Molecular Shape Analysis-Guided Virtual Screening Platform for Adenosine Kinase Inhibitors

Savita Bhutoria[1], Ballari Das[2] and Nanda Ghoshal[1]

[1]Structural Biology and Bioinformatics Division, CSIR-Indian Institute of Chemical Biology, Kolkata, India. [2]Department of Bioinformatics, West Bengal University of Technology, Kolkata, India.

ABSTRACT: We propose a new application of molecular shape descriptors in hierarchical selection during virtual screening (VS). Here, a structure-based pharmacophore and docking-guided VS protocol have been evolved to identify inhibitors against adenosine kinase (AK). The knowledge gained on the shape requirements has been extrapolated in classifying active and inactive molecules against this target. This classification enabled us to pick the appropriate ligand conformation in the binding site. We have suggested a set of hierarchical filters for VS, from a simple molecular shape analysis (MSA) descriptor-based recursive models to docking scores. This approach permits a systematic study to understand the importance of spatial requirements and limitations for inhibitors against AK. Finally, the guidelines on how to select compounds for AK to achieve success have been highlighted. The utility of this approach has been suggested by giving an example of database screening for plausible active compounds.

KEYWORDS: molecular shape analysis, virtual screening, adenosine kinase, docking, structure-based pharmacophore

CITATION: Bhutoria et al. Molecular Shape Analysis-Guided Virtual Screening Platform for Adenosine Kinase Inhibitors. *Bioinformatics and Biology Insights* 2016:10 97–103 doi: 10.4137/BBI.S38430.

TYPE: Original Research

RECEIVED: February 05, 2016. **RESUBMITTED:** June 14, 2016. **ACCEPTED FOR PUBLICATION:** June 16, 2016.

ACADEMIC EDITOR: J. T. Efird, Associate Editor

PEER REVIEW: Four peer reviewers contributed to the peer review report. Reviewers' reports totaled 1,346 words, excluding any confidential comments to the academic editor.

FUNDING: Authors disclose no external funding sources.

COMPETING INTERESTS: Authors disclose no potential conflicts of interest.

CORRESPONDENCE: nghoshal@iicb.res.in

Introduction

Adenosine kinase (AK) is an evolutionary, ancient, and highly conserved enzyme, which is directly related to bacterial ribokinases and fructokinases .[1,2] AK has emerged as a rational therapeutic target for many diseases and pathological conditions.[3,4] Two types of AK inhibitors, nucleosides and non-nucleosides are known to bind in closed and open conformation respectively.[5] Nucleoside inhibitors resemble the adenosine (substrate) molecule in shape. Nucleosides have failed to undergo successful clinical development due to dose-limiting side effects.[6,7] Thus, there is a demand for designing novel scaffolds other than nucleosides. We designed this study to identify novel scaffolds that could function as AK inhibitors.

In an effort to identify eligible inhibitors, a new protocol for VS was evolved in this study. Here, an important issue of inclusion of the constraints imposed by the binding site is discussed and addressed. With the objective of finding new scaffolds, docking and pharamacophore analyses were carried out. Docking is an important strategy in VS that involves the prediction of ligand conformation using electrostatic, hydrophobic, and shape complementarities.[8,9] Numerous VS studies have been carried out using docking methodologies.[10,11] Although most of the docking programs reproduce correct binding modes for crystallographic ligands, docking scores do not give reliable rankings.[12,13] Because of some target-specific peculiarities, the VS method needs to be made specific for a particular target. Previous studies have successfully introduced the shape of the molecule in VS. It has been proved that shape can be considered to measure the performance of VS, as it can provide more specificity than simply docking scores.[14–17]

