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Performance of a docking/molecular dynamics protocol for virtual screening of nutlin-class inhibitors of Mdmx

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Abstract

A virtual screening protocol involving docking and molecular dynamics has been tested against the results of fluorescence polarization assays testing the potency of a series of compounds of the nutlin class for inhibition of the interaction between p53 and Mdmx, an interaction identified as a driver of certain cancers. The protocol uses a standard docking method (AutoDock) with a cutoff based on the AutoDock score (ADscore), followed by molecular dynamics simulation with a cutoff based on root-mean-square-distance (RMSD) from the docked pose. ROC analysis of the experimental and computational results shows modest performance of ADscore alone, but dramatically improved performance when RMSD is also used.

Graphical abstract

Keywords

docking; Molecular dynamics; fluorescence polarization; virtual screening; Mdmx; Mdm4; nutlin

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Supporting Information. Table of compounds, IC₅₀ values, Autodock scores and RMSD values. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

INTRODUCTION

The amplification of the Mdmx gene (also called Mdm4) and overexpression of the Mdmx protein is a major driver of the formation and maintenance of retinoblastoma¹. We have previously shown that the Roche tool compound, nutlin-3a, which targets Mdm2, also binds, albeit weakly, to Mdmx². Unfortunately, the current clinical candidate, RG7112³, does not interact with Mdmx. We therefore sought to develop analogs to nutlin-3a that were selective for Mdmx and more potent than nutlin-3a. Mdmx, like Mdm2, down-regulates the tumor suppressor p53 through a mechanism in which Mdmx binds to the N-terminal transactivation domain (NTD) of p53; inhibitors of Mdmx must be able to displace the p53-NTD from Mdmx. The structure of Mdmx with a bound small-molecule being available⁴, we decided to utilize structure-based virtual screening to discover new nutlin analogs with the desired properties.

Given a target protein structure with an identified binding site, a set of compounds to be screened, and suitable digital representations of these compounds, structure-based virtual screening (SBVS) typically begins with docking and scoring^{5, 6}. This consists of computationally searching for poses of a candidate compound against the binding site so as to achieve an optimal score. The search space includes compound translation and rotation, usually some rotatable bonds of the compound, and sometimes some internal degrees of freedom of the protein. The scoring function is intended to correlate approximately with binding free energy, but to be used in searching, it must be rapidly computable and well integrated with the searching software. A number of different docking/scoring programs are in common use.^{7–12} The end result of these programs is, for each compound, a set of docked conformations and a score for each conformation. Because of the need for very rapid computation, the docking scores are often very rough estimates and a screening cutoff score set at a level that admits most of the genuine hit compounds (true positives) will typically admit a much larger number of miss compounds (false positives).

Where docking score alone does not provide a satisfactory screening criteria it is common to set a fairly permissive docking score cutoff as a preliminary screen and subject compounds passing it to a more computationally intensive, but hopefully more accurate screen. A common second screen is the MM/PBSA method in which a molecular dynamics (MD) simulation is run and snapshots from it are analyzed using the molecular mechanics (MM) force-field for direct target—ligand interactions and the Poisson—Boltzmann and surface-area methods are used to estimate the polar and apolar contributions to solvation, respectively^{13, 14}. Another MD-based method is the linear interaction energy (LIE) method¹⁵ in which the MM force-field is used to calculate the difference in the electrostatic and non-bonded terms between the ligand and its surroundings in simulations of the free and bound states. These energy terms are scaled by empirical factors to obtain an estimate of binding free energy. MD simulations allow for ensemble effects to be considered in terms of both ligand and protein flexibility, effects which are omitted or severely simplified in docking/scoring. Both the MD simulations and the MM/PBSA analysis allow a more rigorous handling of solvent effects than is possible in docking scores. The MM/PBSA method has been used successfully in drug discovery^{16, 17}, although it appears to be more

successful with some targets than others¹⁸. Zhang *et al.* have reported on an automated pipeline for SBVS of compound libraries using a docking screen followed by MM/PBSA calculations¹⁹. Hu *et al.*²⁰ have reported excellent correlation between MM/PBSA and experimental binding free energies for a set of spiro-oxindole inhibitors of Mdm2²¹.

