Impact of Scoring Functions on Enrichment in Docking-Based Virtual Screening: An Application Study on Renin Inhibitors†

Eva M. Krovat‡ and Thierry Langer*

Institute of Pharmacy, Department of Pharmaceutical Chemistry, Innrain 52a, University of Innsbruck, A-6020 Innsbruck, Austria

Received November 21, 2003

The docking program LigandFit/Cerius[2] has been used to perform shape-based virtual screening of databases against the aspartic protease renin, a target of determined three-dimensional structure. The protein structure was used in the induced fit binding conformation that occurs when renin is bound to the highly active renin inhibitor **1** ($IC_{50} = 2$ nM). The scoring was calculated using several different scoring functions in order to get insight into the predictability of the magnitude of binding interactions. A database of 1000 diverse and druglike compounds, comprised of 990 members of a virtual database generated by using the iLib diverse software and 10 known active renin inhibitors, was docked flexibly and scored to determine appropriate scoring functions. All seven scoring functions used (LigScore1, LigScore2, PLP1, PLP2, JAIN, PMF, LUDI) were able to retrieve at least 50% of the active compounds within the first 20% (200 molecules) of the entire test database. A hit rate of 90% in the top 1.4% resulted using the quadruple consensus scoring of LigScore2, PLP1, PLP2, and JAIN. Additionally, a focused database was created with the iLib diverse software and used for the same procedure as the test database. Docking and scoring of the 990 focused compounds and the 10 known actives were performed. A hit rate of 100% in the top 8.4% resulted with use of the triple consensus scoring of PLP1, PLP2, and PMF. As expected, a ranking of the known active compounds within the focused database compared to the test database was observed. Adequate virtual screening conditions were derived empirically. They can be used for proximate docking and scoring application of compounds with putative renin inhibiting potency.

INTRODUCTION

With respect to the rate-limiting function of renin concentration in the RAAS and the high specificity of renin for its substrate angiotensinogen, renin inhibition can provide a promising strategy in hypertension therapy. In this study we present an approach of flexible docking of molecules in order to find new potential leads for the inhibition of the aspartic protease renin using the docking program LigandFit within the software package Cerius[2].[1] The aim of this study was to investigate the enrichment that is provided by each of the different scoring functions for the virtual screening effort by automatic docking of flexible ligands into the protein's active site. Ligand docking is a method in which the conformational selection of compounds is of high impact for the feasibility of the proposed binding poses. Therefore we applied flexible docking on the selection of available chemical structures with proven inhibitory effect on renin.[2,3] Virtual screening of large databases is a useful method in rational lead discovery. A ranking of those compounds with a good chance for high interaction energy with the protein can be of interest concerning the prioritization of subsequent biological testing. Since the docking and scoring runs are performed in an accurate way, the success of the pharmacological implementation of the computational screening approaches increases. The high cost for experimental testing is a motivation for the theoretical approach of high-throughput docking. The main challenge in this field of drug discovery is to cut off binding poses from nonbinding poses. A pose is the conformation and the orientation of a ligand in the binding pocket of the target protein. More accurate force fields as well as ligand and receptor flexibility calculation can optimize the results but, however, increase the computational cost (time). A useful method is to compute interaction energies at grid points instead of using the complete force field interactions. This approximation and interpolation of ligand−protein interactions performs in a reasonable time frame.

DOCKING METHODS

Docking protocols can be understood as a two-part procedure: a search strategy and a subsequent scoring function. The algorithm used in the search process should generate a broad coverage of conformational space of each molecule including the experimentally determined binding poses. A systematic approach would definitively find the poses of interest but affords a high computational expense. Monte Carlo based methods of conformational search allow an exhaustive coverage of the conformational space in reasonable time. Therefore, energetic constraints are used in the generation of conformers to search only in the favorable conformational space to enhance the chance of retrieving local or global minima. An approximation in the early docking methodology was the use of ligand and the protein as rigid structures, exploring only 6° of translational and rotational freedom. A more common approach is to

* Corresponding author phone: +43 512 507 5252; Fax: +43 512 507 5269; e-mail: thierry.langer@uibk.ac.at.

† Abbreviations: MW, molecular weight; RAAS, Renin-Angiotensin-Aldosteron System; RMS, root-mean-square deviation.

‡ Phone: +43 512 507 5264; e-mail: eva.krovat@uibk.ac.at.

10.1021/ci0342728 CCC: $27.50
Published on Web 04/07/2004

consider the flexibility of the ligand but to treat the receptor as rigid.[4] The LigandFit program gives the user the opportunity to perform both rigid or flexible docking, referring to the conformational state of the ligand. The protein is always considered as rigid. LigandFit applies an approach that submits rapid docking of small molecules into protein active sites by considering shape complementarity between the ligand and the protein active site. Generally there are two steps implemented in the LigandFit process:

First, the binding site locations are defined by a shape-based search to find accurate cavities in the protein. The algorithm for cavity detection calculates a rectangular grid enclosing the protein, cavity regions, and bulky water around the complex. The protein is mapped on a grid. All grid points occupied by the protein are not available in the site search. The unoccupied grid points inside the protein are potential binding sites. Every grid point outside the protein is a free point. Cavities will be included in the free points. The free grid points will subsequently be separated in site points that connect the protein and those regions lying outside. This challenge is performed by a cubical-shaped eraser that removes free grid points and stops whenever it comes in contact with the protein atom. The size of the eraser corresponds to the extent of the removed free points. Additionally, a site can be detected in a ligand-based search and enlarged or scaled down automatically or manually.

