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Ligand-Based Virtual Screening Approach Using a New Scoring Function

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Abstract

In this study, we aimed to develop a new ligand-based virtual screening approach using an effective shape-overlapping procedure and a more robust scoring function (denoted by the HWZ score for convenience). The HWZ score-based virtual screening approach was tested against the compounds for 40 protein targets available in the Database of Useful Decoys (DUD) (dud.docking.org/jahn/), and the virtual screening performance was evaluated in terms of the area under the ROC curve (AUC), Enrichment Factor (*EF*), and Hit Rate (*HR*), demonstrating an improved overall performance compared to other popularly used approaches examined. In particular, the HWZ score-based virtual screening led to an average AUC value of 0.84 ± 0.02 (95% confidence interval) for the 40 targets. The average *HR* values at top 1% and 10% of the active compounds for the 40 targets were $46.3\% \pm 6.7\%$ and $59.2\% \pm 4.7\%$, respectively. In addition, the performance of the HWZ score-based virtual screening approach is less sensitive to the choice of the target.

Introduction

Virtual screening approach has been widely used in drug discovery process for lead identification, lead optimization, and scaffold hopping. It provides an inexpensive and fast alternative to high-throughput screening for discovering new drugs.¹ There are two broad categories of computational techniques for virtual screening: (1) Ligand-based drug design (e.g. ligand similarity) and (2) Structure-based drug design (ligand docking) methods. The ligand similarity methods capitalize on the fact that ligands similar to an active ligand are more likely to be active than random ligands, whereas protein-ligand docking attempts to use the three-dimensional (3D) protein structure of the target protein to predict the binding modes and affinities of ligands to the target.

Ligand-based virtual screening methods use the information present in known active ligands rather than the structure of a target protein for both lead identification and optimization. Ligand-based methods are the only chosen when no 3D structure of the target protein is available. This is typically the case, for example, with G-protein-coupled receptor (GPCR) targets² or protein structures resolved in the apo form.³ In practice, without knowing the protein structure for the target of interest, one often knows that a set of ligands are active

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Supporting Information Available. Detailed information about the calibration of the HWZ scoring function. This information is available free of charge *via* the Internet at <http://pubs.acs.org>.

against the target of interest. As such, ligand-based virtual approaches can be employed, i.e. finding new ligands by evaluating the *similarity* between candidate ligands and the known active compounds.⁴ By using a ligand-based method, the known active compounds are collected in order to select a query for virtual screening or alignment in ligand-based design.

A ligand-based computational method involves two essential elements: an efficient similarity measure and a reliable scoring method. In addition, the computational method should be able to screen a large number of potential ligands with reasonable accuracy and speed.

Thus, the similarity measures consist of geometrical information of arbitrary objects defined on the structures. The type of an object varies between different methods and can be classified into three types: pharmacophores, molecular shapes, and molecular fields. Pharmacophore-based methods generate patterns of distances between predefined molecular properties like aromatic systems or hydrogen-bond acceptors/donors⁵ and calculate the similarity value through a comparison of the corresponding patterns. Molecular shape approaches try to maximize the overlap of shapes and determine a similarity value based on the shape overlap. Ballester et al. introduced a non-superposition comparison algorithm for molecular shapes, called Ultrafast Shape Recognition, and applied it to virtual screening experiments.⁶

A scoring method for ligand-based screening should effectively discriminate active compounds from the inactive ones during the ranking phase and can be used to efficiently identify a small number of active compounds from a library including a large number of inactive compounds. See ref.(7) for reviews of molecular similarity methods.

At present, the highly optimized screening platform ROCS (Rapid Overlay of Chemical Structures)⁸ (OpenEye Inc.) is considered as the industry standard for ligand shape-based virtual screening. It uses 3D Gaussian functions to describe the shape of query structures and to screen databases in search of molecular structures with similar shapes.^{8c} It is well-known that ROCS performance is highly dependent on the selection of query molecule.⁹ On the other hand, the current literature suggests that virtual screening performance of ROCS is essentially unaffected by choice of the conformation of a given query compound.^{9–10}

The available range of other shape-based screening tools and algorithms includes the algorithm Cat-Shape as implemented in CATALYST (Accelrys: San Diego),¹¹ the Phase-Shape module as implemented in the Schrödinger software suite (Schrödinger, LLC), PARASHIFT,¹² Hex,¹³ ShaEP,¹⁴ and the USR (ultrafast shape recognition) algorithms.¹⁵ Putta and Beroza provided an excellent review¹⁶ on shape-based screening methods, alignment procedures, and the challenges. However, the performances of these software packages are often inconsistent across different databases and queries.¹⁴ In their recent work, Hawkins et al.¹⁰ pointed out that while docking usually suffers from a high false positive rate (i.e. inactive compounds are sometime identified as active compounds) the problem of shape-based screening is mainly its inferior false negative rate (i.e. a low score is incorrectly assigned to an active compound).¹⁷

Although the field of virtual screening may be maturing,¹⁸ and many accurate methods have been proposed, the promise of the virtual compound library¹⁹ to rapidly increase the number of candidate ligands demands further improvement in terms of the computational efficiency of similarity measure algorithms and the scoring function.

The basic assumption of shape-based similarity screening is that molecules of shape and chemistry comparable to a known active compound (query structure) have a significant probability of also showing activity. Therefore, active ligands with shape differing from the

query structure (which has been known to be active) are likely to be missed during virtual screening. For this purpose, it is necessary to continue the search for novel ligand-based strategy with the ability to identify more potent ligands of a drug target.

Despite many reports of successful applications of the ligand shape-based virtual screening methods, serious issues remain unsolved. For example, the shape (volume)-based screening tools in some cases still cannot reliably identify the active ligands in the early stage of the database screening.²⁰ More importantly, the screening performance measured by the area under the ROC curve (AUC) is for some targets equivalent to the random identification of active ligands from the database.^{14, 20a} Indeed, Vainio *et al.*¹⁴ have compared the performance of the ShaEP and ROCS programs using the 40 targets available in the Database of Useful Decoys (DUD) (<http://dud.docking.org/>)²¹ and found that AUC was below 0.5 (an AUC value representing a failed screening) for 9 targets screened using the ShaEP and 5 targets screened using the ROCS. In most of the cases, the ligand shape (volume)-based virtual screening methods use the Tanimoto scoring function²² or a similar one²³ for ranking ligands, whereas a more sophisticated scoring function has been defined in the ROCS program in which both shape and a color force field are used for optimization of the shape (volume) overlap.²⁴

In this report, we present a novel ligand-based virtual screening approach using an effective and efficient ligand similarity algorithm including an effective shape-overlapping procedure and a more robust scoring function. The novel ligand-based virtual screening approach has been tested against the 40 targets available in the DUD database²¹ in comparison with the performance of existing standard methods reported in literature, demonstrating the effectiveness of the ligand-based virtual screening approach.