In this study, we proposed a novel protocol for VS using shape descriptor-based models. This study was conducted to know the requirement that inhibitors should bear and what will happen if very diverse molecules were selected as hits in VS. We proposed that molecular shape analysis (MSA) can be considered to measure the performance of VS. The utility of using simple shape descriptors was extrapolated in a novel way for the extraction of newer scaffolds from the database. First, GOLD-based scores have been introduced in order to measure the discriminatory power and then compared with that of MSA descriptors. Finally, a combination of scoring and MSA descriptor-based screening technique for the improvement of VS was investigated. A rational quest is proposed as to whether the compounds adopt a conformation consistent with some reasonable commonality with adenosine (ADO); if the answer is yes, the compounds are reported in the output as actives. This study reflects the importance of shape parameters in context to the requirement of a specific volume of ligands in the binding site. Overall, it has been demonstrated that the VS for identifying new leads call for forced requirement of

a characteristic shape or spatial properties. Molecule cannot bind and have activity if devoid of such shape requirements.

The above methodology was based on its ability to discard the inactives from the set of molecular candidates. This composite method seems efficient in that it is able to avoid any false positives before conducting expensive synthesis and activity studies. For example, if a molecule does not fit into the active site of an enzyme because of its size and shape parameters, it should not be considered further, and even no other computed scores of the molecule are needed to make this decision. This finding was considered for better evaluation of hits with new scaffolds for this system. Further, as finding novel active scaffolds is often a more important success criterion than hit rates of virtual screens, emphasis should be given to novel scaffolds. In VS, such hits with even weaker activities can also be considered to be those that can be improved by iterative lead optimization. We believe that this novel protocol can be extrapolated to other enzyme systems also.

Methods

Dataset for derivation of the protocol. Our aim was to construct a test dataset with the known active and diverse decoy molecules. Active compounds contain moderate to high affinity binders. It is known that many large compounds may not bind in a similar manner[5] and our previous results demonstrated that larger compounds bind to semi-open conformation of AK.[18] Therefore, we decided to take lighter-weight AK inhibitors, which are similar in size to the adenosine molecule.[19–22] Finally, 22 active compounds were selected in the test set (Supplementary Table 1). For preparation of the decoy set, the thymidine kinase (TK) decoy set from the directory of useful decoys (DUD) was taken and further processed to make it suitable for this system. This decoy set was selected, as thymidine and adenosine are structurally similar. A set of topologically diverse compounds, having similar molecular weights to that of adenosine, was selected from this dataset. For this purpose, topological descriptors were calculated and principal component analysis (PCA) was used to evaluate the molecular diversity in the dataset. PCA reduces the data set from many variables to a few components with loadings. The purpose is to express the main information of the variables using a lower number of variables called the principal components. Topological descriptors were calculated using Cerius2 and PCA was carried out using Minitab.[23,24] The full set of decoy molecules and descriptors was analyzed by means of PCA. Three principal components PC1, PC2, and PC3 contributed 34.845%, 15.273%, and 10.681% to the total components, respectively. In fact, PC1 and PC2 have made more than 50% of the variances and, therefore, play a major role in the importance of the descriptors. The first and second resulting components (PC) were used to plot the decoy molecules in a three-dimensional space (Supplementary Fig. 1). Finally, 218 diverse molecules (Supplementary Table 2) than the adenosine were selected as the decoy set.

Preparation of the protein model, definition of active site, and docking. Protein coordinates used for docking were taken from the X-ray structure of AK in complex with adenosine (PDB ID: 1BX4). Usually, water molecules are removed from the active site, except the molecules that are known to bind very tightly to the protein or are known to be essential for the interaction with the drug (ligand). Here, we kept two bound water molecules (W1 and W2) in the protein active site (Fig. 1) as also described in our earlier work.[18] Bound ligand and other water molecules were removed. Hydrogen atoms were added and minimized using Chemistry at Harvard Macromolecular Mechanics force field. The active site was defined as the collection of amino acids enclosed at least within a 7 Å radius from each atom of the bound ligand. All the compounds were then docked and scored using GOLD using Goldscore (GS) and a modified form of Chemscore (CS for kinases) as previously described.[18,25] Docking strategy was tested by assessing the ability to reproduce the experimental binding orientation of ADO and correct water (W2) displacement by 5-Iodotubercidin in AK complex (Fig. 1) as also described previously.[18] The outcome of the VS was assessed by the ROC curve analysis. The area under the ROC curve (AUC) is an important indicator of the VS performance and can be calculated as the sum of all rectangles formed by the sensitivity and 1 – specificity values for the different thresholds. VS workflows that perform better than a random discrimination of actives and decoys retrieve an AUC value between 0.5and 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. The closer the AUC to 1, the better is the performance of the classification.