Second, the docking procedures are composed of several substeps: (i) conformational searching for flexible ligands, (ii) pose selection based on shape similarity between site and the ligand conformation, and (iii) grid-based energy calculation to compute the interaction energy between ligand and protein.

(i) The generation of ligand conformations is a randomized process to reduce the chance of getting trapped in local shape minima. In the docking procedure, ligand poses, which are energetically favorable for protein interactions, are calculated. (ii) After the site search and the conformational search have been executed, ligand fitting is performed by a shape comparison between the ligand conformation and the binding site. Accepted candidates for docking include a low shape discrepancy and are further used in docking and the calculation of the interaction energy. In the docking of the ligand into the binding site four axes are considered. The dock energy is composed of two terms, namely, the internal energy of the ligand and the interaction energy, summarized by van der Waals and electrostatic energy terms. (iii) To improve the time-consuming computation of the interaction energy, an approximation by grid-based interpolation was employed. A grid encloses the site, and at each point of the grid the potentials are computed for a given protein and a detected active site. The potentials at the ligand atom locations are subsequently interpolated. The docking functions are not used in ranking ligands corresponding to the expected binding affinities.

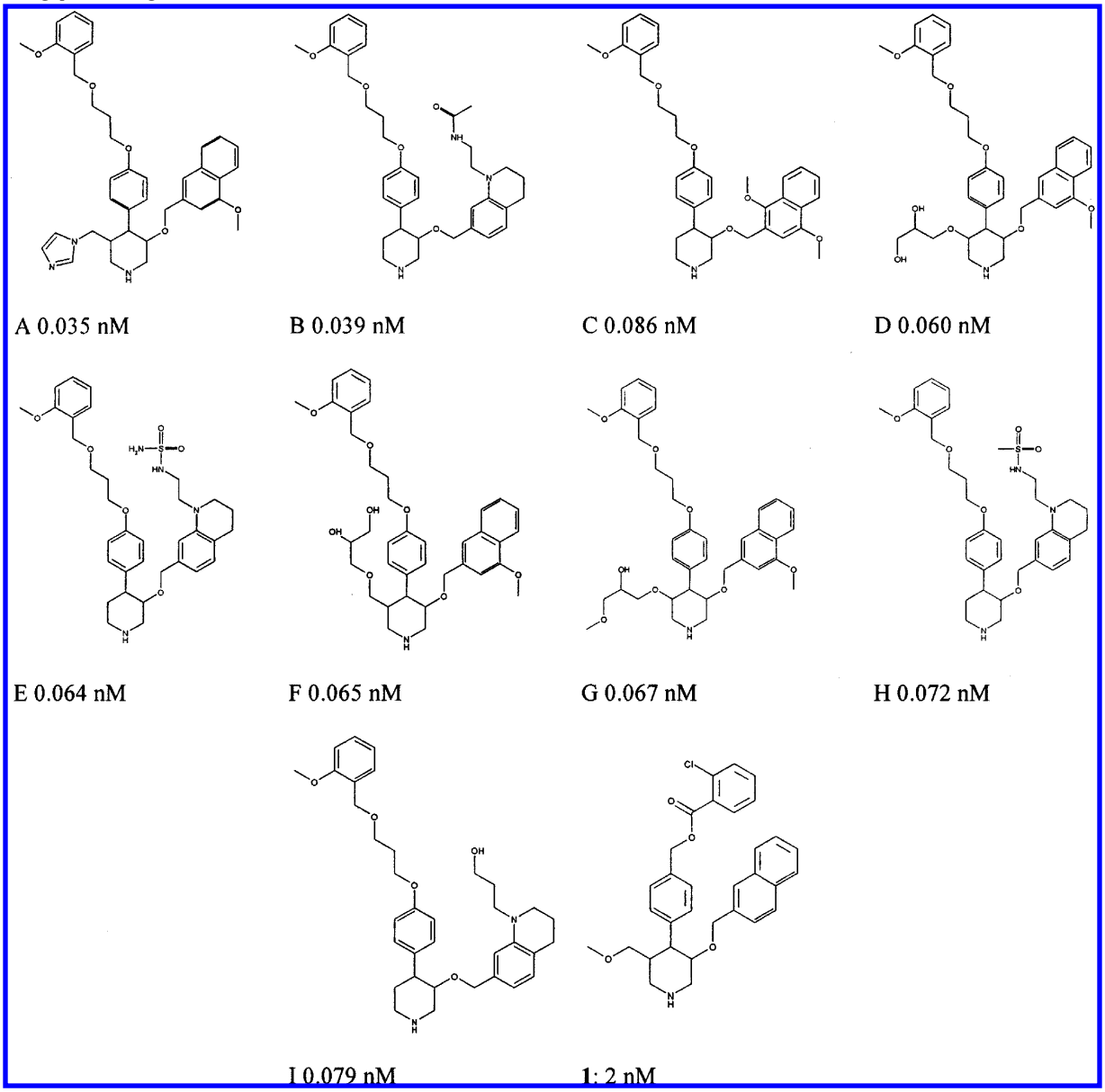
The primary goal of scoring is to outline the border of binders against nonbinders. The prediction of the ranking of ligands corresponding to their activity is of lower interest. Since this is the main importance in result interpretation, scoring functions are implemented in the LigandFit module, e.g. LigScore, Ludi, PLP, PMF, and JAIN. Consensus scoring means the combination of multiple scoring functions. This method may dramatically reduce the number of false positives identified by distinct scoring functions. In the present study, the consensus score was used as an indicator for compounds that likely occur in the top 20% of the scored candidates. The detailed description of used algorithms is presented in a recently published paper by Venkatachalam et al.[5]

