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Targeting Dynamic Pockets of HIV-1 Protease by Structure-Based Computational Screening for Allosteric Inhibitors

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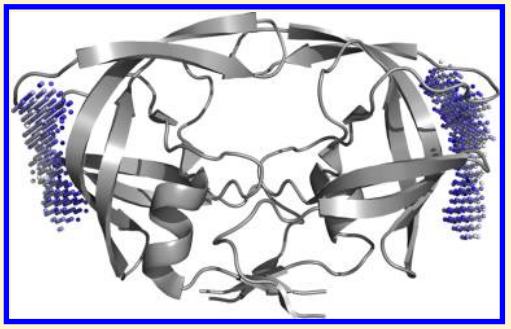
Supporting Information

ABSTRACT: We present the discovery of low molecular weight inhibitors of human immunodeficiency virus 1 (HIV-1) protease subtype B that were identified by structure-based virtual screening as ligands of an allosteric surface cavity. For pocket identification and prioritization, we performed a molecular dynamics simulation and observed several flexible, partially transient surface cavities. For one of these presumable ligand-binding pockets that are located in the so-called "hinge region" of the identical protease chains, we computed a receptor-derived pharmacophore model, with which we retrieved fragment-like inhibitors from a screening compound pool. The most potent hit inhibited protease activity in vitro in a noncompetitive mode of action. Although attempts failed to crystallize this ligand bound to the enzyme, the study provides proof-of-concept for identifying innovative tool compounds for chemical biology by addressing flexible protein models with receptor pocket-derived pharmacophore screening.



In light of reports from the World Health Organization expecting AIDS as one of the major threats of public health by 2030, human immunodeficiency virus (HIV-1) protease remains an important target for drug discovery and development.[1] It has been suggested that the allosteric inhibition of HIV-1 protease by addressing dynamic potentially allosteric ligand binding pockets might provide a viable strategy to reduce the effects of protease resistance against direct active-site inhibitors.[2] Allosterism is a thermodynamic phenomenon of ligand−receptor interaction, and many, if not all, dynamic ligand-accommodating pockets on the surfaces of biological macromolecules might be considered allosteric.[3,4] Allosterism can be achieved by various molecular mechanisms and is not necessarily accompanied by large structural rearrangements of the receptor backbone.[3,5] In fact, as allosteric sites are apparently under low evolutionary pressure, even subtle differences between allosteric binding sites might provide an opportunity for the design of target subtype-selective ligands.[5,6] A common approach to identify allosteric sites is by high-throughput fragment screening in combination with crystallographic, spectroscopic, and kinetic studies.[7,8] More recently, molecular dynamics (MD) simulations have been employed to complement these extensive and often time-consuming methods.[9−11] Here, we present a virtual screening study based on MD simulations of human HIV-1 protease subtype B, in which we computationally targeted an allosteric dynamic ligand-binding pocket and retrieved first-in-class low molecular weight inhibitors. We limited our proof-of-concept study to HIV-1 protease subtype B as the most prominent representative in the Western hemisphere. Our study confirms structure-based virtual screening as suitable for identifying allosteric ligands.

For the MD simulation, we decided to start from a closed form (closed "flaps", residue positions 46−53) of the protease and investigate flap movement, as our idea was to potentially freeze the closed form by a suitable ligand.[9−11] Because of the fact that there was no HIV-1 protease subtype B *apo*-structure with closed flaps available from the Protein Data Bank (PDB),[12] we constructed a comparative protein model as starting point for our MD study. An HIV-1 protease subtype A *apo*-structure with closed flaps (PDB ID: 3ixo[13]) served as template for the program MODELLER 9.9[14] to computationally mutate six residues (R20K, I36M, K57R, K69H, S81P, M89L), which led to a subtype B model with closed flaps. All backbone and conserved side-chain positions were fixed during the modeling process, and only the mutated side-chains were allowed to move. The resulting protein models were audited on the PROCHECK server,[15] and the best model was kept for further study.