Methods

General strategy

Unlike other ligand-based shape-overlapping methods, the main feature of our approach is to first find the finest starting superposition of the candidate structure (B from a database) with the query (A), which might result in an optimal and fast shape-density overlap convergence.

In the first step, a list of chemical (functional) groups, *e.g.* cyclo-hexane, alkyl chain, and halogen substitution, are identified for both the query (named List^A) and each candidate structure (named List^B). Subsequently, if the chemical groups of the List^B are not present in the List^A, the chemical groups will be removed from the candidate structure. If the List^B is entirely distinct from the List^A, the complete candidate structure will be preserved. This process is realized for each candidate structure in the database and a new database is built with “*reduced*” candidate structures (Figure S1 in Sup. Inf.).

Shape-overlapping procedure

The shape overlapping procedure in our algorithm begins by overlapping the center of mass of the *reduced candidate structure* (molecule B) with that of the query structure, and then aligning their principal moments of inertia.

This strategy allows us to explore the whole 3D space with a minimum number of iterations throughout the shape overlapping and optimization procedures and, thus, is expected to produce less false-positive overlaps. Moreover, this approach can significantly reduce the CPU time required for the shape overlapping process. Subsequently, the candidate ligand (molecule B) is replaced by its full structure. Then the molecule B is moved (translation and rotation) as a rigid body to produce a quasi-optimal shape-density overlap V_{AB} with the query structure. Finally, the position and the orientation of molecule B are refined with

respect to the rigid-body translations and rotations of the coordinates using the steepest descent method.²⁵ The optimized pose of molecule B, which should have an optimal shape-density overlap V_{AB} , is then compared to the poses already in a temporary file to determine if the candidate pose should be accepted, and the temporary file is updated accordingly. An efficient algorithm based on quaternions²⁶ is used to compute the rotations of the structure coordinates around the axis of the coordinate system.

Scoring of the shape-overlapped structure

Currently, a major weakness of ligand-based screening approach, i.e. the inability to consistently identify true positives (active compounds), is likely due to the imprecise scoring methods. It has been known that the use of the Tanimoto scoring function²² or a similar one²³ was inadequate for some targets. For example, the recent screening test using the DUD database realized by Vainio et al. and Kirchmair et al. demonstrated that the enrichment factor and AUC metrics were largely dependent on the screened system and even failed for some targets.^{14, 20a}

A crucial issue is the relative scores of a candidate ligand with a size larger than the query ligand and a candidate ligand with a size smaller than the query ligand. To demonstrate the feature of the Tanimoto scoring function, we have illustrated in Figure 1 the concept of density/volume-based shape similarity between two structures (for the query ligand A and candidate ligand B); each is represented by a set of hard atomic spheres. The superimposition of ligands A with B is scored according to the shape-density overlap V_{AB} between the query (ligand A) with volume V_A and testing structure (B) with volume V_B . The commonly occupied volume $V_{A \cap B}$ is normalized by the total volume $V_{A \cup B}$ to arrive at a Tanimoto-like shape similarity:

$$T = \frac{V_{A \cap B}}{V_{A \cup B}} \quad (1a)$$

It is interesting to note the following two possible conditions:

1. When $V_B < V_A$, the volume overlap between ligands A and B is $V_{AB} = V_B - V_e$ (see Figure 1), in which V_e represents the residual volume of ligand B which does not overlap with the query ligand A. Under this condition, Eq (1a) becomes

$$T^{(1)} = \frac{(V_B - V_e)}{[V_A + V_B - (V_B - V_e)]} \quad (1b)$$

Specially, when $V_e = 0$, Eq.(1b) becomes

$$T^{(1)} = V_B / V_A \quad (1c)$$

2. Using the same notations, when $V_B > V_A$ and $V_e = 0$, Eq.(1a) becomes

$$T^{(2)} = \frac{(V_A - V_e)}{[V_A + V_B - (V_A - V_e)]} \quad (1d)$$

Equations (1c) and (1d) clearly reveal that, under these extreme conditions, the ranking of the candidate ligands (B) is determined by two inhomogeneous criteria, i.e. Eqs.(1c) and (1d), according to the Tanimoto scoring function. By using Eqs.(1c) and (1d), one can obtain

the perfect score ($T = 1$) for ligand B when $V_B = V_A$. When $V_B < V_A$ and the volume difference is $x V_A$, we have $V_B = (1 - x) V_A$ and Eq.(1c) gives

$$T^{(1)} = 1 - x. \quad (1e)$$

When $V_B > V_A$ and the volume difference is $x V_A$, we have $V_B = (1 + x) V_A$ and Eq.(1d) gives

$$T^{(2)} = 1 / (1 + x). \quad (1f)$$

Clearly, $T^{(2)}$ in Eq.(1f) is always bigger than $T^{(1)}$ in Eq.(1e) for a given x value, as depicted in Figure 2(A). So, the Tanimoto scoring function always gives the advantage to the candidate ligands whose sizes are larger than the query ligand in comparison with the candidate ligands whose sizes are smaller than the query ligand. In other words, the Tanimoto scoring function might bias to the candidate ligands whose sizes are larger than the query ligand.

We have developed a new scoring approach to solve the scoring function problem. Specifically, the candidate ligands are ranked according to a uniformed scoring function, denoted by Hamza-Wei-Zhan (HWZ) score for convenience:

$$HWZ = \sum_{k=1}^N \left[a_k \left(\frac{V_{AB}}{V_A} \right)^k + b_k \left(\frac{V_{AB}}{V_B} \right)^k + c_k \left(\frac{V_{AB}}{V_A + V_B} \right)^k \right] \quad (2a)$$

in which the coefficients a_k , b_k , and c_k have been calibrated through extensive training and testing against 16 drug targets collected in “The Binding Database” (www.bindingdb.org/bind/index.jsp). See below for the choice of N .