STRATEGY IN LIGAND DOCKING

As outlined before, we were interested in determining under which experimental conditions the LigandFit program would be able to retrieve 10 known active renin inhibitors within a database of 1000 diverse and druglike compounds by attributing top ranking scores to these molecules. The following questions should be answered within this study: Which scoring functions should be used to rank potential hits? How can consensus scoring improve the hit rate? What virtually generated structures are ranked close to known highly active renin inhibitors? This concept was organized into two main steps: (i) a 1000 compound test library, represented by 10 active renin inhibitors and 990 randomly generated molecules, was used to determine the scoring functions for reasonable ligand scoring. (ii) Another 1000 compound database, termed a focused library, was used to compare docking and scoring results with the test database and to suggest potential de novo hits. The sd files of the test and focused database are available on request by the authors. The size of the databases was set to 1000 compounds for each of the libraries to keep the computational cost (time) low. Recently published docking studies were accomplished by means of similar settings considering database size.[5] For the screening of large-scale databases the usage of the pharmacophore approach which provides a rapid and efficient tool for virtual high-throughput screening is more favorable. Pharmacophore-based in silico screening applications are reviewed in the literature.[6,7]

First, we tried to optimize the experimental conditions within the docking process. Flexible docking of the X-ray ligand resulted in conformations close to the definite binding conformation. The site definition was confirmed by this procedure. Then the test library, containing 10 renin inhibitors (activity values range from 0.035 to 2 nM IC_{50}) and 990 compounds generated within the combinatorial library generation software program iLib diverse, was implemented to flexible docking.[8−10] The aim of this step was to optimize the scoring explicitly for a differentiation between active and inactive ligands, rather than for the correct prediction of binding affinities. We cannot guarantee that all 990 compounds are inactive; however, they were generated totally by random without focusing on functional groups and features necessary for renin inhibition. We therefore hypothesize that no highly potent (sub-nanomolar affinity) compounds were present in the 990 members of the test library. The probability that compounds comprising activity in the picomolar range are included in the 990 compounds of the test and focused database is very low. To define the clear borderline between active and inactive renin inhibitors, only highly active compounds were chosen for the docking operation (activity values range from 0.035 to 2 nM IC_{50}). The challenge was to verify a separation of actives from nonactives, using distinct scoring functions. We identified the optimal scoring functions for our active compound set.

Chart 1. Chemical Structures and Activity Data (IC_{50} Values) of the Ten Renin Inhibitors Included in the Test Library and Focused Library Used for High-Throughput Docking[3,9]



Finally, to obtain new ideas for the design of possible renin inhibitors, the focused database containing the same 10 active renin inhibitors and 990 compounds with high topological similarity to the known actives was docked and scored within LigandFit. Highly ranked molecules of this focused library may serve as suggestions for de novo derived new lead candidates.

INPUT DATA AND PARAMETER SETTINGS

Preparation of the Protein. Reference protein coordinates for docking were taken from the X-ray structure of renin in complex with the non-peptide compound **1** (IC_{50} = 2 nM; resolution: 2.9 Å; structure depicted in Chart 1),[9] kindly provided by Dr. H.-P. Märki, (Roche Ltd.). The protein/ligand complex was split into two separate models. All solvent molecules were removed and hydrogen atoms were added using Cerius[2] templates for protein residues. The bond order and the number of hydrogen atoms of the ligand were adjusted and saved. Charges were assigned to all molecules in the protein and the docked ligands using the cff1.02 force field.

Preparation and Definition of the Active Site. The site search was performed in the shape-based mode. The site is a collection of grid points. The largest site covered the X-ray ligand conformation and was verified by the location of the redocked X-ray ligand. The majority of the proteins can be treated with an eraser size of 5−6 Å as reported in a quantitative investigation of protein-ligand complexes.[5] Hence, we did not change the default values of site search preferences (grid resolution, 0.5 Å; opening size of the site, 5 Å). A manual refinement was used to complete the site definition, which consisted of 3794 grid points. The ligand present in the original X-ray coordinate file was redocked flexibly within the defined site to the crystal structure of the enzyme renin to ensure the accuracy of the site model. Diverse conformations were computed using the Monte Carlo algorithm within Cerius[2]. The parameter of maximum saved conformers was set to N_{save} = 20, and the flexible fitting resulted in 16 poses corresponding to 16 diverse conformations and orientations of the ligand. The best alignment of X-ray conformation and the flexible fitted ligand pose yielded