Molecular shape analysis in recursive partitioning (RP)-based classification for VS. In this study, we have derived a novel method for the classification of active and

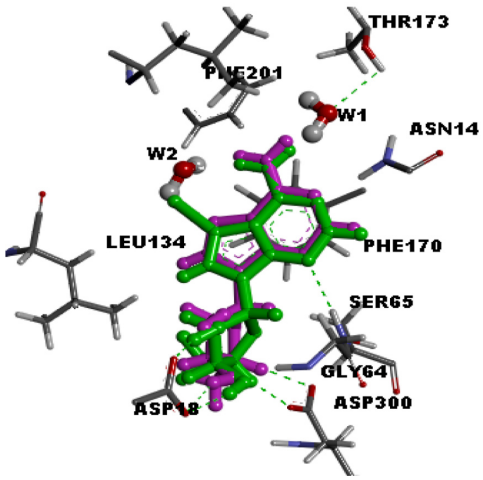

Figure 1. Docked poses of natural substrate ADO (purple) and inhibitor 5-Iodotubercidin (green) in the AK-binding site.
Notes: W1 and W2 are water molecules; W2 is displaced by 5-Iodotubercidin.

inactive molecules using molecular shape analysis (MSA), implemented in Cerius2.[23,26] MSA descriptors, difference volume (DIFFV), common overlap steric volume (COSV), common overlap volume ratio (Fo), and non-common overlap steric volume (NCOSV) belong to an interesting class of molecular descriptors that have been proposed for a number of QSAR type tasks.[27,28] These descriptors represent the commonality and differences of considered molecules in terms of shapes with the reference ligand. The steps involved the selection of shape reference compound (bound ligand from 1BX4) and measuring molecular spatial properties of docked conformations, using MSA descriptors. The MSA descriptors were used to devise the scheme to discriminate active and inactive molecules by the recursive partitioning algorithm in MOE.[29]

This algorithm led to a model called classification tree. The prediction accuracy of this classification tree was evaluated by means of a twofold cross-validation methodology. The final selection of the tree was made by comparison of the misclassification rate R(T). It measures the proportion of cases that are incorrectly classified by a tree. R(T) can be defined as $N_{\text{missclassified}}/N_{\text{total}}$, where $N_{\text{missclassified}}$ is the total number of misclassified cases and N_{total} is the total number of cases in the training set.

Derivation of the VS scheme. The classification schemes were utilized for identification of active (defined as 1) or inactive (defined as 0) conformations and its utilization in scoring of molecules. A consensus prediction score was devised as follows:

$$\text{Consensus Prediction Score (CPS)} = \text{Prediction by classification tree} \times \text{CS/GS}$$

If the conformations are predicted as 0 in classification tree, the CPS will give output as zero or inactive. The purpose of using this classification is to have prediction for binding probability with the hope to eliminate structures, which will not bind to the binding site of AK. To improve the accuracy in prediction of active and inactive molecules, a consensus-ranking approach was devised. The ranks of all the molecules were assigned on the basis of CPS-GS and CPS-CS scores separately. Consensus ranking is based on the average ranks obtained from the above two ranks for each molecule. The strategy is illustrated for the consensus prediction score as follows:

$$\text{Consensus rank} = (\text{Molecule's rank by CPS-GS} + \text{Molecule's rank by CPS-CS})/2$$