In order to obtain motivated structural fluctuations, we then performed an MD simulation with NAMD 2.8[16] and the CHARMM 27 force field.[17,18] The system was simulated in a TIP3P explicit solvent water box[19] and neutralized by adding 63 chlorine counterions. Periodic boundary conditions and Ewald summation for long-range electrostatic interaction were

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applied.[20] The simulation was separated into three parts: (i) minimization for 2000 steps with a fully constrained backbone, followed by 2000 minimization steps with a harmonically restrained backbone, (ii) heating to 310 K over 12 ps and 5 ns equilibration, and (iii) productive simulation for 20 ns without backbone constraints. We analyzed the MD trajectory using VMD,[21] R[22] and PyMOL (v1.4.1; Schrödinger, Cambridge, U.S.A.). After approximately 10 ns of simulation time, the flap opened and slowly returned to the closed state again (Figure 1). This breathing mode had already been observed in previous

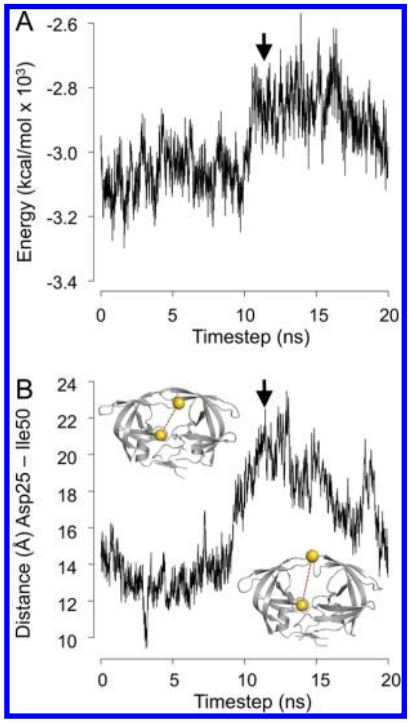

Figure 1. Analysis of the productive MD simulation of HIV-1 protease according to energy (A) and the distance between the catalytic Asp25 (chain A) and Ile50 (chain A) located at the tip of a flap covering the active site (B). The arrow indicates the protein snapshot used in the virtual screening experiments (protein model provided as Supporting Information).

simulations and is in line with the reported flexibility of the protease.[23] We envisaged to target a potential ligand-binding site located at the hinge of the moving flaps and by virtual screening find a ligand that might act as a wedge that could impair the flap movement.

The MD simulation suggested a bipartitioned Exo pocket, with its major part behaving similarly in the two identical chains of the protease. This hypothesis is reflected in the structure of the pocket graphs presented in Figure 2 (upper panel). Changes of the Exo pocket conformations were observed together with a movement of the flaps. For one of the two Exo pockets (Exo I), we found a unique subpocket forming that was not observed for the Exo II site during the MD simulation (clusters 2.6 and 3.8, Figure 2). Consequently, we selected the Exo I pocket snapshot at 11.25 ns of simulated time from "open flap" cluster no. 3.8 for virtual ligand screening (10.7−13.1 ns, Figures 1 and 2). This pocket represents a transient cavity conformation exclusively found during the MD simulation. Of note, in this conformation, the flap of the second protein