Here we report on a test of a two-stage SBVS protocol for a set of 130 nutlin-class compounds against Mdmx, comparing computed quantities against competitive potency of the compounds against the p53 NTD peptide measured by fluorescence-polarization assays. The initial protocol design was docking followed by molecular dynamics and MM/PBSA analysis, but surprisingly, molecular dynamics without MM/PBSA provided an excellent correlation to competitive ability due to the tendency of weakly binding or non-binding compounds to drift away from the initial bound conformation during simulation. Chen *et al.*²² have found that docked structures sometimes become unstable in subsequent MD simulations, but here, it is shown that a measure of this instability, the root-mean-square deviation (RMSD) can serve as a very effective screening parameter.

METHODS

Fluorescence-polarization assay

GST-MDMX (1 – 185) was cloned, expressed, and purified as described previously by Reed *et al.*²³. The fluorescence polarization (FP) assay was performed with 1 μ M GST-MDMX (1 – 185) in 10 mM Tris (pH 8.0), 42.5 mM NaCl, and 0.0125% Tween-20 assay buffer. Increasing concentrations of small molecules dissolved in DMSO, as described above, were pre-incubated with protein for 30 min in black 384-well plates (Corning) and then treated with Texas Red labeled wild-type p53-NTD peptide (15 nM, amino acids 15 – 29: GSGSSQETFSDLWKLLPEN) and incubated for an additional 45 min. After incubation the FP signal was measured on an EnVision plate reader fitted with a 555-nm excitation filter, 632-nm emission filter, and a Texas Red FP dichroic mirror. Unlabeled WT-p53 peptide was used as a positive control, and DMSO was used as a negative control.

Triplicate data was normalized to the positive (100% inhibition) and negative (0% inhibition) controls on the corresponding row of the 384-well plate (the percentage inhibition = $100 \times (\text{sample result} - \text{negative control}) / (\text{positive control mean} - \text{negative control})$). Two to seven independent experiments of normalized data were combined into a data set, and then fit using a non-linear regression in GraphPad Prism with the formula $\log(\text{inhibitor})$ vs. response – Variable slope (four parameters). Additionally the residuals of the curve fit were plotted to determine the fit of the theoretical curve. IC50 and 95% confidence intervals (CI) were determined from these graphs.

Docking calculations

Multiple starting conformations were generated for each of the nutlin derivatives using a knowledge-based anchoring strategy described previously²⁴. These conformers docked into the active site of Mdmx were utilized as an input for molecular docking using the iterated local search method of AutoDock Vina 1.1^{10, 25}. The AutoDock plugin for Pymol²⁶ was used to analyze the binding sites and prepare the target-related input parameters and target

structure information for AutoDock Vina. The grid box parameters were generated with the default selection around the crystallographic ligands. The ligand pdbqt files were generated by utilizing scripts included in the Molecular Graphics Laboratory (MGL) tools²⁷. This docking protocol allows for ligand flexibility, but not receptor flexibility.

Molecular dynamics simulations

Force field parameters for the ligands were created with the use of the Antechamber program²⁸ from the Amber10 package using General Amber Force Field (GAFF)²⁹ and AM1-BCC³⁰ partial charges. All energy minimizations and MD simulations were performed using the pmemd program from the Amber12 molecular dynamics package³¹ and the Amber99SB³² force field for the protein. The protein—ligand complex from docking was energy-minimized in two stages: ligand and hydrogen atoms, followed by all atoms. The system was then inserted in a box of TIP3P extending at least 10 Å from any given protein, and neutralized by the addition of counter-ions. The solvated system was minimized in two stages: water, then all atoms. After heating to 300 K, and equilibration to 1 atm pressure in the NPT ensemble, 3 ns of production MD simulations were carried out in the NPT ensemble with coordinates stored every 2 ps. All explicit-solvent calculations used the particle mesh Ewald (PME) method³³. Drift of the compounds away from their starting pose was measured as an average compound, all-atom RMSD over the last 1 ns of simulation relative to the compound position after in vacuo minimization.