Structure-based pharmacophore modeling. The crystal structure of AK in complex with 5-iodotubercidin (PDB ID: 2I6A) served as a starting point for structure-based inhibitor design by the program catalyst.[30] The amino acids aspartic acid (Asp), asparagine (Asn), glycine (Gly), serine (Ser), phenylalanine (Phe), and leucine (Leu) interact with the ligand. Asp seeks a hydrogen bond, while Asn acts as an H-bond donor as well as an H-bond acceptor. Ser acts as an H-bond donor. Phe and Leu are responsible for hydrophobic interaction with the ligand (Supplementary Figure 2). We selected only a single vector originating from a particular point, as a single point cannot be represented by two vectors in a catalyst. A Glycine–Glycine switch is responsible for a conformational change in AK triggered by the interaction of sugar-like backbone with these glycine residues.[31,32] The specific requirement of the backbone of sugar-like moiety will increase the probability of that scaffold for being active. By giving a shape constraint, the obtained hits would represent a complementary shape of the adenosine-binding site, which includes the information about the shape (position of atoms) of the sugar moiety as well. This pharmacophore query was used as the fast tool for identifying novel hits from the Maybridge database.

Results and Discussion

Virtual screening using ligand docking and Molecular shape analysis. All the dataset compounds were docked using GOLD and scored GS and CS. To assess the ability of scores to discriminate active compounds from decoys, ROC curves were calculated. In both the cases, whether CS or GS, the ROC curves indicated significantly good performance suggesting that docking scores are able to classify the best ranked conformation of known actives and decoy molecules. Inspection of the result evinces that the discrimination obtained with Goldscore surpass those with Chemscore (Fig. 2). In addition, the MSA descriptors were calculated to plot the ROC curves for highest ranked conformations of all the molecules of this dataset (Fig. 2A and 2B). MSA descriptors, especially COSV and DIFFV, are able to differentiate the actives and inactives effectively. The COSV parameter gives ROC curves, comparable to that of Goldscore. The results suggest the importance of these shape-based MSA descriptors, COSV and DIFFV, in discriminating active and inactive molecules.

Some clear trends were apparent from the areas under the respective ROC curves. Analysis of ROC curves in Figure 2 suggested that DIFFV and COSV are two important parameters in the classification of actives and inactives. Comparison with respect to descriptor NCOSV allows us to gain more insight in the information content. ROC curve suggests that the deviation of NCOSV for the known actives and decoys from that of bound ligand (ADO) does not show any trend in distinguishing the actives from inactives. NCOSV can be low or high for inactives, but COSV should be high for actives. Actives also have non-common overlap with ADO, but at the same time they have adequate common overlap with ADO, which makes them active. It was also observed that the decoy molecules, which did not possess a similar shape as that of ADO, showed remarkable difference in their values of descriptors (COSV and DIFFV) than those of ADO or active inhibitors [Supplementary Tables 1 and 2]. This indicated that the molecules should resemble the ADO in terms of shape for being an inhibitor, which again strengthens the fact that the shape of the molecule has some bias toward this protein.

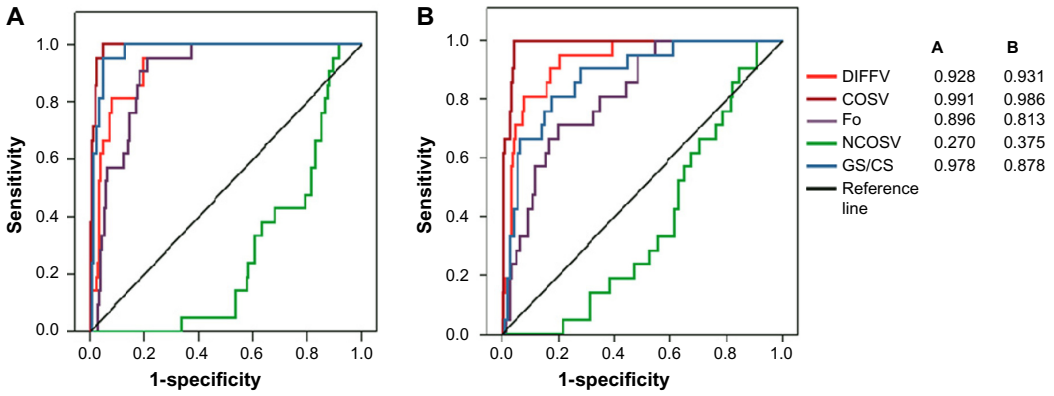

Figure 2. ROC curves for test set: (**A**) GS (**B**) CS. ROC curve parameters are shown in A and B columns for Goldscore (GS) and Chemscore (CS) respectively.