To compare the feature of the HWZ scoring function with that of the Tanimoto scoring function, we also used the aforementioned extreme conditions and notations to analyze the HWZ function. Specifically, when $V_B < V_A$ and $V_e = 0$, Eq.(2a) gives

$$HWZ^{(1)} = \sum_{k=1}^N \left[a_k \left(\frac{V_B}{V_A} \right)^k + b_k \left(\frac{V_B}{V_B} \right)^k + c_k \left(\frac{V_B}{V_A + V_B} \right)^k \right]. \quad (2b)$$

When $V_B > V_A$ and $V_e = 0$, Eq.(2a) gives

$$HWZ^{(2)} = \sum_{k=1}^N \left[a_k \left(\frac{V_A}{V_A} \right)^k + b_k \left(\frac{V_A}{V_B} \right)^k + c_k \left(\frac{V_A}{V_A + V_B} \right)^k \right]. \quad (2c)$$

As depicted in Figure 2(B), the HWZ scoring functions does not significantly bias to the candidate ligands whose sizes are larger than the query ligand when $x < 0.3$. So, it is interesting to examine the performance of this new scoring function in comparison with the Tanimoto (T) scoring function.²²

Calibration of the HWZ scoring function

The empirical parameters (a_k , b_k , and c_k) in the HWZ scoring function represented by Eq. (2a) were determined by iteratively adjusting the parameter values based on the extensive, repeated training and testing tests against 16 targets (listed in Sup. Inf. Table S1) with a total

of 2784 active ligands (see Sup. Inf. Table S2) collected in “The Binding Database” (www.bindingdb.org/bind/index.jsp). The training and testing with each target included all of the active ligands available in the “The Binding Database”. For each target, the available active ligands were randomly partitioned into training set (50%) and testing set (50%). The random partition was repeated for five times, and the average results for the testing were taken. For each time, each of the 50% active ligands in the training set had an opportunity to be used as the query. For the training, the 50% active ligands in the training set were seeded into a decoy set to create a screening database for training. The decoy set consisted of 700 decoys that were selected randomly from the ZINC database (<http://zinc.docking.org/>). Similarly, the rest 50% active ligands in the testing set were also seeded into a decoy set (that consisted of 700 different decoys selected randomly from the ZINC database) to create a screening database for testing.

The screening database for training or testing was conformationally expanded by using OMEGA (OpenEye Scientific Software) with parameters as described before.²⁷ We note that 700 was an arbitrary number for a decoy set. The arbitrary number of 700 decoys representing the structural noise of the database was chosen due to a compromise between the accuracy of the computed parameters and the cumulative computing time. Our additional training and testing indicated that adding more decoys would not significantly change/improve the calibrated scoring function.

For both the training and testing, the ligands were ranked according to the scoring function. The area under the ROC curve (AUC) metric was calculated for each query and the average value, <AUC>, for the active ligands. The optimal parameter values of the HWZ scoring function were reached for the highest possible <AUC> value obtained from the testing on all of the 2784 active ligands for the 16 targets. Technically, we first calibrated the parameters a_k , b_k , and c_k against each of the 16 targets and obtained 16 different sets of parameter values. Then, we took the averages as the initial values to further optimize the parameter values and test the overall performance of the HWZ scoring function against all of the 16 targets.

Molecular shape-density model

As suggested by Grant et al.,^{8a, 28} it is reasonable to approximate the molecular electron density function simply as a superposition of atom-centered Gaussian functions. Thus, the molecular shape-density of a ligand is calculated using the Gaussian function, which uses a representation in which each atom i with coordinates $\mathbf{R}_i = (X_i, Y_i, Z_i)$ is given by a spherical Gaussian:

$$\rho_i(\mathbf{r}) = p_i \exp(-\alpha_i(\mathbf{r} - \mathbf{R}_i)^2) \text{ in which } \alpha_i = \pi \left(\frac{3 p_i}{4 \pi \sigma_i^3} \right)^{2/3} \quad (3)$$

The amplitude is set to $p_i = 2 \sqrt{2}$, and the decay factor α_i is calculated from p_i and σ_i is the van der Waals radius of the atom i .²⁸ The molecular shape-density is the sum of all individual atomic densities. The molecular shape-density is given by

$$V_A = \sum_{i=0}^n \int d\mathbf{r} \rho_i(\mathbf{r}) \quad (4)$$

The shape-density overlap of two molecules A and B is defined as:

$$V_{AB} = \sum_{i \in A} \sum_{j \in B} \int d\mathbf{r} \rho_i(\mathbf{r}) \rho_j(\mathbf{r}) = \sum_{i \in A} \sum_{j \in B} p_i p_j \exp \left(-\frac{\alpha_i \alpha_j d_{ij}^2}{\alpha_i + \alpha_j} \right) \left(\frac{\pi}{\alpha_i + \alpha_j} \right)^{3/2} \quad (5)$$

where i and j refer to atoms in molecules A and B, respectively, and d_{ij} is the distance between atoms i and j . In order to perform the superposition between a pair of molecules, the algorithm translates the molecular shape-density of the moving molecule (B) to that of the query structure (molecule A) and then searches for the rotation that minimizes the “distance” between the corresponding pairs of spherical Gaussian functions.

Validation

The above approach using the HWZ or Tanimoto scoring function has been coded in Fortran90 as a standalone module (denoted by SABRE, or “*Shape-Approach-Based Routines Enhanced*”, for convenience of the use in our lab). The implementation has provided an opportunity for us to calibrate and validate the scoring function, i.e. Eq.(2a). Validation of our approach was based on an appropriate use of the DUD database²⁹ of annotated active compounds and decoys. The DUD is a publicly available virtual screening test database and is the second data set used by Cross et al.³⁰ It consists of 40 pharmaceutically relevant protein targets, each with a set of molecules known to be active against the protein target and many decoys. Currently, the DUD is considered as the industry standard for validation of docking and related protocols, and recently it has also been applied to the evaluation of related structure-based and ligand-based virtual screening methods.^{31,21} The DUD was downloaded from web site (http://dud.docking.org/jahn/DUD_LIB_VS_1.0.tar.gz in sdf file format).²¹

For our virtual screening tests against each target in the DUD database, the ratio of the number of decoys to the number of active ligands was fixed at ~36, i.e. (number of decoys)/(number of active ligands) = ~36, in order to be consistent with the ratio used in the previously published virtual screening tests.

To evaluate the effectiveness of the virtual screening approach, we first generated various molecular orientations and multiple conformations of each ligand (active or decoy) of the 40 targets in the DUD using OMEGA (OpenEye).³² OMEGA sampling is capable of selecting a ligand conformation similar to that of the targeted X-ray crystal structure by using an appropriate option (*default*) including a low-energy cutoff to discard high-energy conformations, a low root-mean-square deviation (RMSD) value below which two conformations are considered to be similar.³³ Atom typing, energy calculations, and geometry optimization in OMEGA were performed using the Merck Molecular Force Field (MMFF). The maximum allowed conformations per compound was set to 200 and the energy window (the value used to discard high-energy conformations) was set to 10 kcal/mol. The default values of the OMEGA program were used for other parameters.