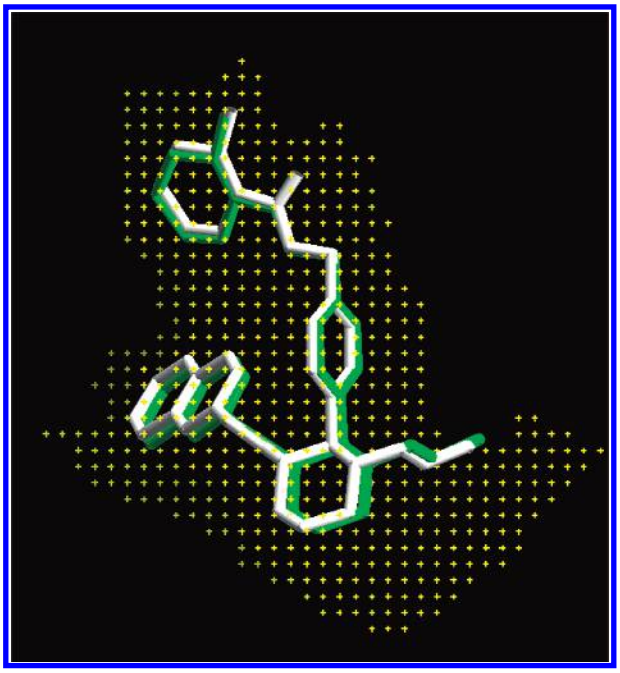


Figure 1. Flexible docking, yielding an RMS value of 0.291 Å between the starting X-ray conformation of renin inhibitor **1** (green) and the docked pose (white) presented in the grid of the protein's active site (yellow).

an RMS deviation of 0.291 (no hydrogen atoms, in angstroms) as shown in Figure 1.

Preparation of Database Molecules. For all ligand structures partial charges were automatically assigned using the Gasteiger calculation implemented in the Cerius[2] docking process. The general protonation state of the database molecules was the same as that for the active inhibitors used for the docking experiment previously described; i.e., compounds were all used in the noncharged form. The setup conditions during flexible ligand fitting were changed to the following settings: $N_{\mathrm{MaxTrials}} = 5000$; $N_{\mathrm{save}} = 20$; CFF force field; grid resolution, 0.5 Å. Docked conformations were clustered using a Leader algorithm in Cerius[2] with an RMS threshold of 1.5 Å. Two data sets were flexibly docked in this study into the protein's active site. First, flexible docking of the test library containing 1000 compounds, enclosing 990 compounds derived from a virtual combinatorial library and 10 known highly active renin inhibitors, was executed. The 990 compounds were randomly generated within the software package iLib diverse[8] and were characterized by a MW between 450 and 600. Data from the 10 renin inhibitors was taken from the literature.[3,9] One of the 10 molecules was the X-ray ligand **1** ($IC_{50} = 2$ nM)[9] complexed with renin previously used in the redocking process. Only the X-ray compound was used in the determined X-ray conformation, whereas for the nine other structures a minimized conformational pose was used. All nine compounds show IC_{50} values in the sub-nanomolar range. No information about the binding conformation of these nine compounds is available. Therefore, the interpretation of the docking poses must be neglected for them. Structure and activity data for the known renin inhibitors are given in Chart 1.

Moreover, a 1000 compound database termed as focused library was docked. This library consisted of 990 virtual compounds, derived from the iLib diverse software package with topological characteristics similar to the active renin inhibitors. This software package has previously been shown to generate highly diverse and druglike compounds starting from a well-balanced set of molecular fragments.[8] For the present experiment, only 33 fragments were selected (a complete list of fragments is available upon request by the authors), and compounds were forced to exhibit at least one hydrogen bond acceptor, one hydrogen bond donor, and one positive ionizable function. Compounds were built from a combination of six fragments; the molecular weight constraint was set to 400−650. Structural diversity was assessed by calculating a set of topological and thermodynamical descriptors and performing PCA. To visualize the results, the first two principal component scores derived are plotted in Figure 2. This library should constitute an idea pool for de novo generated ligands of the aspartic protease renin. The library design concerned the approach of privileged motifs which were part of the biologically active molecules used in this study. The core structures and more fragments occur in the database in a certain user-defined extent. The trend to move from huge random combinatorial databases toward smaller and focused druglike subsets is reviewed in a recent article.[11]

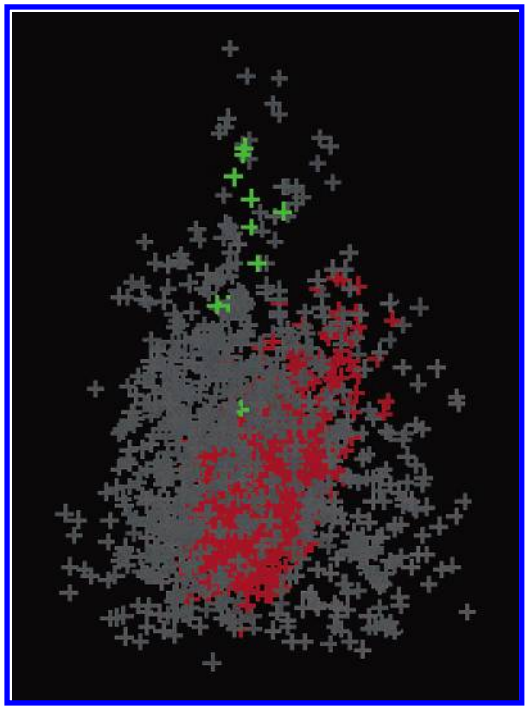


Figure 2. Diversity analysis of database compounds by the PCA of molecular descriptors: Scores of first versus second principal components (gray, test library; red, focused library; green, active renin inhibitors).