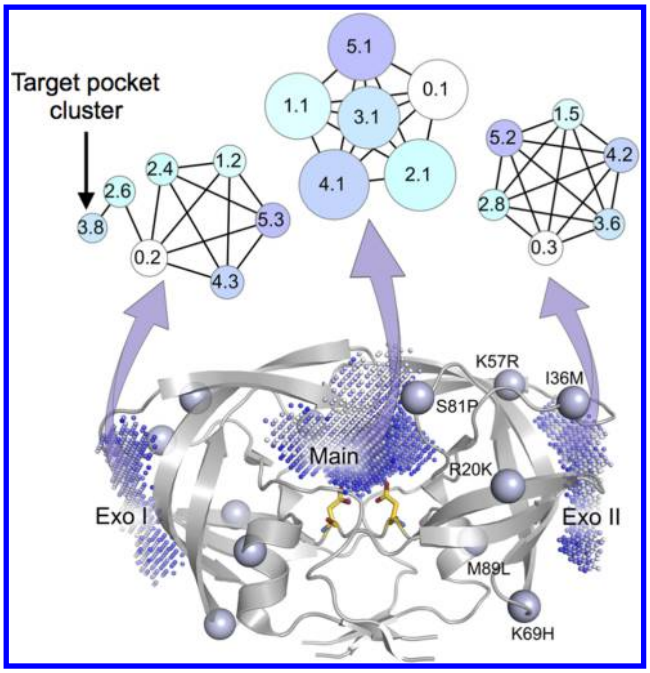

Figure 2. Pocket analysis of HIV-1 protease MD snapshots. The protein dimer is depicted in a cartoon representation with the mutated residues highlighted as large spheres and the catalytic Asp25 as stick models. The three largest pockets (main = active site, Exo I, Exo II; volumes >100 Å^3) were extracted using the software PocketPicker[24] and are shown as local clusters of grid points. Color intensity codes the grid point buriedness. The relative similarities of representative pocket snapshots from the MD simulation[25] are shown as graphs (top panel), in which circle size represents pocket volume and edges denote similar pocket forms. Numbers denote the cluster and respective pocket index (cluster 0 = homology model; higher numbers: MD cluster representatives). The networks were generated with Cytoscape 2.8 software.[39]

monomer is in a closed conformation, implying that the two chains of HIV-1 protease might behave differently.

We performed structure-based virtual screening in two steps. We first computed a protein-derived pharmacophore model of the Exo I pocket with our software VirtualLigand.[26] This software generates a receptor-derived pharmacophore model that contains idealized ligand pharmacophore features. For model generation, geometry-based interaction rules between all pocket grid points, and the surrounding amino acid residues were evaluated. The resulting point sets were further translated into Gaussian pharmacophore features. The computed model essentially is a mirror image of potential receptor−ligand interactions in the Exo I site. This approach enables the usage of pharmacophore descriptors to computationally screen a compound library by similarity searching. The descriptors of the screening compounds were calculated with the software LIQUID.[27] Herein, potential pharmacophore points are defined based on atom type and local environment rules. A LIQUID representation of a pharmacophore model is computed as a cross-correlation vector of the potential pharmacophore points. This leads to the same number of corresponding descriptor values for both the VirtualLigand model and each compound in the screening library. Our screening pool consisted of more than three million structurally standardized compounds, for which we computed single conformations using CORINA 3.46 (Molecular Networks, Erlangen, Germany).[28,29] The top-scoring candidate compounds from pharmacophore similarity

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searching were docked into the Exo I pocket. Ligand docking was done with GOLD v5.0.1,[30] and poses were evaluated by the *ChemPLP* fitness function. From the top-ranking compounds, we selected five candidates for in vitro activity determination based on a compromise between high pharmacophore similarity and favorable *ChemPLP* values. This final selection was done manually.

Compounds **1** and **2** exhibited moderate concentration-dependent inhibition of HIV-1 protease (Figure 3). Having

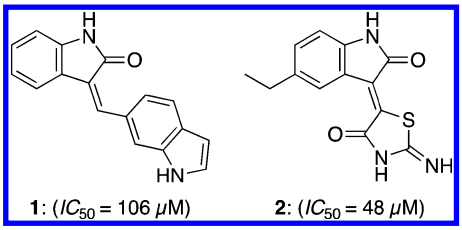

Figure 3. Compounds selected for the Exo I pocket of HIV-1 protease by virtually screening for molecules that satisfy a receptor-derived "fuzzy" pharmacophore model.

compound **2** as a promising starting point (IC_{50} = 48 μM, ligand efficiency[31] = 0.32, *ChemPLP* score = 47), we tested five structural analogues to obtain a preliminary structure–activity relationship (SAR). Four of them showed micromolar IC_{50} values (Figure 4). Positioning of the ethyl substituent at the

