Supporting Information

Assay for fluorescence polarization (FP) assay to identify small-molecule inhibitors of the Keap1-Nrf2 interaction.

All fluorescence polarization (FP) assays were performed on a SpectraMax Multi-Mode Microplate Reader (Molecular Devices) using the excitation and emission filters appropriate for each fluorophore used in the binding experiment. The plates used for the FP measurements were the black nonbinding surface Corning 3676 384-well plates, loaded with 40μL of assay solution per well, consisted of 10 μL of 4 nM FITC-9mer Nrf2 peptide amide and 10 μL of 12 nM Keap1 Kelch domain protein, 10 μL of HEPES buffer, and 10 μL of an inhibitor sample of varying concentrations. The binding experiments were performed in triplicates, with initial concentration of the inhibitor typically set at 100μM and serially diluted twofold to give the next concentration. The plate was covered and rocked for 30 min at room temperature prior to FP measurements. For fluorescein, 485 nm excitation and 535 nm emission filters were used. FP was determined by measuring the parallel and perpendicular fluorescence intensity ($F_{\parallel}$ and $F_{\perp}$) with respect to the linearly polarized excitation light. We elected to use polarization in our quantitative analysis. The percentage inhibition of the competitor at each concentration point was determined by using equation %inhibition = 1- (Pobs-Pmin) / (Pmax-Pmin). The values of Pmax, Pmin, and Pobs in the equations refer to the polarization of the wells containing Keap1 and the probe, the polarization of the free probe, and the observed polarization for the wells containing the inhibitors at a range of concentrations under the assay conditions. The EC50 of an inhibitor was determined from the plot of %inhibition against inhibitor concentration analyzed by Origin8.5 sofware.

Assay for ARE luciferase reporter activity

HepG2 cells stably transfected with ARE luciferase reporter (HepG2-ARE-C8) were kindly provided by Professor Dr. A. N. Tony Kong (Rutgers University, Piscataway, NJ). HepG2-ARE-C8 cells were plated in 96-well plates at a density of 4 × 104 cells/well and incubated overnight. The cells were exposed with different

concentrations of test compounds, with tBHQ serving as a positive control, DMSO as a negative control and the luciferase cell culture lysis reagent as a blank. After 12 h of treatment, the medium was removed and 100 μL of cold PBS was added into each well. Then the cells were harvested in the luciferase cell culture lysis reagent. After centrifugation, 20 μL of the supernatant was used for determining the luciferase activity according to the protocol provided by the manufacturer (Promega, Madison, WI). The luciferase activity was measured by a luminoskan ascent (Thermo scientific, USA). The data were obtained in triplicates and expressed as fold induction over control. Inductivity = (RLU test – RLU blank)/ (RLU control – RLU blank). RLU = relative light unit.

Validation the docking method

In order to further validation the docking method, we used the reported hit with known co-crystal structure (PDB ID: 4IQK)[1] to carry out the Ligandfit docking experiment. As shown in the following Figure A, the binding modes from the co-crystal structure and the docking are nearly the same. The RMSD of the heavy atom is only about 1.834 Å. It indicated that the Ligandfit docking method is fit for the Keap1-small molecular ligand system. It has been included in the Supporting Information.

