>
<td>Anti-inflammatory (5) Inhibits IκB kinase (5)</td>
</tr>
<tr>
<td>EA</td>
<td>Synthetic</td>
<td></td>
<td>Diuretic (6) Anti-cancer (7)</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Indian spice tumeric (8)</td>
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<td>Anti-inflammatory (8) Anti-cancer (9)</td>
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<tr>
<td>Dimethyl fumarate</td>
<td>Derivative of fumaric acid (13)</td>
<td></td>
<td>Anti-psoriasis (13) Inhibits NFκB activation (13)</td>
</tr>
<tr>
<td>α-β saturated carbonyl</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Hypoestoxide reduced</td>
<td></td>
<td></td>
<td>N/A</td>
</tr>
<tr>
<td>EA-reduced</td>
<td></td>
<td></td>
<td>N/A</td>
</tr>
<tr>
<td>linomide</td>
<td>Synthetic</td>
<td></td>
<td>Anti-cancer (14)</td>
</tr>
<tr>
<td>Capsaicin</td>
<td>Chili peppers (15)</td>
<td></td>
<td>Activation of sensory neurons (15)</td>
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<tr>
<td>Compound class/ name</td>
<td>Structure</td>
<td>Source</td>
<td>Pharmacological properties</td>
</tr>
<tr>
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<tr>
<td>Isothiocyanates</td>
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<tr>
<td>PEITC</td>
<td><img src="image" alt="PEITC Structure" /></td>
<td>Cruciferous vegetables (16, 17)</td>
<td>Anti-cancer (17)</td>
</tr>
<tr>
<td>Sulfuraphane</td>
<td><img src="image" alt="Sulfuraphane Structure" /></td>
<td>Cruciferous vegetables (16, 17)</td>
<td>Anti-cancer (17)</td>
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<td>Sulphydral reactive metals</td>
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<td>PAO</td>
<td><img src="image" alt="PAO Structure" /></td>
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<td>Binds to vicinal thiol residues of proteins (18)</td>
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<tr>
<td>FlAsh</td>
<td><img src="image" alt="FlAsh Structure" /></td>
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<td>Fluorescent Designed to bind vicinal thiol residues (19, 20)</td>
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<tr>
<td>ReAsh</td>
<td><img src="image" alt="ReAsh Structure" /></td>
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<td>Mersalyl acid</td>
<td><img src="image" alt="Mersalyl Acid Structure" /></td>
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<td>Mercury containing diuretics (21)</td>
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<td>Flavonoids</td>
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<td>Tangeretin</td>
<td><img src="image" alt="Tangeretin Structure" /></td>
<td>Peel of citrus fruits (22)</td>
<td>Anti-cancer (22)</td>
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<tr>
<td>Apigenin</td>
<td><img src="image" alt="Apigenin Structure" /></td>
<td>Parsley and celery (23, 24)</td>
<td>Anti-cancer (25)</td>
</tr>
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<td>Luteolin</td>
<td><img src="image" alt="Luteolin Structure" /></td>
<td>Celery and many vegetables (24, 26)</td>
<td>Anti-oxidant (26) Anti-inflammatory (26) Anti-microbial (26) Anti-cancer (25, 26)</td>
</tr>
</tbody>
</table>
Table S1. Cont.

<table>
<thead>
<tr>
<th>Compound class/name</th>
<th>Structure</th>
<th>Source</th>
<th>Pharmacological properties</th>
</tr>
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<tbody>
<tr>
<td>Quercetin</td>
<td><img src="image1" alt="Quercetin Structure" /></td>
<td>Citrus fruit, grapes, and red onions (27)</td>
<td>Anti-oxidant (27) Lowering blood pressure (27) Ameliorating hyperglycemia-related diseases (27)</td>
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<td>β-naphthoflavone</td>
<td><img src="image2" alt="β-naphthoflavone Structure" /></td>
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<td>Agonist of aryl hydrocarbon receptor (28)</td>
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<td>Polyphenols</td>
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<tr>
<td>TBHQ</td>
<td><img src="image3" alt="TBHQ Structure" /></td>
<td>Synthetic</td>
<td>Aryl hydrocarbon receptor ligand (29)</td>
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<td>Gossypol</td>
<td><img src="image4" alt="Gossypol Structure" /></td>
<td>Cotton plant (30)</td>
<td>Anti-leukemia (30) Possible inhibitor of Bcl-2 and Bcl-XL (30)</td>
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<td>Resveratrol</td>
<td><img src="image5" alt="Resveratrol Structure" /></td>
<td>Grapes, red wine (31, 32)</td>
<td>Cancer preventive (31–33)</td>
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<td>Piceatannol</td>
<td><img src="image6" alt="Piceatannol Structure" /></td>
<td>Metabolite of resveratrol found in red wine (34)</td>
<td>Anticancer (35)</td>
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<td>Hydroquinone</td>
<td><img src="image7" alt="Hydroquinone Structure" /></td>
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<td>Carcinogen (36)</td>
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<td>Sudan-1</td>
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<td>Carcinogen (37)</td>
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