Modeling and System preparation

System preparation, modeling and initial docking calculations were performed using the Schrödinger molecular modeling suite (version 2015-4), unless otherwise noted. The protein structure was obtained from the Protein Data Bank (PDB) and prepared using the Protein Preparation Wizard. In this step, force field atom types and bond orders are assigned, missing atoms are added, tautomer/ionization states are assigned, water orientations are sampled, Asn, Gln, and His residues are flipped to optimize the hydrogen bond network, and a constrained energy minimization is performed. All resolved crystal water molecules were retained during the preparation.

Potential binding sites were explored and characterized using the SiteMap tool. Ligands which have shown activity in experiments together with known inactives where docked in the putative binding sites using Glide SP in order to test enrichment of known actives. Reasonable scores for the ‘Ro’ series was shown for the ‘Gossypol’ binding site described by Lan *et al*.

Since receptor structure may not be in the optimal conformation to bind small molecule inhibitors, induced fit docking of ligand Ro 08-2750 was performed to this binding pocket. Induced fit docking results were validated with the Metadynamics protocol described by Clark *et al*. The pose ranked second using the Induced Fit Docking score came out best. This receptor configuration was furthermore validated towards a virtual screening using a Glide SP docking of known actives and inactives. Furthermore, a WaterMap calculation was done for this receptor.

The virtual screening was then preformed with this receptor conformation using Glide SP by docking the March 2016 collection of the eMolecules dataset. All ligand structures were prepared with LigPrep including a minimization with the OPLS3 force field. One low energy ring conformation per compound was generated. Ionization states and tautomer forms were enumerated at pH 7.0 ± 2.0 with Epik.

The top 5000 hits from virtual screening were filtered by applying filters according to Lipinski’s rule of five, flagging REOS and PAINS.

The hitlist was ranked in addition to the Glide SP DockingScore also by a Pareto ranking of DockingScore and number of WaterMap hydration sites with Δ*G* > 2 kcal/mol which overlap with the ligand pose. The top 200 ranked hits from both lists were combined. Finally, a leader-follower clustering using dendridic fingerprints was performed using Canvas resulting in 243 unique cluster hits.

Induced Fit Docking of Ro-A6 and Ro-OH compounds

Induced Fit Docking was performed against the receptor pose from the selected Ro 08-2750 pose, using Schödinger molecular modeling suite (version 2017-4). Poses for Ro-A6 and Ro-OH were selected that most closely matched the Ro 08-2750 pose, the top and second scored poses respectively.

Alchemical Free Energy Calculations

*System Preparation* *and modeling*. The systems prepared according to the above protocol were used as input for these calculations.

*Parameterization.* An OpenMM ForceField was instantiated using AMBER14SB force field parameters for the protein and TIP3P water model. The ligand was assigned charges using the Am1-Bcc implementation in OpenEye and parametrized in the Gaff forcefield.

*Minimization.* Minimization was perform using the implementation of the L-BFGS algorithm in OpenMM 7.1.1 with a tolerance of 1kJ/(M\*nm).

*Production Simulation.* The ligand was restrained using a Harmonic restraint centered on the following residues in the receptor: 2, 4, 46, 76, 78, and 80. The calculation was performed using an explicit PME solvent, with a nonbonded forces cutoff using a 9Å cutoff and neutralizing NaCl. The calculation was carried out at 300K, 1 atm pressure and using a 2.0 fs timestep. Ro 08-2750 and Ro A6 were run for 10000 iterations with 2500 timesteps per iteration, while Ro-OH was run for 15000 iterations with 2500 timesteps per iteration. The alchemical pathway was automatically determined for each compound using the YANK autoprotocol feature.

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