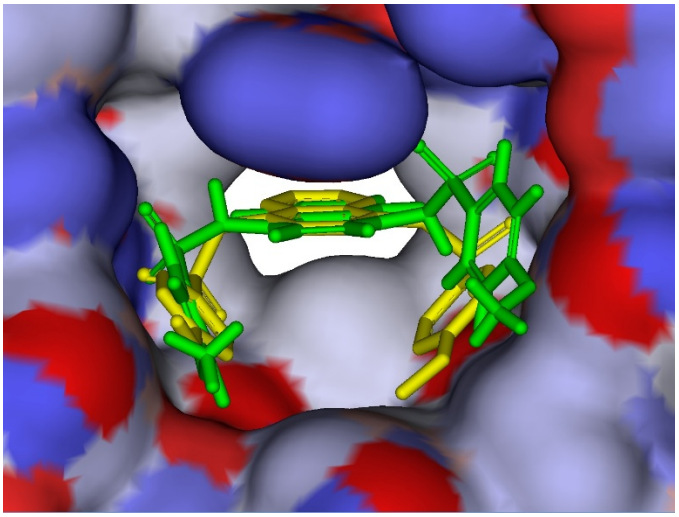
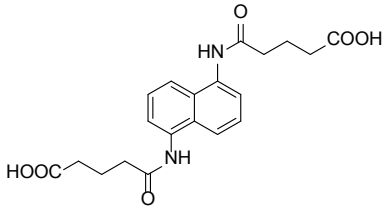
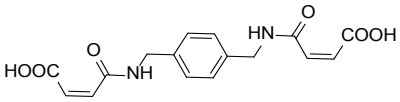
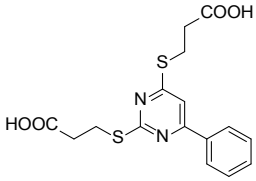
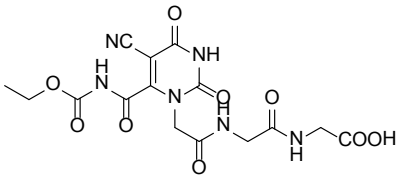
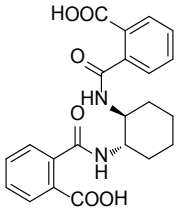
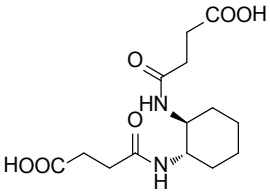
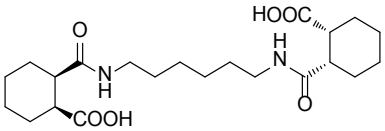
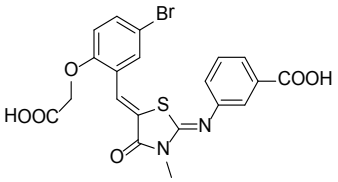
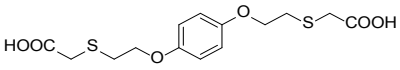
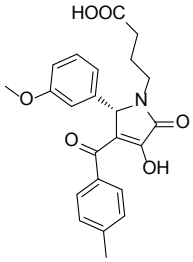
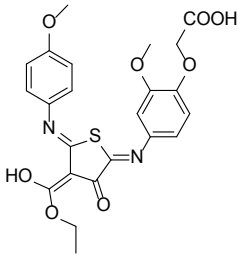
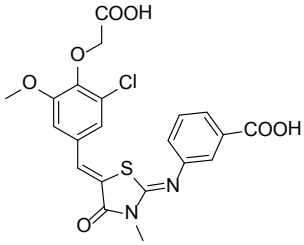
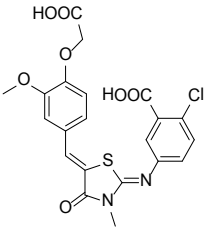
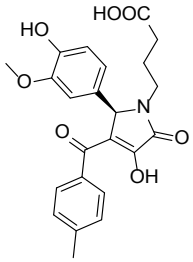
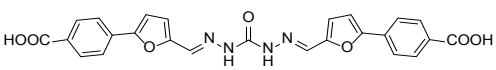


Figure A. Validation of the Ligandfit docking tool. The ligand conformation from the crystal structure was colored as yellow and the ligand conformation was colored as green. The RMSD of the heavy atom is only about 1.834 Å. The Keap1 surface

was colored as partial charge.

Compound List

Compound	Structure	Binding energy (kcal/mol)	%inhibitory 100 μM
01 AG-205/37175008		-23.3939	1.55
02 AG-205/37175018		-15.2931	-2.67
03 AG-690/15434541		-47.1998	2.22
04 AJ-030/12105933		-17.5515	7.18
05 AN-329/40826196		-33.7842	1.65
06 AN-329/40826197		-40.7625	4.66
07 AN-329/40942995		-30.4702	4.28

08 AN-698/40704715		-10.2370	-1.08
09 AO-638/40907188		-22.8176	1.19
10 AQ-149/13889398		-57.6746	25.19
11 AN-989/14669072		-52.4767	-2.95
12 AN-698/40705118		-47.2037	16.64
13 AN-989/40683903		-47.2037	10.84
14 AQ-149/13889205		-47.2037	1.33
15 AN-465/144580		-46.277	EC50=9.8μM

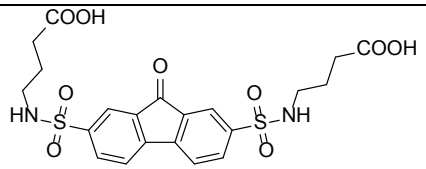
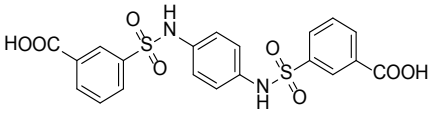
16 AG-690/11665658		-46.1416	8.36
17 AJ-292/40927066		-38.3674	21.6

Table. Chemical structures and Nrf2 induction activities of selected compounds from virtual screening. The table summarizes, for each compound, SPECS code, chemical structure, binding energy and %inhibitory at 100 μM

Figure S1. Workflow for in silico screening