Rule-based classification model/scheme. As the first objective in any VS is the identification of binders or non-binders, rules were formulated for selecting the conformations, which would fulfill the criteria for successful binders. Recursive partitioning (RP) was done using MSA descriptors as the pool of potential splitting (active or inactive; Table 1) to understand whether these fit or do not fit the criteria for binding.

The statistical analysis of models showed similar trends of overall good accuracy. In the first case (GS-based conformations of actives and decoys), the misclassification rate of 0.017 was observed, while in the second case (CS-based conformations of actives and decoys) the misclassification rate was 0.038. It is interesting to look at the descriptors in the RP model and to extract the properties, which differentiate actives and inactives in the data set. COSV was found in both the RP trees, again signifying the importance of shape similarity to adenosine. The comparison of volumes occupied by ADO and a decoy molecule, ZINC03923460, illustrates this more clearly (Fig. 3). Although decoys occupy the ADO-binding site, they do not possess sufficient common overlap. The best-ranked conformation of this molecule has a COSV value of 118.04 (with GS-based best-ranked conformation) and 110.45 (with CS-based best-ranked conformation). These values are less than the required values obtained either by GS-or CS-based RP tree. Thus, this decoy molecule possesses lesser values than the required values for molecules to be classified as active. If we see the COSV values for active molecules (Supplementary Table 1), we find that they possess values that are higher than the minimum values required by the RP classification scheme.

Hence, from this analysis it was realized that the preliminary step is shape recognition. The molecules, which do not have similarity in shape, will not exhibit binding and

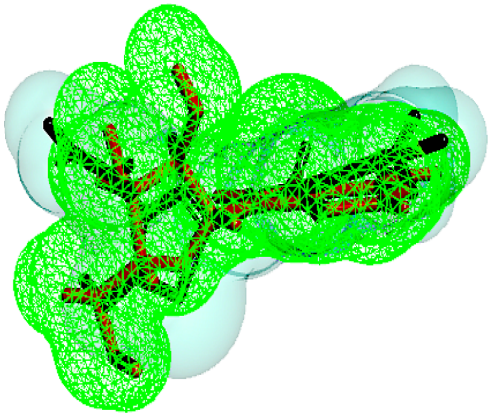

Figure 3. COSV occupied by a decoy set molecule (ZINC03923460) in comparison with ADO in the adenosine-binding site, green mesh surface: ADO (red), blue transparent surface: decoy compound (black).

Table 1. Binary classification tree.

MODE OF CONFORMATIONS	RP TREE	R(T) (MISCLASSIFICATION RATE)
GS	ROOT <0> (0.005) DIFFV <=29.1 DIFFV >29.1 <0> (0) COSV <=141 <1> (0.13) COSV >141	0.017
CS	ROOT <0> (0) COSV <=147 <1> (0.3) COSV >147	0.038

Note: Rule-based classification with MSA descriptors for selecting actives (1) and inactives (0).
Abbreviations: GS, Goldscore; CS, Chemscore.

thus there is no room for activity. The results reinforce that the MSA descriptors are able to correctly classify the dataset in active and inactive molecules against this target and thus certainly offer a useful set of descriptors for VS tasks. It is important to note that information of true binders and subsequent scoring of the ligands can be recognized as a ranking tool for identifying true actives from virtual libraries.

Virtual screening. *Pharmacophore perception and screening of hits.* Based on all the desired interactions (Supplementary Fig. 1), a structure-based pharmacophore hypothesis was created with the help of catalyst. The pharmacophore model has three hydrogen bond acceptor (HBA) features, one hydrophobic, one hydrogen bond donor (HBD) feature, and a ring aromatic feature (Fig. 4). The model also includes the shape constraint feature, which provides an insight regarding the allowed topography in the binding site and thus makes the pharmacophore more specific. The resulting pharmacophore model identified 116 compounds as hits from the Maybridge database.