RESULTS AND DISCUSSION

Docking of the Test Library. The 1000 compounds were saved as a minimized single conformer sd file data format and subsequently docked into the binding site previously defined by the shape of the protein. This resulted in 998 docked molecules and 14 916 different poses; only two compounds could not be docked by the program. The ranking of the active compounds within the 998 molecules corresponding to diverse scoring functions was then investigated. Each scoring function was considered separately. Only the highest ranked conformation of each compound was recognized in Figure 3.

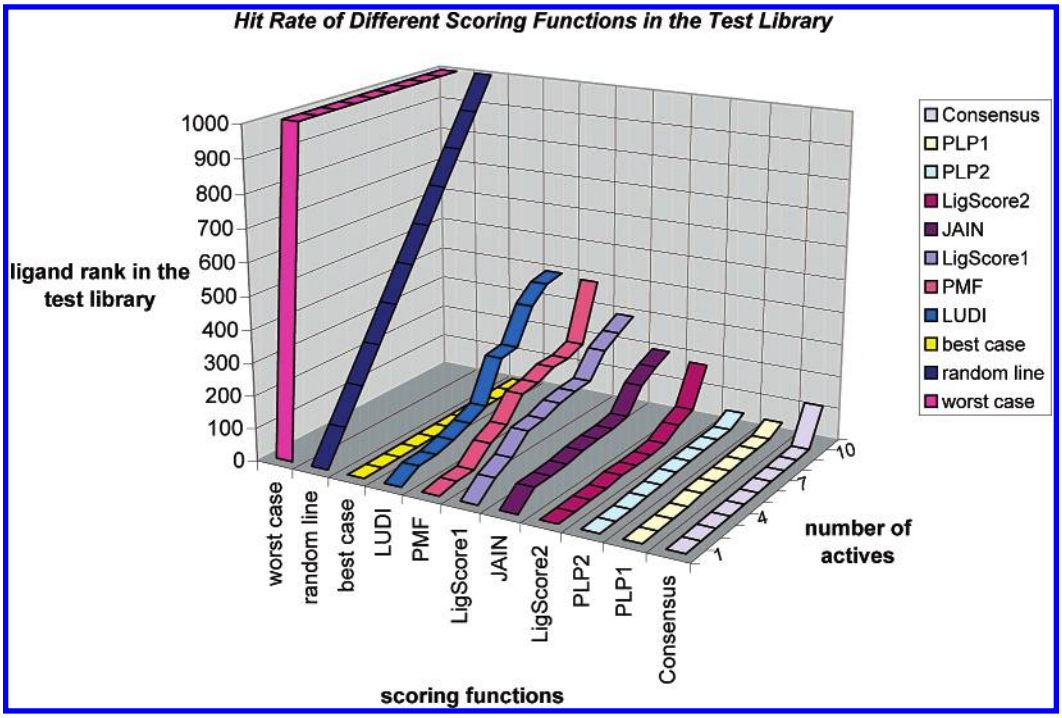


Figure 3. Number of actives plotted against ligand rank corresponding to distinct scoring functions in the test library.

The number of actives plotted against the ligand rank corresponding to the seven scoring functions LigScore1, LigScore2, PLP1, PLP2, PMF, JAIN, and LUDI are shown in Figure 3. Only those scoring functions that were able to retrieve all 10 renin inhibitors within the first 20% (200 molecules) of the test library were considered useful in further evaluation of potential candidates for renin inhibition. This request was fulfilled by four of the seven scoring functions: LigScore2, PLP1, PLP2, and JAIN. A hit rate of 100% in the top 20% scorers was achieved by these four scoring functions (Figure 3). The RMS deviation between the X-ray ligand and the created pose of the flexibly docked X-ray ligand was found to be 0.291, depicted in Figure 1. The remaining three scoring functions LigScore1, PMF, and LUDI performed comparibly well. They identified at least 50% of the active compounds in the top 20% scorers of the entire database (Figure 3). In the next step, consensus scoring was performed with the four previously selected scoring functions. A value for consensus scoring of 4 was achieved for 14 compounds. Nine of the 10 renin inhibitors were included in these 14 top compounds by considering the consensus score. A hit rate of 90% in the top 1.4% resulted with use of the quadruple consensus scoring of LigScore2, PLP1, PLP2, and JAIN. The top 20% of each selected scoring function was included in the consensus, and only the best conformer for each score was considered. This enrichment is presented in Figure 3.

Docking of the Focused Library. The second flexible docking run was performed by employing the focused library, derived from a virtual library created within iLib diverse. The aim of using a focused library is the enrichment of the virtual screening effort. In other words, more active compounds will be revealed in a focused than in a random library. The position of the 10 renin inhibitors in the entire focused library is depicted in Figure 4.