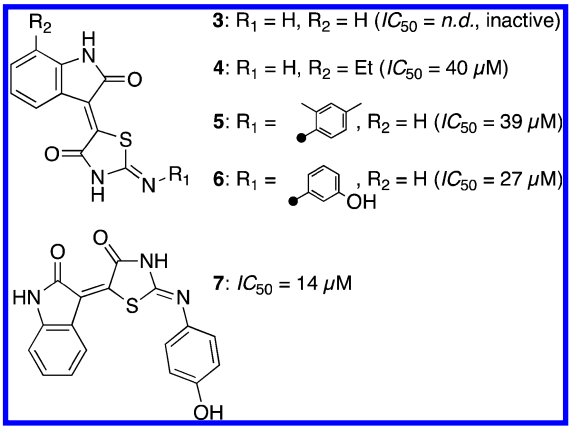

Figure 4. Analogues of primary hit **2** tested for inhibition of HIV-1 protease. n.d.: not detectable.

benzene ring (R_2) appears to have little influence on enzyme inhibition (**2** vs **4**), but its absence led to the complete loss of potency (**3**). Apparently, the Michael acceptor system itself has no influence on activity, as the nonsubstituted scaffold in **3** turned out to be inactive, without affecting the protease in the assay. Potency was restored by an aryl substitution at R_1 (**5**) and was most pronounced when a hydroxyl group was *meta*-positioned in this aromatic system (**6**). Moreover, a change of the exocyclic double bond configuration from *E* (**6**) to *Z* (**7**) led to further activity improvement. Compound **7** (IC_{50} = 14 μM, *ChemPLP* score = 47, ligand efficiency = 0.28) achieved 3-fold stronger inhibition than the mother compound **2**.

The computed docking pose of **7** suggests that the R_2 moiety points toward the solvent (Figure 5), which is in partial disagreement with the structure–activity data (R_2 = Et retains activity, but R_2 = H is inactive). Thus, the presented docking pose has to be treated with caution. We cannot exclude the possibility that compound **7** binds as a *trans*-tautomer instead of the modeled *cis*-tautomer (as designated by the supplier). On the basis of these preliminary results, the optimization of the inhibitory potency and the lead-likeness of compound **7** are reasonable next steps, for example, by introducing bioisosters or preferably by extended virtual screens aiming at scaffold hops.

Finally, we tested biochemically whether compound **7** actually noncompetitively inhibits HIV-1 protease. We employed the Yonetani–Theorell method for examining subsites of enzyme active centers[32,33] and measured substrate cleavage in the presence of active site inhibitor pepstatin A (IC_{50} = 0.3 μM, Figure 6A) at varying concentrations (0, 0.02 to 0.1 μM), while keeping the concentration of compound **7** fixed at 0, 15, 20, or 30 μM, respectively. Enzyme activity was monitored as a time-course measurement of the increase in the fluorescence signal from fluorescently labeled peptide substrate. The initial linear portion of the slope (signal × min^{-1}) was analyzed to determine reaction rates in the absence (v_o) and the presence (v_i) of inhibitors. The result of this experiment clearly suggests that compound **7** inhibits HIV-1 protease activity in a noncompetitive fashion (Figure 6B). In an aspartic protease panel screen (human cathepsin D, cathepsin E, pepsin A, BACE, renin), compound **7** turned out to be inactive (non-inhibiting) at a concentration of 10 μM. Preliminary attempts at obtaining a crystal structure of **7** bound to human HIV-1 protease failed, as soaking of HIV protease crystals with our compound led either to empty structures or the destruction of the crystal. This observation actually suggests that the compound destabilizes the *apo*-form of the enzyme and binds to a different conformation, which would be in agreement with our MD simulation.

Our virtual screening study exceeds an earlier high-throughput X-ray campaign that led to the identification of 2-methylcyclohexanol that was found to bind to the elbow region, yet turned out to be functionally inert.[34] Recently, Chang et al. synthesized and tested a 44,000 member compound library for HIV-1 protease inhibition and found one presumable Exopocket binder (IC_{50} = 17 μM, ligand efficiency = 0.18).[35] Here, we independently validate this surface region of the enzyme as functionally important for HIV-1 protease and demonstrate that virtual screening has the ability to deliver selective and innovative ligand-efficient binders.