Evaluation of the virtual screening efficiency

For evaluating the effectiveness of a virtual screening of all compounds in a database against a given protein target, a popularly used and the simplest metric method is the enrichment at a given percentage of the database screened.³⁴ The Enrichment Factor (EF) is defined as:

$$EF^{x\%} = \frac{Hits_{selected}^{x\%} / N_{selected}^{x\%}}{Hits_{total} / N_{total}} \quad (6)$$

where $Hits_{selected}^{x\%}$ is the number of hits (active ligands) found at top $x\%$ of the database screened, $N_{selected}^{x\%}$ is the number of compounds screened at top $x\%$ of the database, $Hits_{total}$

is the number of active ligands in entire database, and N_{total} is the number of compounds in entire database. The $EF^{x\%}$ metric relies on cutoffs made at various points through the ranking and so can be sensitive to small changes in ranking. A disadvantage of the EF is its high dependency on the ratio of active compounds of the database screened.³⁵ This obstacle makes the descriptor unsuitable for the direct comparison of performance investigations and screening runs that are based on test sets of different ratios between the active and inactive compounds. To avoid this problem and to appropriately compare the effectiveness of our approach with other well-known ligand-based approaches, we have also compared the obtained actual $EF^{x\%}$ value (denoted by $EF_{actual}^{x\%}$) with the desirable ideal $EF^{x\%}$ value (denoted by $EF_{ideal}^{x\%}$) for each of the 40 targets in the DUD database. This defines another way that portrays the effectiveness at fixed percentages through the database, named the Hit Rate ($HR^{x\%}$) corresponding to the ratio of the known hits:³⁶

$$HR^{x\%} = \frac{EF_{actual}^{x\%}}{EF_{ideal}^{x\%}} * 100 \quad (7)$$

The efficiency metric was computed by using the enrichment factor $EF^{x\%}$ and hit rate $HR^{x\%}$ at 1%, 5%, and 10% for each of the 40 DUD targets.

A variant of the enrichment statistic was developed to avoid the sensitivity of the small changes in ranking. The Receiver Operator Characteristic (ROC) curve approach has been used extensively in the life sciences and social sciences arenas since its inception.³⁷ For a recent application of the ROC curve in virtual screening, see the work of Tribelleau.^{35a} The ROC curve is a graphical plot of the sensitivity (true positive rate) vs 1-specificity (false positive rate). The sensitivity (Se) and specificity (Sp) are defined as

$$Se = \frac{TP}{TP + FN} \quad (8)$$

$$Sp = \frac{TN}{TN + FP} \quad (9)$$

where TP represents the number of correctly identified actives (true positives), TN the number of correctly identified inactives (true negatives), FP the number of inactives incorrectly predicted as actives (false positives), and FN the number of actives incorrectly predicted to be inactive (false negatives).

The area under the ROC curve (AUC) represents another descriptor for virtual screening performance and can be calculated as the sum of all rectangles formed by the Sensitivity (Se) and Specificity ($1 - Sp$) values for different thresholds.^{35a} The AUC of a ROC plot gives an objective measure of query performance which is essentially independent of the actual number of positive and negative instances. For the ideal distributions of actives and decoys, an AUC value of 1 would be obtained; completely random distributions would lead to an AUC value of 0.5. Practical virtual screening workflows that perform better than a random discrimination of the actives and decoys retrieve an AUC value between 0.5 and 1, whereas an AUC value lower than 0.5 represents the unfavorable case of a method that has a higher probability to assign higher scores to decoys than to actives. But a rough guide for classifying the accuracy of a screening test is the traditional academic point system known as: 0.9 AUC 1 is excellent; 0.80 AUC < 0.9 is good; 0.70 AUC < 0.8 is fair; 0.50 AUC < 0.7 is poor; and AUC < 0.5 is a failure.

The numerical results below are reported with the 95% confidence interval.

Results and Discussion

As noted above, the HWZ score has been calibrated against 16 drug targets corresponding to a total of 2784 active ligands and 6960 training tests (see Sup. Inf. Table S2 concerning the individual numbers for each target), and the finally calibrated parameters a_k , b_k , and c_k are listed in Table 1. As noted above, we also calibrated the parameters a_k , b_k , and c_k against each of the 16 targets and obtained 16 different sets of parameter values. It is interesting to note (in Sup. Inf. Table S2) that the obtained 16 sets of parameter values are very close to each other, and there is no difference between the finally calibrated parameter values and the corresponding average values. These data (see in Sup. Inf. Table S2) suggest that the optimal values of the HWZ parameters are nearly independent of the target.

As seen in Table 1, we elected to use $N=3$ in the present study, because the results obtained from using $N=3$ are good enough based on the calibration against the 16 targets. Below, we first compare the performance of our shape-filtering approach using the calibrated HWZ score in comparison with approaches using the Tanimoto score and other popularly used scoring approaches for their applications to the compounds for the same 40 targets in the DUD database. All of the computational results discussed below for the other approaches (the ROCS and ShaEP programs) were obtained from literature cited.

Concerning the practical application of a given ligand-based virtual screening to each target, there were always multiple active compounds available in the DUD database. The virtual screening was carried out for multiple times, as each active compound had an equal opportunity to serve as the query. The numerical results reported below all refer to the average. To examine the effectiveness of the HWZ scoring function, we have analyzed the results by using four different indicators, including three discussed below and an additional one, i.e. the Güner-Henry score (GH score) metrics (provided in Sup. Inf. Figure S2).³⁸

Area under the ROC Curve (AUC) Values

The performance of our shape-filtering approach was first tested using the Tanimoto scoring function on the 40 targets in the DUD database. As seen in Table 2, based on our shape-filtering approach using the Tanimoto score, the obtained average AUC value, denoted by $\langle \text{AUC} \rangle$, for the 40 targets was 0.70 ± 0.03 . For comparison, the $\langle \text{AUC} \rangle$ values reported in literature were 0.73 ± 0.06 ^{20a} and 0.69 ± 0.05 ¹⁴ obtained by using the ROCS program^{8c, 24} and 0.64 ± 0.05 ¹⁴ by using ShaEP program. The ROCS uses the Combo Scoring function (Shape-Tanimoto and Scaled Color score), whereas the ShaEP uses the Hodgkin²³ similarity index. It is interesting to note that the three scoring functions demonstrated the close overall performance in the tests on the 40 targets based on the AUC metric.

Further, based on our shape-filtering approach using the HWZ scoring function, the average AUC value, denoted by $\langle \text{AUC} \rangle$, for the 40 targets was 0.84 ± 0.02 , demonstrating a “good” virtual screening performance. A detailed analysis of the AUC values obtained for individual targets (Table 3) revealed that the AUC value was never below 0.6 when the HWZ score was used for any target, whereas the AUC was below 0.6 for 9 of the 40 targets (~23%) when the Tanimoto score was used (see Figure 3 and Table 2). Overall, when the HWZ score was used, we obtained AUC ≥ 0.8 for 29 targets (72%). In comparison, in the reported use of the ROCS, AUC ≥ 0.8 for only 17 targets (42% targets)^{20a} or 12 targets (30%).¹⁴ Further, when the HWZ score was used, we obtained AUC ≥ 0.6 for all of the 40 targets (100%). In comparison, in the reported use of the ROCS, AUC ≥ 0.6 for only 28 targets (70%)^{20a} or 26 targets (65%).¹⁴ As AUC < 0.6 (cutoff) is considered to be associated with a “poor-fair” screening, the reported screening tests using the ROCS failed for 12

targets (30%)^{20a} or 14 targets (35%),¹⁴ whereas the use of the HWZ score did not fail for any of the 40 targets.