RESULTS

FP assays

A set of 130 nutlin analogs whose synthesis we have previously reported³⁴, were assayed for disruption of the p53-Mdmx interaction by monitoring the fluorescence polarization of a TexasRed-labeled p53-NTD peptide in the presence of increasing concentration of inhibitor. A table of the compounds and their corresponding IC₅₀ values with 95% confidence intervals generated using a non-linear regression curve fit is provided in Supplemental Materials (Table S1). The compounds will be referred to here by the SJ identifiers as provided in that table. In order to determine IC₅₀ values, compounds were tested in a concentration range from 1 nM to 40 µM, using a 3-fold dilution scheme, which defines the experimental limits of the assay. No compound disrupted the p53-MDMX interaction with an IC₅₀ value more potent than 1 µM. Confidence intervals ranged from a factor of 4 for the more tightly bound compounds to a factor of 50 for compounds having less potent IC₅₀ values. For most of the tested compounds, the inhibition was too weak for an IC₅₀ value to be determined and have been designated as IC₅₀ > 40 µM. Examples of FP dose-response plots and curve fitting for a potent inhibitor of the p53-MDMX interaction (low IC₅₀ value) as compared to a compound that showed no inhibitory response (IC₅₀ > 40 µM) are provided in Figure 1. The threshold for designating a compound as a “hit” according to FP was set to 30 µM. As expected from a designed library, 14 of the 130 compounds were found to be hits (10.8%).

Docking and MD

All 130 compounds were docked against the Mdmx protein model generated from the crystal structure of Mdmx bound to a small molecule inhibitor (WK298/Novartis-101, PDB id: 3LBJ)⁴ using our previously developed method of compound conformer generation²⁴, followed by docking of the conformers using AutoDock Vina¹⁰. Visual inspection of the docking results showed poses very similar to those of the crystallographic Mdm2/nutlin-2 complex.³⁵ The normal selection criterion for virtual screening with AutoDock Vina is the Dock score (ADscore), which is meant to correlate to binding free energy; more negative ADScores represent tighter binding. These scores are given in Supplemental Table S1 alongside the experimental IC₅₀ values. The performance of ADscore as a screening parameter is analyzed by a ROC plot and an enrichment factor (EF) plot in Figure 2. Both methods of analysis illustrate the behavior of a scoring parameter as a discriminator of hits and misses as the threshold value for the parameter is varied. ROC plots illustrate the tradeoff between *sensitivity*, measured as the fraction genuine hits among the accepted compound (true positives), and $1 - \textit{selectivity}$, measured as the fraction of genuine hits among the rejected compounds (false positives). The overall performance of the parameter is then measured as the area under the ROC curve (AUC); a perfect classification parameter would have AUC=1, while a random classifier, which would lead to a diagonal ROC plot would have AUC=0.5. The enrichment factor (EF) is defined in terms of a subset of compounds selected by some threshold value of the screening parameter. EF is then the hit ratio of that subset divided by the hit ratio of the whole set, where hit ratio means the number of hits in a set divided by its population. The ROC plot for the ADscore (solid line in Figure 2a) has an AUC of 0.65, only modestly better than random choice. Strikingly, it has a gap between 0 and 0.24 followed by a steep climb between 0.24 and 0.51. This reflects the fact that all hit compound have ADScores between -7.6 and -7.1 . However, there were 26 “misses” with scores < -7.6 . This is also reflected in the EF plot (Figure 2b) where *no* enrichment is seen for the first 20% of the database and maximum enrichment factor is 2.28 at 41% of the database.

A cutoff of ADscore -7.0 was chosen for passage to the second, MD-based, filter. This includes 83 compounds: all 14 experimental hits and 69 misses. For each of these compounds, explicit-solvent MD was performed in the NPT ensemble at $P = 1$ atm. And $T=300$ K. The original intent had been to perform MM/PBSA analysis of the trajectories, but visual inspection showed that many of the experimental miss compounds with the most favorable ADScores had drifted away from the original docked pose after only 3 ns of simulation. This conformational drift precluded reliable MM/PBSA analysis, so it was decided to test the RMSD of the compounds from their starting poses over the last ns of simulation as a potential screening parameter. These RMSD values are tabulated in supplemental Table S1.