Hierarchical selection and scoring. The pharmacophore model picked many active hits and lots of them possess good docking scores (Supplementary Table 3). A question was raised whether all are probable inhibitors of AK. As the MSA descriptor-based classification scheme was successful in differentiating the active and inactive molecules, we decided to use these RP models in identifying real hits. To predict real hits using the RP models, the MSA descriptors were computed for ten best conformations of all the screened molecules. The consensus prediction score was calculated for all the conformations of all the hits. The consensus-ranking approach was then applied for identifying final hits with respective CPS scores (Table 2). Out of 116 compounds, only three compounds were screened, which are likely to represent the prospective lead candidates against AK.

Binding mode analysis of hits. Molecules with lesser affinity than the expected (at the same time having a diverse structure) would be interesting leads, as they can be optimized further. Three compounds (SCR 00361, NRB 04489, and SCR 00980) are predicted as novel AK inhibitors. The hits have been further investigated through few validation measures such as visual examination of how well they dock into the binding groove and how the hits interact with the important amino acid residues of the binding site. A scaffold should form the key interactions and the binding modes should be conserved. These hits represent very similar binding conformations in both the docking methods (GS and CS), thus substantiating their candidature as an AK inhibitor (in a closed binding site; Fig. 5).

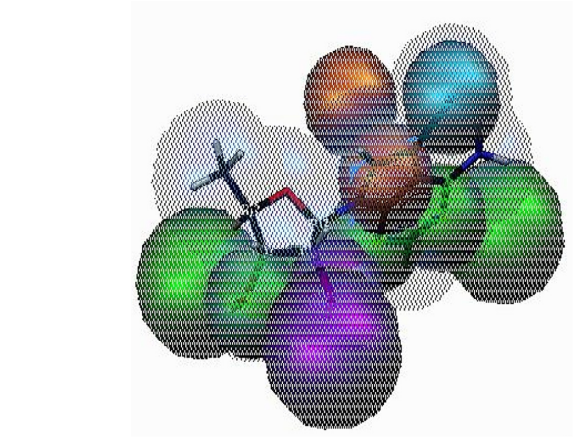

Figure 4. Structure-based pharmacophore.
Notes: green: HBA, purple: HBD, brown: ring aromatic, blue: hydrophobic, gray: Shape constraint.

Table 2. Selected hits with their compound ID and their respective score as obtained from our virtual screening method.

CONSENSUS RANK	COMPOUND NAME	CPS CHEMSCORE	CPS GOLDSCORE
1	SCR 00361	29.9776	57.1475
2	NRB 04489	25.3959	53.1071
3	SCR 00980	19.743	50.1106

The outcome interaction data show that the residues, Gly63, Gly64, Leu138, Leu141, Phe170, and Asn14, are critical for the binding of these inhibitors. In selected hits, these residues form a pocket between the inhibitor and AK framework region. The selected hits represented the conserved interactions with the active site residues, which are quite consistent with the adenosine interaction. The hits were found to interact well with the critical residues, despite their modest scores. These inhibitors mimic two most important interactions of ADO, first with Gly63-64 switch (like sugar backbone) and with Phe170, (like nucleotide core), in addition to other interactions. It is also noted that they have the potency associated with the removal of water molecules (Fig. 6). GOLD docking has the functionality to keep or displace water molecules from the binding site. All the three hits displaced the W2 molecule from the binding site and SCR00980 displaced W1 as well (Fig. 6). 5-Iodotubercidin is known to displace this water molecule (W2) from the AK-binding site. Displacement of W2 from the AK-binding site increases the candidature of these hits as inhibitors. This again strengthens the possible success of this methodology in VS.