The elbow region of HIV-1 protease apparently undergoes notable changes during flap movement and together with the low mutation rate in this area has previously been suggested as an allosteric regulation site.[2] We here present the computer-assisted discovery of a functional allosteric inhibitor of HIV-1 protease supposedly binding to the Exo pocket. Although all indications point to the fact that the compound binds to the presumed allosteric site, there is no definitive proof that this actually is the case. Future studies will also have to address the actual selectivity of the inhibitors found to compare the results obtained for the other HIV-1 protease subtypes.

Our study demonstrates that structure-based computational screening in combination with MD simulation and pocket identification may help to reduce synthetic and biochemical efforts, thereby saving time and minimizing the costs of experiment. Our approach extends existing structure-based virtual screening methods for allosteric inhibitors,[35−37] offers a complementary approach to the substrate envelope approach for direct inhibitor design,[38] and might serve as a prototype for similar exercises in chemical biology and early drug discovery

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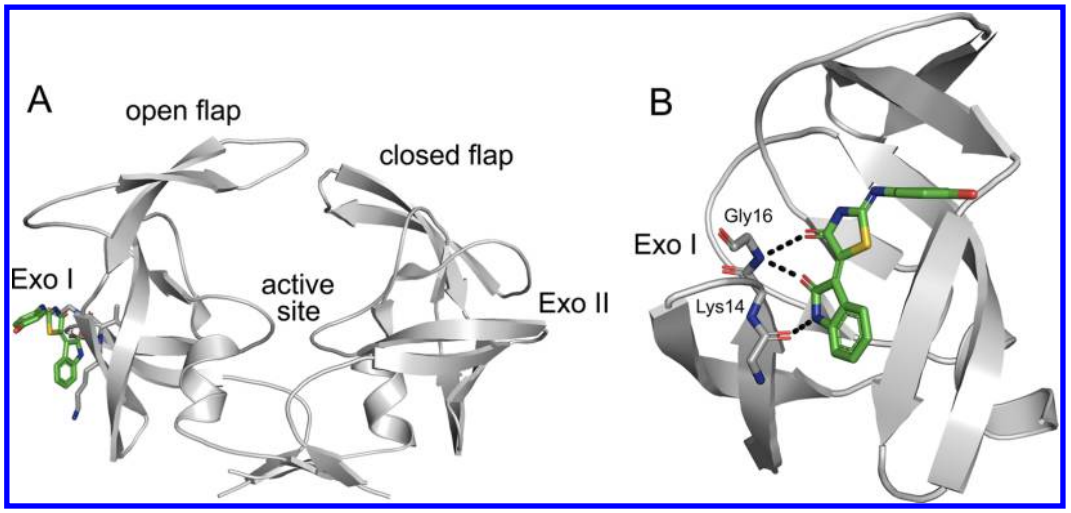

Figure 5. (A) Computed docking pose of inhibitor compound **7** in the Exo I pocket of HIV-1 protease in an "open flap" state (snapshot observed after 11.25 ns of simulated time). The "closed flap" state of the Exo II pocket in this protein snapshot is shown for comparison. (B) Close-up of the presumable Exo I binding site. Dashed lines indicate potential hydrogen bridges (lengths: 2.8−3.1 Å) between the ligand and the protein backbone of Lys14 and Gly16.