The result of a ROC analysis for the RMSD parameter is shown by the dashed line in Figure 2a. Overall performance of RMSD as a second screen gave an AUC of 0.91 (95% confidence interval 0.86 to 0.98), which is surprisingly close to the perfect value of 1.0. The EF plot shows that if compounds are rank ordered in terms of RMSD, compounds at the top of the list comprise a highly enriched subset. With 14 of the 83 compounds being hits, the

maximum possible enrichment is $83/14 = 5.93$. This maximum is achieved as the first three compounds in the RMSD rankings are all hits. If only the top 10 are taken, seven of them are hits for an EF of 4.15.

DISCUSSION

A two-step virtual screening protocol, docking with a screen by docking score followed by MD with a screen by RMSD from the docked conformation has been implemented for a set of nutlin-like compounds as potential inhibitors of the binding of Mdmx to p53-NTD. All compounds have been assayed by FP for their ability to disrupt the targeted protein—protein interaction. Even before virtual screening, the compound library was quite focused, exploiting knowledge of the activities of nutlin-2 and nutlin-3a and the crystallographic structures of nutlin-2 bound to Mdm2, which is very similar to Mdmx, and the structures of both Mdm2 and Mdmx bound to the p53-NTD.^{36–38} A comparison of virtual screening scores from the two stages showed that the second stage, MD and RMSD, provided a dramatic improvement of overall performance. The EF analysis is particularly pertinent for practical situations where the purpose of virtual screening is to select a relatively small number of compounds for experimental testing, and these are to be selected from the high-ranking compounds in the virtual screen. In the present case, if only the docking score were used, the EF plot shows that many misses would be selected before the first hit, because a number of miss compounds nevertheless have more favorable scores than any hit compound. For the same reason the ROC plot has gap between 0.0 and 0.24 that is rather unusual in docking results. The MD/RMSD screen does an excellent job of screening these out.

These results are a contrast to those of a recent study of Lauro et al.³⁹ in which an MD-based method, LIE,¹⁵ failed to provide significant improvement over docking. In that study, a compound database of known ligands of trypsin and decoy compounds was screened with 4 different single-stage methods: LIE and 3 different docking programs. It was found that while LIE's performance was good, it was no better than the best docking programs. These results contrast from our present results in two ways: Our docking stage performed much worse than theirs even when the same program (AutoDock Vina) was used, and our MD-based method performed much better. This could be due to the difference between an enzyme active-site target in their case and the protein—protein interaction in the present case. More specifically, Mdmx has a highly hydrophobic p53-NTD binding site, and in past efforts to dock nutlins into either Mdmx or Mdm2 it has proved very difficult to get a correct pose.^{24, 40} In the present study, our previously developed nutlin conformation-generation method has allowed AutoDock to find good poses but the associated scores do not provide a very satisfactory ranking of compounds. Another factor for the difference in docking vs. MD methods is the chemical variety of the compound database. The study of Lauro *et al.* used a chemically diverse database, while the present compound set is much more narrowly focused. This may be the reason that the ADscores of the true hits lie in a relatively narrow band — all successful nutlin-like compounds bind Mdmx in the same way, while failures fail in many different ways, and some of these ways are not captured by the ADscore. They are captured, however, by MD simply because their defects tend to push them away from a nutlin-like binding pose.