The PLP1, PLP2, and PMF scoring functions were used for a triple consensus scoring considering the best conformer of the top 20% of each selected scoring function. A set of 84 compounds of the focused library was characterized by a consensus score value of 3, which means that these 84 compounds are ranked in the top 20% of all three scoring functions PLP1, PLP2, and PMF. All 10 known actives were included in the 84 compounds showing the highest consensus score value of 3. A hit rate of 100% in the top 8.4% resulted with use of the triple consensus scoring of PLP1, PLP2, and PMF. Four diverse compounds ranked as part of the top 84 compounds with a consensus score value of 3 are presented as potential new structural scaffolds to inhibit renin (Figure 5). Furthermore, these four example structures satisfy the demands of a chemical feature-based pharmacophore model derived from the X-ray data of the renin/compound **1** complex. (unpublished data, not shown) This three-dimensional hypothesis was characterized by five chemical functions (four hydrophobic features; one hydrogen bond donor or positive ionizable feature) and several excluded volume spheres, which present the surrounding atoms in the binding pocket of the X-ray complex.

In docking of the test library and the focused library, the PLP1 and PLP2 scoring functions provide good scoring results with respect to the enrichment of the libraries. In the test library, only 39 compounds (3.9%) instead of 1000 compounds need to be screened in order to detect all included active compounds when PLP1 is regarded. In the focused library 158 (15.8%) compounds need to be screened to retrieve all actives when the PLP1 scoring function is considered. A suggestion for the selection of scoring functions in further docking studies for renin inhibitors in the described protein's active site could be the use of PLP1 or PLP2 considering the data obtained in this study. The good performance of the PLP scoring functions may be explained by the structural homology and similarities of the viral (HIV-1 protease) and mammalian aspartic proteases (renin): The HIV-1 protease complex had been used among other complexes in the development of the molecular

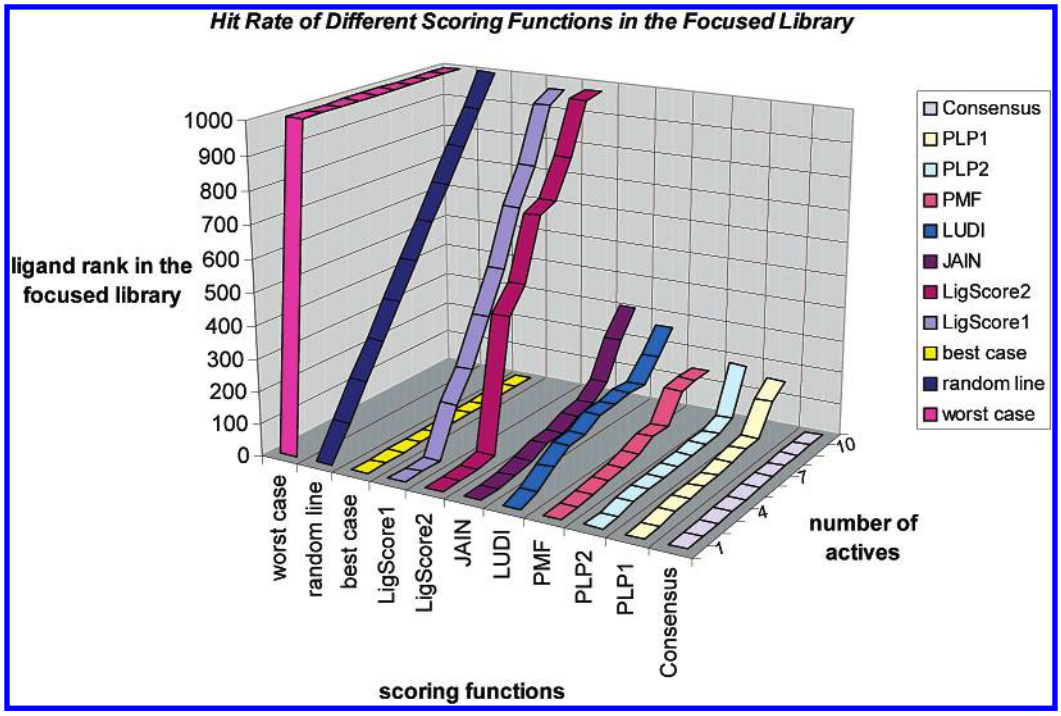


Figure 4. Number of actives plotted against ligand rank corresponding to distinct scoring functions in the focused library.