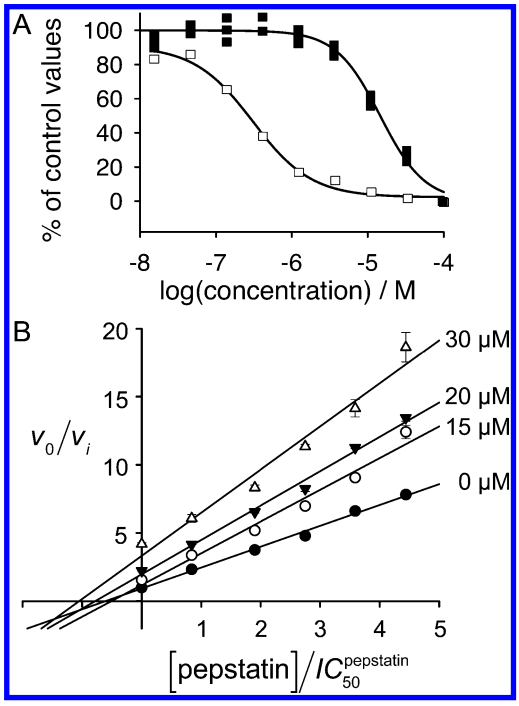

Figure 6. (A) Concentration-dependent inhibition of HIV-1 protease by pepstatin A (open squares, $n = 1$) and compound **7** (filled squares, $n = 3$). Regression by fitting the four-parameter Hill equation. (B) Yonetani−Theorell plot for compound **7** vs pepstatin A showing a noncompetitive inhibitory mode of action ($n = 3$, mean ± SEM). The reasonable convergence of the regression lines suggests independent binding sites.

aiming at ligand-efficient functional modulators with innovative modes of action.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Coordinate file of the HIV-1 protease subtype B model (MD state observed after 11.25 ns of simulated time) in PDB format. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

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Notes

The authors declare the following competing financial interest(s): G. Schneider and P. Schneider are shareholders of inSili.com LLC, Zürich; G. Schneider is a shareholder of AlloCyte Pharmaceuticals Ltd., Basel, and acts as a scientific consultant of the pharmaceutical industry.

HIV-I activity determination and cross-competitive inhibitor measurements were performed by ReactionBiology Corp. (Malvern, U.S.A.) on a fee-for-service basis. Screening compounds were compiled from Asinex Gold, Platinum and Synergy 09/2011 (Asinex, Moscow, Russia), ChemBridge 05/2011 (ChemBridge Corporation, San Diego), Enamine HTS collection 05/2011 (Enamine, Kiev, Ukraine), Interbioscreen natural compound library and synthetic compound collection 08/2010 (Interbioscreen, Moscow, Russia), and Specs natural products and screening collection 08/2010 (Specs, Delft, The Netherlands). All compounds were pretreated by the "wash" function (deprotonation of strong acids and protonation of strong bases) in the Molecular Operating Environment (MOE 2011.10) software (Chemical Computing Group, Montreal, Canada). Compounds **3**−**7** were purchased from Inter-BioScreen, Specs, ChemBridge. The protease panel screen was performed at Sanofi-Aventis Deutschland GmbH R&D, Frankfurt, Germany, using in-house assays.

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■ REFERENCES

(1) Qiu, X.; Liu, Z. P. Recent developments of peptidomimetic HIV-1 protease inhibitors. *Curr. Med. Chem.* **2011**, *18*, 4513−4537.

(2) Hornak, V.; Simmerling, C. Targeting structural flexibility in HIV-1 protease inhibitor binding. *Drug Discovery Today* **2007**, *12*, 132−138.