A possible lesson is that when a docking score does not appear to give good performance, MD-based methods should be considered as a refinement. A short MD simulation scored by RMSD would be a good first try because of the relatively short simulations needed and the simplicity of the RMSD calculation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

EF	Enrichment factor
FP	Fluorescence polarization
LIE	Linearized Interaction Energy
MM/PBSA	Molecular Mechanics / Poisson—Boltzmann Surface Area
NPT	Number-Pressure-Temperature
NTD	N-terminal Transactivation Domain
RMSD	Root Mean Square Deviation
ROC	Receiver Operator Characteristic

References

1. Laurie NA, Donovan SL, Shih CS, Zhang J, Mills N, Fuller C, Teunisse A, Lam S, Ramos Y, Mohan A, Johnson D, Wilson M, Rodriguez-Galindo C, Quarto M, Francoz S, Mendrysa SM, Guy RK, Marine JC, Jochemsen AG, Dyer MA. Inactivation of the p53 pathway in retinoblastoma. *Nature*. 2006; 444:61–6. [PubMed: 17080083]
2. Zhang F, Tegen M, Throm S, Mallari J, Miller L, Guy RK, Dyer MA, Williams RT, Roussel MF, Nemeth K, Zhu F, Zhang J, Lu M, Panetta JC, Boulos N, Stewart CF. Whole-body physiologically based pharmacokinetic model for nutlin-3a in mice after intravenous and oral administration. *Drug metabolism and disposition: the biological fate of chemicals*. 2011; 39:15–21. [PubMed: 20947617]
3. Andreeff M, Kojima K, Padmanabhan S, Strair R, Kirschbaum M, Maslak P, Hillmen P, O'Brien S, Samaniego F, Borthakur G, Konopleva M, Vassilev L, Nichols G. Open-Label, Phase I Study of Single Agent RG7112, A First In Class p53-MDM2 Antagonist, In Patients with Relapsed/Refractory Acute Myeloid and Lymphoid Leukemias (AML/ALL) and Refractory Chronic Lymphocytic Leukemia/Small Cell Lymphocytic Lymphomas (CLL/SCLL). *Blood*. 2010; 116:287–288. [PubMed: 20435883]
4. Popowicz GM, Czarna A, Wolf S, Wang K, Wang W, Domling A, Holak TA. Structures of low molecular weight inhibitors bound to MDMX and MDM2 reveal new approaches for p53-MDMX/MDM2 antagonist drug discovery. *Cell cycle*. 2010; 9:1104–11. [PubMed: 20237429]
5. Braga RC, Alves VcM, Silva AC, Nascimento MN, Silva FC, Li??o LM, Andrade CH. Virtual Screening Strategies in Medicinal Chemistry: The State of the Art and Current Challenges. *Curr Topics Med Chem*. 2014; 14:1899–1912.