Because of the similar binding conformations of these scaffolds in both the docking runs and presence of crucial interactions with the binding site residues, further studies could be initiated to use such scaffolds as the new starting point for lead optimization. These scaffold interaction data can be used to design new inhibitors with increased affinity toward AK. The new actives contained different underlying chemical architecture than nucleosides, indicating successful scaffold-hopping. This study was dedicated for correctly identifying ligands or scaffolds that will act as binders, which is of primary interest in lead optimization. Furthermore, the application of such docking and MSA-based hierarchical

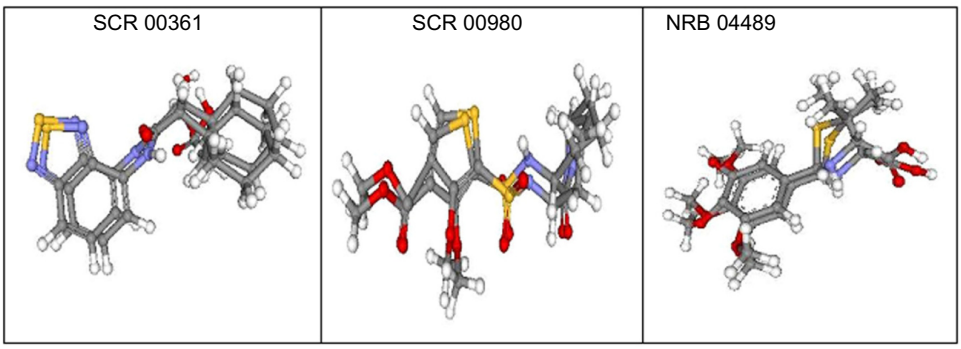

Figure 5. Binding (docked) conformations of hits selected by GS and CS. Best conformations are shown.

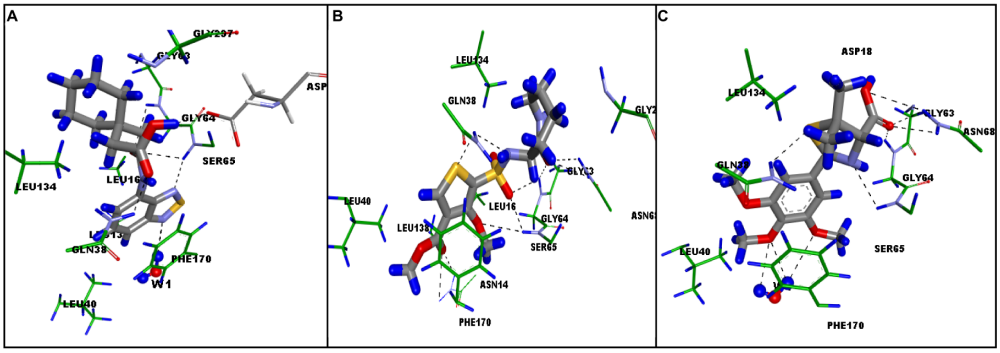

Figure 6. Interactions of selected hits with the binding site; (**A**) SCR 00890 (**B**) SCR 00361 (**C**) NRB 04489. Dotted lines represent H bonds with the interacting residues. A and C have water molecule (W1), which could not be displaced by hits.

selection scheme has the potential for rejecting false positives in VS at early stages of lead discovery for promising targets. Finally, the proposed methodology can be applied to any other protein ligand system with specific requirement of inhibitors. This will be particularly advantageous for those targets for which no, or only structurally similar, active molecules are known. Looking at it more broadly, the integration of this protocol will be extremely useful for therapeutic targets such as methylases and acetyltransferases, where the binding site is very specific for their substrates.

Conclusions

One of the key steps in the early stage drug discovery is finding active compounds, which can be further optimized into potential drug candidates. VS is an excellent strategy for generating such compounds as hits. In this study, a new method for VS of AK inhibitors has been presented. It is known that the AK-binding site poses some spatial constraints for the binding of inhibitors. We have devised a novel protocol to identify true binders from a database using MSA-guided hierarchical hit selection methodology. This strategy, as discussed, can be used to reduce the number of false positives. The MSA descriptors are particularly suited, as they can quickly calculate the necessary information for the ligand's volume, essential for binding in closed conformation of AK. This study of exploring VS with docking and shape-based descriptors has demonstrated its importance in identifying active conformations in the binding site. This knowledge is expected to be useful in the abstraction of novel inhibitors from compound libraries, before further investigations are implemented. This method could also be adapted to tackle similar protein ligand systems.