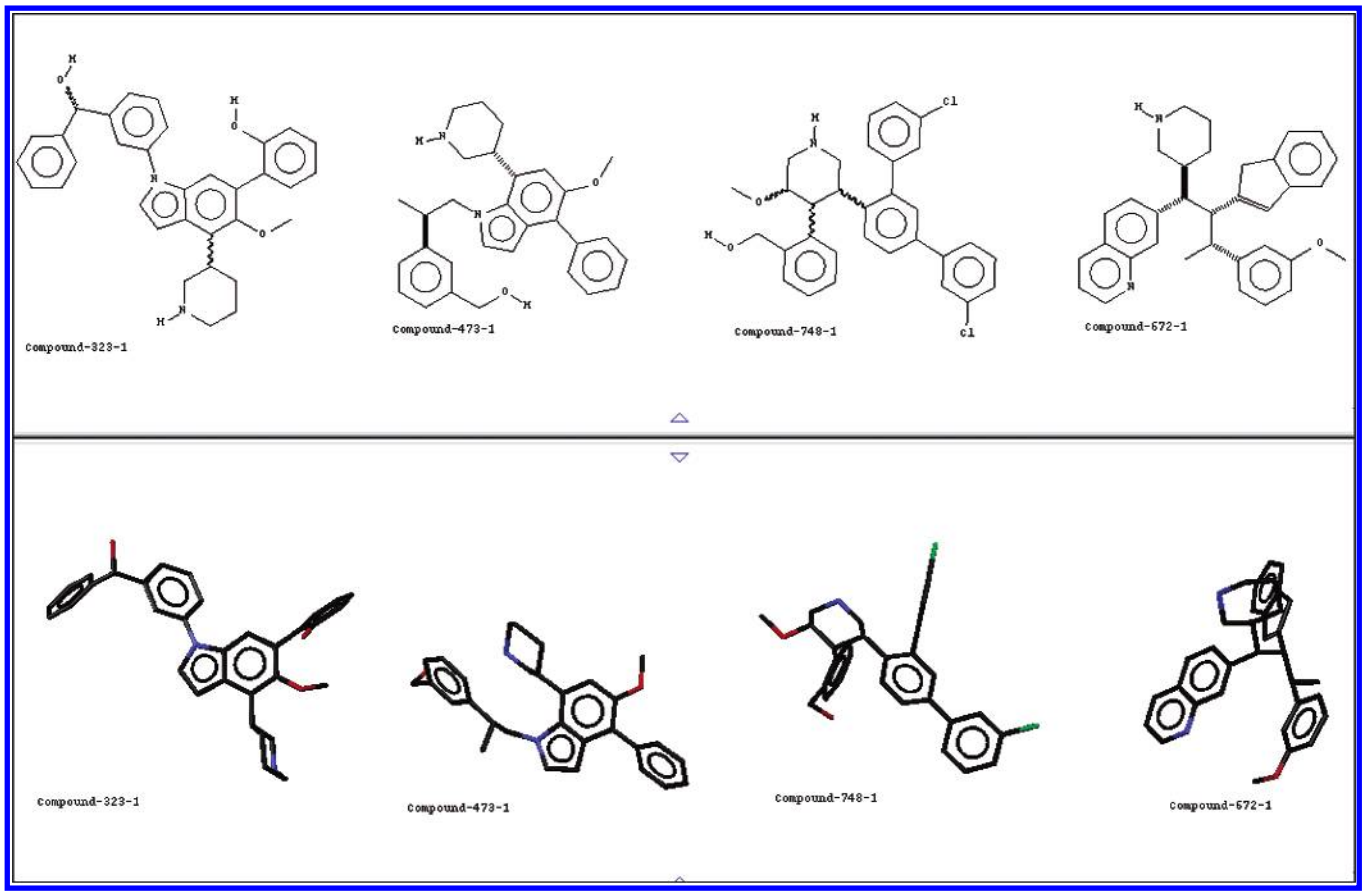


Figure 5. Four examples of virtually generated compounds which were characterized by a high rank in consensus scoring and by the pharmacophoric pattern yielded in a structure-based pharmacophore modeling approach.

recognition model and the evolutionary programming of the PLP scoring functions.[12]

CONCLUSION

For the target described in this study, the aspartic protease renin, flexible docking of two data sets plus subsequent scoring was performed. Two 1000 compound libraries were fitted into the protein's active site. Moreover, the most suitable scoring functions were selected to discriminate active from inactive compounds. The scoring function which detected all 10 active renin inhibitors within the top 20% of the test and focused database, respectively, were used in

consensus scoring. Five of the seven scoring functions implemented in Cerius[2] presented themselves as applicable for detection of the renin inhibitors within the two libraries. They can further be used in scoring potential hit candidates for biological testing. A hit rate of 100% was achieved in the top 20% scorers of the test library, when only the best pose of each scoring function (LigScore2, PLP1, PLP2, JAIN) was included. Consensus scoring improved the hit rate to 100% in the top 11.6% of the entire test library. A hit rate of 100% in the top 8.4% scorers resulted using the triple consensus scoring of PLP1, PLP2, and PMF in the focused library. These results are applicable for compounds with the same or a similar mode of interaction as the 10 known renin inhibitors. The implementations of the scoring functions mentioned earlier in the consensus scoring of test and focused library, (especially PLP1 and PLP2) can give a basis for further docking studies in this protein's active site. The docking was performed regarding the conformation of the non-peptide inhibitor bound to the protein (Figure 1). This is not eventually a stable conformation as other X-ray conformations of renin with several inhibitors show.[13,14] Some virtually generated structures are presented to highlight their structural diversity in comparison to the known renin inhibitors. The piperidine moiety was included in every structure of the focused library, because the conformational change of the protein may be based on the presence of this structure. The reported examples of Figure 4 can give some ideas for new compounds acting as renin inhibitors.

EXPERIMENTAL SECTION

All molecular modeling studies were performed using Cerius[2] software package 4.8[1] installed on a Silicon Graphic Octane desktop workstation equipped with two 175 MHz MIPS R10000 processors (512 MB RAM) running the Irix 6.5 operating system. All 2D chemical structures were produced within the ISIS/Draw2.1 drawing program.[15]

ACKNOWLEDGMENT

We thank Dr. H.-P. Märki (Roche Ltd.) for providing us X-ray data and Dr. C. M. Venkatachalam (Accelrys Inc.) for support considering LigandFit parameters.

REFERENCES AND NOTES

(1) *Cerius[2]*, Version 4.8; Accelrys Inc.: San Diego, CA, 2003, http://www.accelrys.com.