(3) Tsai, C. J.; Del Sol, A.; Nussinov, R. Allostery: Absence of a change in shape does not imply that allostery is not at play. *J. Mol. Biol.* **2008**, *378*, 1−11.
(4) Gunasekaran, K.; Ma, B.; Nussinov, R. Is allostery an intrinsic property of all dynamic proteins? *Proteins* **2004**, *57*, 433−443.
(5) Laskowski, R. A.; Gerick, F.; Thornton, J. M. The structural basis of allosteric regulation in proteins. *FEBS Lett.* **2009**, *583*, 1692−1698.
(6) Christopoulos, A. Allosteric binding sites on cell-surface receptors: Novel targets for drug discovery. *Nat. Rev. Drug Discovery* **2002**, *1*, 198−210.
(7) Hardy, J. A.; Lam, J.; Nguyen, J. T.; O'Brien, J.; Wells, J. A. Discovery of an allosteric site in the caspases. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 12461−12466.
(8) Scott, E.; Coyne, A. G.; Hudson, S. A.; Abell, C. Fragment-based approaches in drug discovery and chemical biology. *Biochemistry* **2012**, *51*, 4990−5003.
(9) Louis, J. M.; Ishima, R.; Torchia, D. A.; Weber, I. T. HIV-1 protease: Structure, dynamics, and inhibition. *Adv. Pharmacol.* **2007**, *55*, 261−298.
(10) Levy, Y.; Caflisch, A.; Onuchic, J. N.; Wolynes, P. G. The folding and dimerization of HIV-1 protease: Evidence for a stable monomer from simulations. *J. Mol. Biol.* **2004**, *340*, 67−79.
(11) Broglia, R.; Levy, Y.; Tiana, G. HIV-1 protease folding and the design of drugs which do not create resistance. *Curr. Opin. Struct. Biol.* **2008**, *18*, 60−66.
(12) Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. The Protein Data Bank. *Nucleic Acids Res.* **2000**, *28*, 235−242.
(13) Robbins, A. H.; Coman, R. M.; Bracho-Sanchez, E.; Fernandez, M. A.; Gilliland, C. T.; Li, M.; Agbandje-McKenna, M.; Wlodawer, A.; Dunn, B. M.; McKenna, R. Structure of the unbound form of HIV-1 subtype A protease: Comparison with unbound forms of proteases from other HIV subtypes. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2010**, *66*, 233−242.
(14) Eswar, N.; Eramian, D.; Webb, B.; Shen, M. Y.; Sali, A. Protein structure modeling with MODELLER. *Methods Mol. Biol.* **2008**, *426*, 145−159.
(15) Laskowski, R. A.; MacArthur, M. W.; Moss, D. S.; Thornton, J. M. PROCHECK: A program to check the stereochemical quality of protein structures. *J. Appl. Crystallogr.* **1993**, *26*, 283−291.
(16) Phillips, J. C.; Braun, R.; Wang, W.; Gumbart, J.; Tajkhorshid, E.; Villa, E.; Chipot, C.; Skeel, R. D.; Kale, L.; Schulten, K. Scalable molecular dynamics with NAMD. *J. Comput. Chem.* **2005**, *26*, 1781−1802.
(17) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. CHARMM: A program for macromolecular energy, minimization, and dynamics calculations. *J. Comput. Chem.* **1983**, *4*, 187−217.
(18) Brooks, B. R.; Brooks, C. L., 3rd; Mackerell, A. D., Jr.; Nilsson, L.; Petrella, R. J.; Roux, B.; Won, Y.; Archontis, G.; Bartels, C.; Boresch, S.; Caflisch, A.; Caves, L.; Cui, Q.; Dinner, A. R.; Feig, M.; Fischer, S.; Gao, J.; Hodoscek, M.; Im, W.; Kuczera, K.; Lazaridis, T.; Ma, J.; Ovchinnikov, V.; Paci, E.; Pastor, R. W.; Post, C. B.; Pu, J. Z.; Schaefer, M.; Tidor, B.; Venable, R. M.; Woodcock, H. L.; Wu, X.; Yang, W.; York, D. M.; Karplus, M. CHARMM: The biomolecular simulation program. *J. Comput. Chem.* **2009**, *30*, 1545−1614.
(19) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys.* **1983**, *79*, 926−935.
(20) Ewald, P. The Berechnung optischer und elektrostatischer Gitterpotentiale. *Ann. Phys.* **1921**, *369*, 253−287.
(21) Humphrey, W.; Dalke, A.; Schulten, K. VMD: Visual molecular dynamics. *J. Mol. Graph.* **1996**, *14*, 33−38.