It should also be pointed out that, despite the better performance of the HWZ scoring function for most targets, our virtual screening using the HWZ scoring function had unsatisfactory performance for few targets (exceptions). The worst performance of the virtual screening using the HWZ score was associated with the ER antagonist target, for which $AUC = 0.6$ using the HWZ score and $AUC = 0.59$ using the Tanimoto score. In comparison, for the same ER antagonist target, $AUC = 0.98$ or 0.78 for the reported virtual screening tests using the ROCS, as seen in Table 3. Carefully checking the molecular structures of the active ligands, we noted that these exceptions, including the ER antagonist target, might be associated with some highly flexible structures of the active ligands and the use of different superimposition approaches. For example, many of the ER antagonists include alkyl chains with several rotatable bonds and it is tricky to overlap the highly flexible alkyl chains with other more rigid structures like aromatic rings. Therefore, the worst result of our virtual screening for this particular target is likely attributed to the superimposition approach implemented in the algorithm. Further development of the virtual screening method should be focused on improvement of the superimposition approach.

As seen in Table 3, the virtual screening performance of the ROCS was strongly influenced by the choice of the target. In comparison, the performance of our HWZ score-based virtual screening was least sensitive to the choice of the target.

To further examine the effectiveness of the HWZ scoring function, we have also compared our results with the other published data³⁹ for the same targets in the DUD database, but we have only found nine common targets. Summarized in Table 4 are the results reported for the nine common targets involving five distinct programs/scoring functions, in comparison with the corresponding results obtained from using the HWZ scoring function. As seen in Table 4, the HWZ score provided the highest AUC values for all of the targets, except for DHFR. For DHFR, the best result ($AUC = 0.95$) is associated with the AutoDoc_{Rigorous} program, but the AutoDoc_{Rigorous} program performed very poorly for the other targets ($AUC = 0.35$ to 0.58), as seen in Table 4. The data in Table 4 suggest that the HWZ scoring function has the overall best performance within the common targets available for comparison. The highest average AUC value, 0.83 ± 0.05 , is associated with the HWZ scoring function. On the other hand, we are reluctant to make a final conclusion on this simply based on the data in Table 4 due to the very limited number of available common targets for comparison.

Enrichments Factor

The results of our screening tests were further analyzed using the enrichment factor (EF). The EF values at 1% and 5% of the data set describe the early enrichment, whereas the EF value at 10% represents the late-stage database screening. The EF values are summarized in Table 5 and depicted in Figure 4 for the 40 targets. As seen in Table 5, our HWZ score-based virtual screening led to an average $EF^1\%$ value of 23.7 ± 2.6 , compared to the reported average $EF^1\%$ value^{20a} of 19.4 ± 4.0 obtained by using the ROCS program.

In addition, the data in Figure 4 and Table 5 indicate that our HWZ score-based virtual screening never completely failed as no target was associated with $EF^1\% = 0$ within the 40 targets, whereas the reported virtual screening^{20a} using the ROCS could not identify any active ligand for four targets (GART, Trypsin, FGFR1, and VEGFR2).

Concerning the average enrichment at top 5% for the 40 targets, our HWZ score-based virtual screening led to an average $EF^5\%$ value of 10.4 ± 1.2 , compared to the reported

average $EF^{5\%}$ value of 8.4 ± 1.9 obtained by using the ROCS.^{20a} For the late-stage screening database screening enrichment factor ($EF^{10\%}$) for the 40 targets, our HWZ score-based virtual screening led to an average $EF^{10\%}$ value of 5.9 ± 0.5 , compared to the reported average $EF^{10\%}$ value^{20a} of 5.1 ± 0.9 obtained by using the ROCS.

Concerning the $EF^{10\%}$ values for individual targets, the data in Table 5 indicate that the $EF^{10\%}$ values in our HWZ score-based virtual screening range from 3.1 to 9.2, whereas the reported $EF^{10\%}$ values associated with the ROCS range from 0.4 to 9.8. So, our HWZ score-based virtual screening was less sensitive to the choice of target. Overall, our HWZ score-based virtual screening demonstrated an improved performance.

We also tried to compare the EF values from the HWZ score-based virtual screening with those from other virtual screening tests for the same targets in the DUD database, but we have only found 10 common targets (see Table 6 for comparison with the ligand-based methods and Table 7 for comparison with the structure-based methods). As seen in Tables 6 and 7, the obtained $EF^{1\%}$ and $EF^{10\%}$ values associated with the HWZ score are least-sensitive to the target, whereas the data obtained from the other methods are rather sensitive to the target and, therefore, the relative $EF^{1\%}$ or $EF^{10\%}$ values are dependent on the target when different methods are compared. In comparison with the ligand-based methods, the largest $EF^{1\%}$ value is associated with the HWZ score for 8 targets, and the largest $EF^{10\%}$ value is associated with the HWZ score for 6 targets, as seen in Table 6. In comparison with the structure-based methods, the largest $EF^{1\%}$ value is associated with the HWZ score for 9 targets, and the largest $EF^{10\%}$ value is associated with the HWZ score for 7 targets, as seen in Table 7. Overall, the largest average $EF^{1\%}$ and $EF^{10\%}$ values are all associated with the HWZ score within the 10 common targets available for comparison. Nevertheless, we are reluctant to make a final conclusion on this simply based on the data in Tables 6 and 7 due to the very limited number of available common targets for comparison.

Hit Rate

Summarized in Table 8 and depicted in Figure 5 are the Hit Rates obtained from our HWZ score-based virtual screening and the ROCS-based virtual screening reported in literature for the 40 targets in the DUD database. As seen in Table 8, the average HR value at top 1% ($HR^{1\%}$) was $46.3\% \pm 6.6\%$ for our HWZ score-based virtual screening and $41.9\% \pm 9.5\%$ for the ROCS-based virtual screening reported by Kirchmair *et al.*^{20a} The average $HR^{5\%}$ value was $52.2\% \pm 5.9\%$ for our HWZ score-based virtual screening and $42.2\% \pm 9.5\%$ for the ROCS-based virtual screening reported by Kirchmair *et al.*^{20a} The average $HR^{10\%}$ value was $59.1\% \pm 4.7\%$ for our HWZ score-based virtual screening and $51.4\% \pm 9.4\%$ for the ROCS-based virtual screening reported by Kirchmair *et al.*^{20a} All of these HR values indicate that our HWZ score-based virtual screening demonstrated an improved performance.