(2) Güller, R.; Binggeli, A.; Breu, V.; Bur, D.; Fischli, W.; Hirth, G.; Jenny, C.; Kansy, M.; Montavon, F.; Müller, M.; Oefner, C.; Stadler, H.; Vieira, E.; Wilhelm, M.; Wostl, W.; Märki, H. P. Piperidine-Renin Inhibitors Compounds with Improved Physicochemical Properties. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1403−1408.

(3) Märki, H. P.; Binggeli, A.; Bittner, B.; Bohner-Lang, V.; Breu, V.; Bur, D.; Coassolo, P.; Clozel, J. P.; D'Arcy, A.; Doebeli, H.; Fischli, W.; Funk, C.; Foricher, J.; Giller, T.; Grüninger, F.; Guenzi, A.; Güller, R.; Hartung, T.; Hirth, G.; Jenny, C.; Kansy, M.; Klinkhammer, U.; Lave, T.; Lohri, B.; Luft, F. C.; Mervaala, E. M.; Müller, D. M.; Müller, M.; Montavon, F.; Oefner, C.; Qui, C.; Reichel, A.; Sanwald-Ducray, P.; Scalone, M.; Schleimer, M.; Schmid, R.; Stadler, H.; Treiber, A.; Valendaire, O.; Viera, E.; Waldmeier, P.; Wiegand-Chou, R.; Wilhelm, M.; Wostl, W.; Zell, M.; Zell. R. Piperidine Renin Inhibitors: from Leads to Drug Candidates. *Farmaco* **2001**, *56*, 21−27.

(4) Taylor, R. D.; Jewsbury, P. J.; Essex, J. W. A Review of Protein-Small Molecule Docking Methods. *J. Comput.-Aided Mol. Des.* **2002**, *16*, 151−166.

(5) Venkatachalam, C. M.; Jiang, X.; Oldfield, T.; Waldman, M. LigandFit: A Novel Method for the Shape-Directed Rapid Docking of Ligands to Protein Active Sites. *J. Mol. Graphics Modell.* **2003**, *21*, 289−307.

(6) Kurogi, Y.; Güner, O. F. Pharmacophore Modeling and Three-Dimensional Database Searching for Drug Design Using Catalyst. *Curr. Med. Chem.* **2001**, *8*, 1035−1055.

(7) Langer, T.; Krovat, E. M. Chemical Feature-Based Pharmacophores and Virtual Library Screening for Discovery of New Leads. *Curr. Opin. Drug Discovery Dev.* **2003**, *6*, 370−376.

(8) ILib diverse is available from Inte:Ligand (www.inteligand.com) and is based on the CombiGen software described in: Wolber, G.; Langer, T. In *Rational Approaches to Drug Design, Proceedings of the 13th European Symposium on Quantitative Structure−Activity Relationships*, Hoeltje, H. D., Sippl, W., Eds.; Prous Science: Duesseldorf, Germany, **2000**; pp 390−399.

(9) Oefner, C.; Binggeli, A.; Breu, V.; Bur, D.; Clozel, J.; D'Arcy, A.; Dorn, A.; Fischli, W.; Grüninger, F.; Güller, R.; Hirth, G.; Märki, H. P.; Mathews, S.; Müller, M.; Ridley, R. G.; Stadler, H.; Viera, E.; Wilhelm, M.; Winkler, F. K.; Wostl, W. Renin Inhibition by Substituted Piperidines: a Novel Paradigm for the Inhibition of Monomeric Aspartic Proteinases? *Chem. Biol.* **1999**, *6*, 127−131.

(10) Vieira, E.; Binggeli, A.; Breu, V.; Bur, D.; Fischli, W.; Güller, R.; Hirth, G.; Märki, H. P.; Müller, M.; Oefner, C.; Scalone, M.; Stadler, H.; Wilhelm, M.; Wostl, W. Substituted Piperidines−Highly Potent Renin Inhibitors Due to Induced Fit Adaption of the Active Site. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1397−1402.

(11) Bleicher, K. H.; Böhm, H.-J.; Müller, K.; I., A. A. Hit and Lead Generation: Beyond High-Throughput Screening. *Nat. Rev. Drug Discovery* **2003**, *2*, 369−378.

(12) Gehlhaar, D. K.; Verkhivker, G. M.; Reijto, P. A.; Sherman, C. J.; Fogel, D. B.; Fogel, L. J.; Freer, S. T. Molecular Recognition of the Inhibitor AG-1343 by HIV-1 Protease: Conformational Flexible Docking by Evolutionary Programming. *Chem. Biol.* **1995**, *2*, 317−324.

(13) Tong, L.; Pav, S.; Lamarée, D.; Simoneaou, B.; Lavallèe, P.; Jung, G. Crystallographic Studies on the Binding Modes of P2−P3 Butanediamide Renin Inhibitors. *J. Biol. Chem.* **1995**, *270*, 29520−29524.

(14) Rahuel, J.; Rasetti, V.; Maibaum, J.; Rüeger, H.; Göschke, R.; Cohen, N. C.; Stutz, S.; Cumin, F.; Fuhrer, W.; Wood, J. M.; Grütter, M. G. Structure-Based Drug Design: The Discovery of Novel Nonpeptide Orally Active Inhibitors of Human Renin. *Chem. Biol.* **2000**, *7*, 493−504.

(15) *ISIS/Draw 2.1*; MDL Information Systems, Inc.: 1990−1996, http://www.mdl.com.

CI0342728