SI Methods

Molecular docking

To explore the possible Hsp90-ligand binding mode, the first step was to identify the residues in the interface between Hsp90 and Cdc37 in the Hsp90-Cdc37 complex and to dock celastrol to the Hsp90 N-terminal domain. We aimed to find where celastrol could be inserted most comfortably. The molecular docking for each possible Hsp90-celastrol binding mode was carried out in the same way as we recently performed for studying other protein-ligand binding systems (1). Briefly, a celastrol-binding site was defined as the residues at the interface of Hsp90-Cdc37 complex and centered on the lid segment of Hsp90. Celastrol was initially positioned at ~10 Å in front of an attempted binding site. The initial docking calculations were performed on celastrol with the N-terminal Hsp90 binding site using the '*automatic docking*' Affinity module of the InsightII package (Accelrys, Inc., San Diego, CA). The Affinity methodology uses a combination of Monte Carlo type and Simulated Annealing (SA) procedure to dock the guest molecule (celastrol) to the host (Hsp90) (2). The Hsp90-ligand binding structure obtained from the initial docking was further refined by performing a molecular dynamics (MD) simulation in water.

Molecular dynamic simulation in water

Each binding complex simulated in this study was neutralized by adding an appropriate number of sodium counterions and was solvated in a rectangular box of TIP3P water molecules with a minimum solute-wall distance of 10 Å (3). The partial atomic charges for celastrol were obtained after geometry optimizations at the HF/6-31G* level and subsequent single-point calculations of the electrostatic potential, to

which the charges were fitted using the RESP procedure (4, 5). Force field parameters of celastrol were assigned based on the atom types of the force field model developed by Cornell *et al*. (5). Gaussian03 program was used to optimize the geometries and generate electrostatic potentials. The MD simulation was performed by using the Sander module of the Amber8 program (University of California, San Francisco) in a way similar to what we performed for other protein-ligand systems (1). Each of the solvated systems was carefully equilibrated before a sufficiently long MD simulation in room temperature. The MD simulations were performed with a periodic boundary condition in the NPT ensemble at T = 298.15 K with Berendsen temperature coupling and constant pressure (P = 1 atm) with the isotropic molecule-based scaling. The SHAKE algorithm was applied to fix all covalent bonds containing a hydrogen atom, a time step of 2 fs was used, and the non-bond pair list was updated every 10 steps (6). The particle mesh Ewald (PME) method was used to treat long-range electrostatic interactions. A residue-based cutoff of 10 Å was applied to the non-covalent interactions (7).

The obtained stable MD trajectory was used to estimate the binding free energy (ΔG_{bind}) by using the molecular mechanics/Poisson-Boltzmann surface area (MM-PBSA) free energy calculation method (8). 100 snapshots were used to perform the MM-PBSA calculation. Our MM-PBSA calculation for each snapshot was carried out in the same way as we did for other protein-ligand systems (1). The finally calculated binding free energy was taken as the average of the ΔG_{bind} values with the 100 snapshots.

Most of the MD simulations in water were performed on a HP supercomputer, Superdome, at the Center for Computational Sciences, University of Kentucky. The other computations were carried out on SGI Fuel workstations and a 34-processors IBM x335

Linux cluster in our own lab.

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SI Figure legends

Figure 7. Molecular docking of celastrol with Hsp90. **A**, Stick model of the Hsp90-celastrol binding pocket. Only amino acid residues close to celastrol are displayed for clarity. **B**, Plots of MD-simulated internuclear distances *versus* the simulation time for Hsp90 binding with celastrol. D1 refers to the H···O distance in the hydrogen bond between the hydroxyl group of celastrol and the carboxyl oxygen atom of the Glu-33 residue. D2 refers to the distance between the carbonyl oxygen of the ligand and hydrogen backbone of Gly-118 residue. D3 represents the H···N$^{\delta}$ distance in the hydrogen bond between the hydroxyl of the carboxylic moiety of celastrol and the His197 residue. D4, represent the internuclear distance between the hydrogen of the guanidinium sidechain of Arg-32 and the carbonyl oxygen of the carboxylic group of celastrol.