Conclusion

In the present report, we have developed and implemented a robust new scoring function, i.e. the HWZ score, for ligand-based (shape-filtering) virtual screening method. Compared to other popularly used shape-filtering virtual screening approaches (using different scoring functions) examined for their applications to the compounds for the same 40 protein targets in the DUD database, our HWZ score-based virtual screening approach led to an improved overall performance based on the obtained AUC, Enrichment Factor, and Hit Rate values. In addition, the performance of the HWZ score-based virtual screening approach was less sensitive to the choice of the target. The generation of a novel and robust scoring function is not only useful for ligand-based virtual screening algorithms, but it may also open the door for the ranking of docking results.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Classical representation of the shape based shape similarity between two molecules A and B (*Left*). Representation of the optimal shape overlap V_{AB} , i.e. $V_\epsilon = 0$, when $V_B > V_A$ (*Top right*) and $V_B < V_A$ (*Bottom right*).

Figure 2.

(A) Plots of the Tanimoto score *versus* x in Eqs.(1e) and (1f) when $V_e = 0$ and $V_B = (1 \pm x) V_A$. (B) The plots of the corresponding HWZ score *versus* x when $V_e = 0$ and $V_B = (1 \pm x) V_A$ in Eqs.(2b) and (2c).

Figure 3. Area under the ROC curve (AUC) for the 40 targets in the DUD database, reflecting the performance of the ligand-based virtual screening using the HWZ and Tanimoto scoring functions.

Figure 4. (Top) The Enrichment Factor (EF) values at top 1%, 5%, and 10% obtained from the HWZ score-based virtual screening. We have also reported the $EF^{x\%}_{ideal}$ values for comparison (dashed lines). For clarity, the enrichment factor $EF^{1\%}$ was sorted from the highest EF_{ideal} to the lowest one. (Bottom) The ideal enrichment factor at top 1% and 5% are 10 (red dashed line) and 20, respectively.

Figure 5. Hit Rate (*HR*) values at 1%, 5%, and 10% obtained from the HWZ score-based virtual screening. For clarity, the Hit Rate was sorted from the highest $HR^{1\%}$ value to the lowest one.

Table 1Calibrated coefficients of HWZ scoring function (with $N = 3$).

k	a_k	b_k	c_k
1	0.02	0.02	0.98
2	0	0	1.09
3	0	0	-2.98

Areas under the ROC curves ($\langle \text{AUC} \rangle$) for the 40 DUD targets (N_{target}) using the HWZ and Tanimoto scoring functions and the previously published results using the ROCS and ShaEP programs. The ROCS uses the Combo Scoring function (Shape-Tanimoto and Scaled Color score) and the ShaEP uses the Hodgkin similarity index.

Table 2

Range of $\langle \text{AUC} \rangle$		0	AUC	1	0.8	AUC	1	0.6	AUC	< 0.8	0.6	< AUC
		$\langle \text{AUC} \rangle$	N_{target}	$\langle \text{AUC} \rangle$	N_{target}	$\langle \text{AUC} \rangle$	N_{target}	$\langle \text{AUC} \rangle$	N_{target}	$\langle \text{AUC} \rangle$	N_{target}	$\langle \text{AUC} \rangle$
This work-HWZ		0.84 ± 0.02	40	0.88 ± 0.02	29 (72%)	0.74 ± 0.01	11 (28%)					0
This work-Tanimoto		0.70 ± 0.03	40	0.84 ± 0.02	10 (25%)	0.70 ± 0.02	21 (52%)	0.59 ± 0.02	9 (23%)			
Kirchmair-ROCS^(a)		0.73 ± 0.06	40	0.92 ± 0.02	17 (42%)	0.72 ± 0.03	11 (28%)	0.47 ± 0.05	12 (30%)			
Vainio-ROCS^(b)		0.69 ± 0.05	40	0.90 ± 0.04	12 (30%)	0.70 ± 0.03	14 (35%)	0.50 ± 0.03	14 (35%)			
Vainio-ShaEP^(b)		0.64 ± 0.05	40	0.86 ± 0.01	8 (20%)	0.70 ± 0.03	16 (40%)	0.47 ± 0.05	16 (40%)			

^(a) Data from ref. (20a)

^(b) Data from ref. (14)

Table 3

Areas under the ROC curves (AUC) for the 40 targets in the DUD database based on the HWZ and Tanimoto scoring functions in comparison with the previously published data using the ROCS program.

Target	HWZ score	Tanimoto score	ROCS ^(a)	ROCS ^(b)
ACE	0.84 ± 0.04	0.59 ± 0.04	0.70	0.76
ACHE	0.77 ± 0.06	0.68 ± 0.06	0.77	0.75
ADA	0.93 ± 0.01	0.72 ± 0.01	0.86	0.85
ALR2	0.77 ± 0.05	0.65 ± 0.05	0.57	0.52
AMPC	0.87 ± 0.06	0.81 ± 0.06	0.82	0.86
AR	0.79 ± 0.06	0.60 ± 0.06	0.79	0.48
CDK2	0.74 ± 0.06	0.67 ± 0.06	0.68	0.61
COMT	0.78 ± 0.04	0.59 ± 0.04	0.32	0.39
COX-1	0.80 ± 0.03	0.62 ± 0.03	0.53	0.54
COX-2	0.88 ± 0.04	0.81 ± 0.04	0.93	0.68
DHFR	0.87 ± 0.01	0.83 ± 0.01	0.92	0.95
EGFR	0.77 ± 0.02	0.73 ± 0.02	0.95	0.62
ER agonist	0.87 ± 0.03	0.57 ± 0.03	0.94	0.92
ER antagonist	0.60 ± 0.01	0.59 ± 0.01	0.98	0.78
FGFR1	0.88 ± 0.08	0.79 ± 0.08	0.49	0.57
FXA	0.88 ± 0.03	0.73 ± 0.03	0.39	0.52
GART	0.92 ± 0.04	0.82 ± 0.04	0.93	0.79
GPB	0.72 ± 0.03	0.52 ± 0.03	0.92	0.94
GR	0.85 ± 0.03	0.66 ± 0.03	0.79	0.73
HIVPR	0.87 ± 0.05	0.59 ± 0.05	0.56	0.55
HIVRT	0.70 ± 0.08	0.51 ± 0.08	0.66	0.62
HMGA	0.91 ± 0.02	0.82 ± 0.02	0.92	0.78
HSP90	0.90 ± 0.06	0.69 ± 0.06	0.66	0.80
INHA	0.77 ± 0.05	0.72 ± 0.05	0.72	0.69
MR	0.92 ± 0.02	0.69 ± 0.02	0.87	0.82
NA	0.94 ± 0.01	0.82 ± 0.01	0.97	0.98
P38	0.80 ± 0.09	0.74 ± 0.09	0.52	0.43
PARP	0.91 ± 0.08	0.76 ± 0.08	0.58	0.62
PDE5	0.80 ± 0.05	0.72 ± 0.05	0.53	0.52
PDGFRB	0.87 ± 0.04	0.69 ± 0.04	0.34	0.40
PNP	0.93 ± 0.04	0.88 ± 0.04	0.91	0.90
PPAR γ	0.93 ± 0.04	0.82 ± 0.04	0.92	0.65
PR	0.92 ± 0.02	0.54 ± 0.02	0.67	0.53
RXR α	0.97 ± 0.01	0.84 ± 0.01	0.96	0.98
SAHH	<u>0.95 ± 0.01</u>	0.92 ± 0.01	<u>0.97</u>	0.99
SRC	0.80 ± 0.06	0.74 ± 0.06	0.38	0.47
Thrombine	0.87 ± 0.04	0.67 ± 0.04	0.66	0.57
TK	0.87 ± 0.04	0.75 ± 0.04	0.86	0.86