6. Ferreira LG, dos Santos RN, Oliva G, Andricopulo AD. Molecular Docking and Structure-Based Drug Design Strategies. *Molecules*. 2015; 20:13381–13421.
7. Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT, Repasky MP, Knoll EH, Shelley M, Perry JK, Shaw DE, Francis P, Shenkin PS. Glide: A new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *J Med Chem*. 2004; 47:1739–1749. [PubMed: 15027865]
8. Halgren TA, Murphy RB, Friesner RA, Beard HS, Frye LL, Pollard WT, Banks JL. Glide: A new approach for rapid, accurate docking and scoring. 2. Enrichment factors in database screening. *J Med Chem*. 2004; 47:1750–1759. [PubMed: 15027866]
9. Jones G, Willett P, Glen RC, Leach AR, Taylor R. Development and validation of a genetic algorithm for flexible docking. *J Mol Biol*. 1997; 267:727–248. [PubMed: 9126849]
10. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem*. 2010; 31:455–461. [PubMed: 19499576]
11. Allen WJ, Balias TE, Mukherjee S, Brozell SR, Moustakas DT, Lang PT, Case DA, Kuntz ID, Rizzo RC. DOCK 6: Impact of New Features and Current Docking Performance. *J Comput Chem*. 2015; 36:1132–1156. [PubMed: 25914306]
12. Goodsell DS, Olson AJ. Automated Docking of Substrates to Proteins by Simulated Annealing. *Proteins*. 1990; 8:195–202. [PubMed: 2281083]
13. Kollman PA, Massova I, Reyes C, Kuhn B, Huo S, Chong L, Lee M, Lee T, Duan Y, Wang W, Donini O, Cieplak P, Srinivasan J, Case DA, Cheatham TE. Calculating Structures and Free Energies of Complex Molecules: Combining Molecular Mechanics and Continuum Models. *Acc Chem Res*. 2000; 33:889–897. [PubMed: 11123888]
14. Srinivasan J, Cheatham TE, Kollman PA, Case DA. Continuum solvent studies of the stability of DNA, RNA, and phosphoramidate-DNA helices. *J Am Chem Soc*. 1998; 120:9401–9409.
15. Aqvist J, Medina C, Samuelsson J-E. A new method for predicting binding affinity in computer-aided drug design. *Protein Eng*. 1994; 7:385–391. [PubMed: 8177887]
16. Huo S, Wang J, Cieplak P, Kollman PA, Kuntz ID. Molecular and Free Energy Analysis of Cathepsin D-Inhibitor Interactions: Insight into Structure-based ligand design. *J Med Chem*. 2002; 45:1412–1419. [PubMed: 11906282]
17. Shan JF, Shi D-L, Zheng JWJ. Identification of a specific inhibitor of the dishevelled PDZ domain. *Biochemistry*. 2005; 44:15495–15503. [PubMed: 16300398]
18. Pearlman DA. Evaluating the Molecular Mechanics Poisson-Boltzmann Surface Area Free Energy Method Using a Congeneric Series of Ligands to p38 MAP Kinase. *J Med Chem*. 2005; 48:7796–7807. [PubMed: 16302819]
19. Zhang X, Wong SE, Lightstone FC. Toward Fully Automated High Performance Computing Drug Discovery: A Massively Parallel Virtual Screening Pipeline for Docking and Molecular Mechanics/Generalized Born Surface Area Rescoring to Improve Enrichment. *J Chem Inf Model*. 2014; 54:324–337. [PubMed: 24358939]
20. Hu G, Wang D, Liu X, Zhang Q. A computational analysis of the binding model of MDM2 with inhibitors. *J Comput Aided Mol Des*. 2010 Online 21 May.
21. Ding K, Lu Y, Nikolovska-Coleska Z, Wang G, Qiu S, Shangary S, Gao W, Qin D, Stuckey J, Krajewski K, Roller PP, Wang S. Structure-based design of spiro-oxindoles as potent, specific small-molecule inhibitors of the MDM2-p53 interaction. *Journal of medicinal chemistry*. 2006; 49:3432–5. [PubMed: 16759082]
22. Chen YC. Beware of docking! *Trends Pharmacol Sciences*. 2015; 36:78–95.
23. Reed D, Shen Y, Shelat AA, Arnold LA, Ferreira AM, Zhu F, Mills N, Smithson DC, Regni CA, Bashford D, Cicero SA, Schulman BA, Jochemsen AG, Guy RK, Dyer MA. Identification and characterization of the first small-molecule inhibitor of MDMX. *J Biol Chem*. 2010; 285:10786–10796. [PubMed: 20080970]
24. Bharatham N, Bharatham K, Shelat AA, Bashford D. Ligand Binding Mode Prediction by Docking: Mdm2/Mdmx Inhibitors as a Case Study. *J Chem Inf Model*. 2014; 54
25. Baxter J. Local Optima Avoidance in Depot Location. *J Oper Res Soc*. 1981; 32:815–819.
26. DeLano, WL. The PyMOL molecular graphics system, 1.5.0.4. Schrödinger, LLC;

27. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of computational chemistry*. 2009; 30:2785–91. [PubMed: 19399780]
28. Wang JM, Wang W, Kollman PA, Case DA. Automatic atom type and bond type perception in molecular mechanical calculations. *J Mol Graphics & Modelling*. 2006; 25:247–260.
29. Wang JM, Wolf RM, Caldwell JW, Kollman PA, Case DA. Development and testing of a general Amber force field. *J Computat Chem*. 2004; 25:1157–1174.
30. Jakalian A, Bush BL, Jack DB, Bayly CI. Fast, efficient generation of high-quality atomic charges. AM1-BCC model: I. Method. *J Comput Chem*. 2000; 21:32–146.
31. Case, DA., Darden, TA., Cheatham, TE., III, Simmerling, CL., Wang, J., Duke, RE., Luo, R., Crowley, M., Walker, RC., Zhang, W., Merz, KM., Wang, B., Hayik, S., Roytberg, A., Seabra, G., Kolossvary, I., Wong, KF., Paesani, F., Vanicek, J., Wu, X., Brozell, SR., Steinbrecher, T., Gohlke, H., Yang, C., Tan, J., Mongan, J., Hornak, V., Cui, G., Matthews, DH., Seetin, MG., Sagui, C., Babin, V., Kollman, PA. AMBER 12. University of California; San Francisco: 2012.
32. Hornak V, Abel R, Okur A, Strockbine B, Roitberg A, Simmerling C. Comparison of multiple Amber force fields and development of improved protein backbone parameters. *Proteins*. 2006; 65:712–725. [PubMed: 16981200]
33. Darden T, York D, Pedersen L. Particle mesh Ewald: An $N^2 \log(N)$ method for Ewald sums in large systems. *J Chem Phys*. 1993; 98:10089.
34. Vara BA, Mayasundari A, Tellis JC, Danneman MW, Arredondo V, Davis TA, Min J, Finch K, Guy RK, Johnston JN. Organocatalytic, Diastereo- and Enantioselective Synthesis of Nonsymmetric *cis*-Stilbene Diamines: A Platform for the Preparation of Single-Enantiomer *cis*-Imidazolines for Protein-Protein Inhibition. *J Org Chem*. 2014; 79:6913–6938. [PubMed: 25017623]
35. Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, Kong N, Kammlott U, Lukacs C, Klein C, Fotouhi N, Liu EA. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science*. 2004; 303:844–848. [PubMed: 14704432]
36. Popowicz GM, Czarna A, Holak TA. Structure of the human Mdmx protein bound to the p53 tumor suppressor transactivation domain. *Cell cycle*. 2008; 7:2441–3. [PubMed: 18677113]
37. Popowicz GM, Czarna A, Rothweiler U, Szwagierczak A, Krajewski M, Weber L, Holak TA. Molecular basis for the inhibition of p53 by Mdmx. *Cell cycle*. 2007; 6:2386–92. [PubMed: 17938582]
38. Kussie PH, Gorina S, Marechal V, Elenbaas B, Moreau J, Levine AJ, Pavletich NP. Structure of the MDM2 Oncoprotein Bound to the p53 Tumor Suppressor Transactivation Domain. *Science*. 1996; 274:948–953. [PubMed: 8875929]
39. Lauro G, Ferruz N, Fulle S, Harvey MJ, Finn PW, Fabritiis aGD. Reranking Docking Poses Using Molecular Simulations and Approximate Free Energy Methods. *J Chem Inf Model*. 2014; 54:2185–2189. [PubMed: 25046765]
40. Lu Y, Nikolovska-Coleska Z, Fang X, Gao W, Shangary S, Qiu S, Qin D, Wang S. Discovery of a Nanomolar Inhibitor of the Human Murine Double Minute 2 (MDM2)-p53 Interaction through an Integrated, Virtual Database Screening Strategy. *J Med Chem*. 2006; 49:3759–3762. [PubMed: 16789731]

Figure 1.

Examples of FP dose response data, curve fitting and interpretation. Fitting residuals are displayed in insets. (A) Data for compound SJ000558295, which was fit to a curve with IC_{50} 7.0 μM , 95% confidence interval 4.6 to 10.6, and is designated a “hit”. (B) Data for compound SJ000558303, which could not be reliably fit and is designated as a “miss” with $\text{IC}_{50} > 40 \mu\text{M}$.

Figure 2.

(A) ROC plots and (B) EF plots for ADscore (solid line) or RMSD (dashed line) as the cutoff parameter. The ADscore plot includes all 130 compounds studied. The RMSD plot includes the 83 compounds with ADscore -7.0 kcal/mol. Area under curve (AUC) is 0.65 for ADscore and 0.91 for RMSD. The diagonal dotted line in (A) represents the theoretical outcome of random choice (AUC=0.5).