(22) Team, R. *A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2008.
(23) Pokorná, J.; Machala, L.; Řezáčová, P.; Konvalinka, J. Current and novel inhibitors of HIV protease. *Viruses* **2009**, *1*, 1209−1239.
(24) Weisel, M.; Proschak, E.; Schneider, G. PocketPicker: Analysis of ligand binding-sites with shape descriptors. *Chem. Cent. J.* **2007**, *1*, 7.
(25) Lele, L.; Richtsmeier, J. T. *An Invariant Approach to the Statistical Analysis of Shapes*; Chapman & Hall/CRC Press: London, 2001.
(26) Löwer, M.; Geppert, T.; Schneider, P.; Hoy, B.; Wessler, S.; Schneider, G. Inhibitors of *Helicobacter pylori* protease HtrA found by 'virtual ligand' screening combat bacterial invasion of epithelia. *PloS One* **2011**, *6*, e17986.
(27) Tanrikulu, Y.; Nietert, M.; Scheffer, U.; Proschak, E.; Grabowski, K.; Schneider, P.; Weidlich, M.; Karas, M.; Göbel, M.; Schneider, G. Scaffold hopping by "fuzzy" pharmacophores and its application to RNA targets. *ChemBioChem* **2007**, *8*, 1932−1936.
(28) Renner, S.; Schwab, C. H.; Gasteiger, J.; Schneider, G. Impact of conformational flexibility on three-dimensional similarity searching using correlation vectors. *J. Chem. Inf. Model.* **2006**, *46*, 2324−2332.
(29) Klenner, A.; Hartenfeller, M.; Schneider, P.; Schneider, G. 'Fuzziness' in pharmacophore-based virtual screening and de novo design. *Drug Discovery Today Technol.* **2010**, *7*, e237−e244.
(30) Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. Development and validation of a genetic algorithm for flexible docking. *J. Mol. Biol.* **1997**, *267*, 727−748.
(31) Hopkins, A. L.; Groom, C. R.; Alex, A. Ligand efficiency: A useful metric for lead selection. *Drug Discovery Today* **2004**, *9*, 430−431.
(32) Yonetani, T.; Theorell, H. Studies on liver alcohol hydrogenase complexes. 3. Multiple inhibition kinetics in the presence of two competitive inhibitors. *Arch. Biochem. Biophys.* **1964**, *106*, 243−251.
(33) Yonetani, T. The Yonetani-Theorell graphical method for examining overlapping subsites of enzyme active centers. *Methods Enzymol.* **1982**, *87*, 500−509.
(34) Perryman, A. L.; Zhang, Q.; Soutter, H. H.; Rosenfeld, R.; McRee, D. E.; Olson, A. J.; Elder, J. E.; Stout, C. D. Fragment-based screen against HIV protease. *Chem. Biol. Drug Des.* **2010**, *75*, 257−268.
(35) Chang, M. W.; Giffin, M. J.; Muller, R.; Savage, J.; Lin, Y. C.; Hong, S.; Jin, W.; Whitby, L. R.; Elder, J. H.; Boger, D. L.; Torbett, B. E. Identification of broad-based HIV-1 protease inhibitors from combinatorial libraries. *Biochem. J.* **2010**, *429*, 527−532.
(36) Nalam, M. N.; Ali, A.; Reddy, G. S.; Cao, H.; Anjum, S. G.; Altman, M. D.; Yilmaz, N. K.; Tidor, B.; Rana, T. M.; Schiffer, C. A. Substrate envelope-designed potent HIV-1 protease inhibitors to avoid drug resistance. *Chem. Biol.* **2013**, *20*, 1116−1124.
(37) Grienke, U.; Schmidtke, M.; Kirchmair, J.; Pfarr, K.; Wutzler, P.; Dürrwald, R.; Wolber, G.; Liedl, K. R.; Stuppner, H.; Rollinger, J. M. Antiviral potential and molecular insight into neuraminidase inhibiting diarylheptanoids from *Alpinia katsumadai*. *J. Med. Chem.* **2010**, *53*, 778−786.
(38) Heh, C. H.; Othman, R.; Buckle, M. J.; Sharifuddin, Y.; Yusof, R.; Rahman, N. A. Rational discovery of dengue type 2 non-competitive inhibitors. *Chem. Biol. Drug Des.* **2013**, *82*, 1−11.
(39) Smoot, M. E.; Ono, K.; Ruscheinski, J.; Wang, P. L.; T. Ideker, T. T. Cytoscape 2.8: New features for data integration and network visualization. *Bioinformatics* **2011**, *27*, 431−432.