Target	HWZ score	Tanimoto score	ROCS ^(a)	ROCS ^(b)
Trypsin	0.88 ± 0.06	0.70 ± 0.06	0.78	0.69
VEGFR2	0.78 ± 0.06	0.58 ± 0.06	0.43	0.50
Average	0.84	0.70	0.73	0.69
95% confidence	0.02	0.03	0.06	0.02

^(a)Data from ref.(20a)

^(b)Data from ref.(14)

Table 4

Comparison of the Areas under the ROC curves (AUC) obtained with HWZ score and from five distinct programs/scoring functions using nine DUD targets. The highest AUC value for each target is highlighted in bold.

Target	HWZ score	NNscore 1.0(a)	NNscore 2.0(a)	AutoDock _{fast} (a)	AutoDock _{Rigorous} (a)	Vina(a)
ACHE	0.77 ± 0.06	0.55	0.57	0.48	0.53	0.67
COX-2	0.88 ± 0.04	0.74	0.49	0.38	0.43	0.31
DHFR	0.87 ± 0.01	0.72	0.83	0.83	0.95	0.76
EGFR	0.77 ± 0.02	0.47	0.51	0.51	0.49	0.61
FGFR1	0.88 ± 0.08	0.58	0.55	0.41	0.35	0.46
FXA	0.88 ± 0.03	0.76	0.52	0.44	0.47	0.63
P38	0.80 ± 0.09	0.75	0.58	0.4	0.37	0.54
PDGFRB	0.87 ± 0.04	0.6	0.62	0.50	0.36	0.53
SRC	0.80 ± 0.06	0.58	0.63	0.65	0.58	0.69
Average	0.83	0.64	0.59	0.51	0.50	0.58
Std. Dev.	0.05	0.10	0.10	0.15	0.18	0.14

(a) The AUC values from ref.(39)

Table 5

Enrichment Factor EF at top 1%, 5%, and 10% obtained from our HWZ score-based virtual screening in comparison with the data published by Kirchmair *et al.*^{20a} using the ROCS program.

target	EF 1%			EF 5%			EF 10%		
	HWZ score	ROCS(a)	HWZ score	ROCS(a)	HWZ score	ROCS(a)	HWZ score	ROCS(a)	ROCS(a)
ACE	18.7	27.7	7.0	8.2	3.1	4.7			
ACHE	27.1	26.8	8.5	12.6	5.8	7.4			
ADA	29.1	13.8	13.5	3.5	6.4	3.9			
ALR2	15.6	8.1	6.9	3.1	4.9	3.1			
AMPC	27.3	35.9	11.0	13.6	5.2	7.2			
AR	26.1	27.0	7.2	9.5	5.1	5.1			
CDK2	18.7	26.4	5.1	6.4	4.0	4.0			
COMT	16.3	30.1	8.3	5.5	4.6	2.7			
COX-1	7.6	17.5	4.8	5.7	4.1	2.8			
COX-2	8.9	35.7	4.0	17.1	7.2	8.7			
DHER	29.2	33.5	15.5	14.0	8.9	8.2			
EGFR	30.5	33.0	11.8	16.9	5.3	8.9			
ER agonist	25.1	27.1	12.1	16.1	6.2	9.0			
ER antagonist	21.0	5.3	8.6	15.0	5.6	9.8			
FGFR1	21.7	0.0	11.2	2.4	5.5	2.0			
FXA	33.5	4.3	14.2	1.0	6.8	0.8			
GART	12.3	0.0	12.9	3.9	8.1	5.7			
GPB	17.6	36.6	7.8	14.6	5.2	8.1			
GR	23.2	15.8	7.9	5.2	4.5	5.7			
HIVPR	28.1	5.8	7.5	2.3	5.6	1.5			
HIVRT	19.1	21.1	5.4	4.6	4.2	2.5			
HMGA	30.2	36.5	16.8	15.6	7.9	8.3			
HSP90	33.1	36.8	10.5	9.2	5.7	4.6			
INHA	7.7	36.7	6.7	9.9	4.7	5.1			
MR	32.2	36.7	15.1	16.3	8.4	8.0			
NA	26.8	32.3	13.1	18.5	7.6	9.6			

target	EF 1%		EF 5%		EF 10%	
	HWZ score	ROCS ^(a)	HWZ score	ROCS ^(a)	HWZ score	ROCS ^(a)
P38	24.6	10.2	10.7	2.8	5.1	2.0
PARP	34.0	3.1	14.3	3.1	6.6	3.7
PDE5	22.4	16.2	11.1	4.0	5.6	2.4
PDGFRB	12.5	7.7	7.2	1.7	4.4	1.1
PNP	29.6	20.2	14.8	16.9	7.4	9.3
PPAR γ	41.0	21.6	15.7	15.8	6.5	8.3
PR	32.1	16.4	12.4	3.0	7.1	2.6
RXR α	36.3	26.0	18.5	17.2	9.2	8.6
SAHH	26.5	26.3	16.1	14.7	8.8	9.4
SRC	18.9	2.0	9.7	1.6	5.0	1.0
Thrombine	21.2	1.8	7.3	1.1	5.6	0.7
TK	19.7	13.7	8.7	4.6	4.9	6.0
Trypsin	32.5	0.0	12.7	0.0	5.9	2.8
VEGFR2	8.8	0.0	5.0	0.3	3.9	0.4
Average	23.7	19.4	10.4	8.4	5.9	5.1
95% confidence	2.6	4.0	1.2	1.9	0.5	0.9

^(a)Data from ref.(20a)

Table 6

Enrichment Factor at top 1% and 10% obtained with HWZ score and several distinct programs corresponding to ligand/structural-based virtual screenings. The highest values of the enrichment factor EF are highlighted in bold (EF1%) and underlined (EF10%), respectively, for each DUD target. For comparison, the EF10% results obtained by HWZ score are multiplied by a factor of 10.

Target	HWZ score		Suflex-sim ^(a)		FlexS ^(a)		ICMsim ^(a)	
	1%	10%	1%	10%	1%	10%	1%	10%
ADA	29.1	<u>64</u>	5.1	23.1	15.4	30.8	10.3	28.2
CDK2	18.7	<u>40</u>	2.8	8.3	5.6	11.1	9.7	30.6
DHFR	29.2	<u>89</u>	4.9	25.6	8.0	27.8	20.2	80.7
FXA	33.3	<u>68</u>	1.4	2.7	5.5	19.9	11.6	46.6
HIVRT	19.1	42	9.3	18.6	27.9	<u>58.1</u>	18.6	39.5
NA	26.8	<u>76</u>	16.3	55.1	20.4	69.4	16.3	73.5
P38	24.6	<u>51</u>	9.0	21.1	12.6	26.2	11.2	17.8
Thrombin	21.2	56	1.4	15.3	2.8	12.5	2.8	<u>76.4</u>
TK	19.7	49	22.7	50	9.1	54.5	22.7	<u>63.6</u>
Trypsin	32.5	59	0	59.2	18.4	18.4	10.2	<u>95.9</u>
Average	25.4	59.4	7.3	27.9	12.6	32.8	13.36	55.3
Standard Dev.	5.5	15.3	7.3	19.8	7.9	20.5	5.6	26.3

^(a) Enrichment Factor values are from ref.(40)

Enrichment Factor at top 1% and 10% obtained with HWZ score and several distinct programs corresponding to structural-based virtual screenings. The highest values of the enrichment factor EF are highlighted in bold ($EF^{1\%}$) and underline ($EF^{10\%}$), respectively, for each DUD target. For comparison, the $EF^{10\%}$ results obtained by HWZ score are multiplied by a factor of 10.

Table 7

Target	HWZ score	Suflex-dock ^(a)		FRED ^(a)		FlexX ^(a)		ICM ^(a)		DOCK ^(a)	
		1%	10%	1%	10%	1%	10%	1%	10%	1%	10%
ADA	29.1	<u>64</u>	7.7	48.7	5.1	12.8	5.1	33.3	0	2.6	15
CDK2	18.7	40	6.9	<u>44.4</u>	18.1	31.9	11.1	29.2	13.9	38.9	10
DHFR	29.2	<u>89</u>	17.6	52.9	5.8	17.6	11.9	36.8	19.0	66.8	25
FXA	33.3	<u>68</u>	17.1	59.6	4.1	13.7	14.4	44.5	7.5	45.2	5
HIVRT	19.1	<u>42</u>	18.6	27.9	18.6	32.6	0	4.6	11.6	37.2	5
NA	26.8	76	22.4	67.4	0	4.1	4.1	69.4	34.7	<u>85.7</u>	10
P38	24.6	<u>51</u>	3.7	18.1	7.9	19.6	1.1	21.6	4.6	7.5	2
Thrombin	21.2	<u>56</u>	5.6	47.2	1.4	11.1	6.9	52.8	5.6	33.3	5
TK	19.7	<u>49</u>	0	13.6	18.2	22.7	0	13.6	0	0	0
Trypsin	32.5	59	18.4	<u>81.6</u>	2.0	26.5	0	51.0	2.0	18.4	0
Average	25.4	59.4	11.8	46.1	8.1	19.3	5.5	35.7	9.9	33.6	7.7
Standard dev.	5.6	15.3	7.8	21.4	7.4	9.3	5.4	19.6	10.7	27.9	7.7

^(a) Enrichment Factor values are from ref.(40)

Table 8

Hit Rates at top 1%, 5%, and 10% using our HWZ score-based method in comparison with the corresponding reported by Kirchmair *et al.* using the ROCS program.

target	HR 1%			HR 5%			HR 10%		
	HWZ score	ROCS(<i>a</i>)	HWZ score	ROCS(<i>a</i>)	HWZ score	ROCS(<i>a</i>)	HWZ score	ROCS(<i>a</i>)	ROCS(<i>a</i>)
ACE	47.8	70.9	35.0	41.0	31.0	47.0			
ACHE	68.9	68.2	42.5	63.0	58.2	74.0			
ADA	72.2	34.2	67.6	17.5	64.0	39.0			
ALR2	40.8	21.2	34.4	15.5	48.5	31.0			
AMPC	73.1	96.0	54.9	68.0	51.5	72.0			
AR	62.2	64.3	36.1	47.5	50.6	51.0			
CDK2	42.5	59.9	25.6	32.0	39.8	40.0			
COMT	38.4	70.7	41.5	27.5	45.7	27.0			
COX-1	19.2	44.2	24.1	28.5	40.5	28.0			
COX-2	14.2	57.0	20.2	85.5	72.3	87.0			
DHFR	66.4	76.1	77.5	70.0	89.0	82.0			
EGFR	69.7	75.3	58.8	84.5	52.9	89.0			
ER agonist	61.6	66.5	60.5	80.5	62.1	90.0			
ER antagonist	26.1	6.6	43.0	75.0	56.1	98.0			
FGFR1	33.8	0.0	56.1	12.0	54.7	20.0			
FXA	37.3	4.8	71.2	5.0	68.2	8.0			
GART	12.3	0.0	64.4	19.5	81.0	57.0			
GPB	41.2	85.6	39.1	73.0	52.4	81.0			
GR	25.1	17.2	39.5	26.0	45.1	57.0			
HIVPR	28.1	5.8	37.5	11.5	56.2	15.0			
HIVRT	42.8	47.2	27.1	23.0	41.5	25.0			
HMG A	51.0	61.7	84.0	78.0	79.2	83.0			
HSP90	77.8	86.5	52.7	46.0	57.4	46.0			
INHA	13.4	64.1	33.3	49.5	47.3	51.0			
MR	65.9	75.0	75.5	81.5	84.3	80.0			
NA	70.3	84.7	65.5	92.5	76.3	96.0			

target	HR 1%		HR 5%		HR 10%	
	HWZ score	ROCS ^(a)	HWZ score	ROCS ^(a)	HWZ score	ROCS ^(a)
P38	36.8	15.3	53.4	14.0	51.2	20.0
PARP	78.1	7.1	71.5	15.5	66.1	37.0
PDE5	29.4	21.3	55.7	20.0	55.6	24.0
PDGFRB	25.8	16.0	35.8	8.5	44.3	11.0
PNP	71.3	48.7	74.0	84.5	73.7	93.0
PPAR γ	41.0	21.6	78.5	79.0	65.3	83.0
PR	67.9	34.7	62.2	15.0	71.0	26.0
RXR α	87.2	62.4	92.5	86.0	92.0	86.0
SAHH	65.0	64.5	80.5	73.5	88.1	94.0
SRC	29.3	3.1	48.6	8.0	49.8	10.0
Thrombine	21.2	1.8	36.5	5.5	55.7	7.0
TK	48.6	33.8	43.5	23.0	49.1	60.0
Trypsin	32.5	0.0	63.6	0.0	59.2	28.0
VEGFR2	14.5	0.0	24.9	1.5	39.1	4.0
Average	46.3	41.9	52.2	42.2	59.2	51.4
95% confidence	6.7	9.5	5.9	9.5	4.7	9.4

^(a)Data from ref.(20a)