Acknowledgments

NG thank Council of Scientific and Industrial Research (CSIR), New Delhi, for funding. SB thanks UGC for Senior Research Fellowship.

Author Contributions

Conceived and designed the experiments: SB, NG. Analyzed the data: SB, BD. Wrote the first draft of the manuscript: SB. Contributed to the writing of the manuscript: SB, BD, NG. Agree with manuscript results and conclusions: SB, BD, NG. Jointly developed the structure and arguments for the paper: SB, NG. Made critical revisions and approved final version: SB, NG. All authors reviewed and approved of the final manuscript.

Supplementary Materials

Supplementary Figure 1.
Supplementary Figure 2.

Supplementary Table 1.
Supplementary Table 2.
Supplementary Table 3.

REFERENCES

1. Spychala J, Datta NS, Takabayashi K, et al. Cloning of human adenosine kinase cDNA: sequence similarity to microbial ribokinases and fructokinases. *Proc Natl Acad Sci U S A*. 1996;93:1232–7.
2. Park J, Gupta RS. *Adenosine Metabolism, Adenosine Kinase, and Evolution, in Adenosine: A Key Link between Metabolism and Central Nervous System Activity*. New York: Springer; 2012:23–54.
3. Boison D. Adenosine kinase: exploitation for therapeutic gain. *Pharmacol Rev*. 2013;65:906–43.
4. Boison D. Adenosine kinase, epilepsy and stroke: mechanisms and therapies. *Trends Pharmacol Sci*. 2006;27:652–8.
5. Muchmore SW, Smith RA, Stewart AO, et al. Crystal structures of human adenosine kinase inhibitor complexes reveal two distinct binding modes. *J Med Chem*. 2006;49:6726–31.
6. Ugarkar BG, Castellino AJ, DaRe JS, et al. Adenosine kinase inhibitors. 3. Synthesis, SAR, and antiinflammatory activity of a series of L-lyxofuranosyl nucleosides. *J Med Chem*. 2003;46:4750–60.
7. Gomtsyan A, Didomenico S, Lee CH, et al. Synthesis and biological evaluation of pteridine and pyrazolopyrimidine based adenosine kinase inhibitors. *Bioorg Med Chem Lett*. 2004;14:4165–8.
8. Gabb HA, Jackson RM, Sternberg MJE. Modeling protein docking using shape complementarity, electrostatics and biochemical information. *J Mol Biol*. 1997;272:106–20.
9. Markt P, Petersen RK, Flindt EN, et al. Discovery of novel PPAR ligands by a virtual screening approach based on pharmacophore modeling, 3D shape, and electrostatic similarity screening. *J Med Chem*. 2008;51:6303–17.
10. Sarvagalla S, Singh VK, Ke YY, et al. Identification of ligand efficient, fragment-like hits from an HTS library: structure-based virtual screening and docking investigations of 2H- and 3H-pyrazolo tautomers for aurora kinase A selectivity. *J Comput Aided Mol Des*. 2015;29:89–100.
11. Ke YY, Singh VK, Coumar MS, et al. Homology modeling of DFG-in FMS-like tyrosine kinase 3 (FLT3) and structure-based virtual screening for inhibitor identification. *Sci Rep*. 2015;5:11702.
12. Kontoyianni M, Sokol GS, McClellan LM. Evaluation of library ranking efficacy in virtual screening. *J Comput Chem*. 2005;26:11–22.
13. Perola E, Walters WP, Charifson PS. A detailed comparison of current docking and scoring methods on systems of pharmaceutical relevance. *Proteins*. 2004;56:235–49.
14. Kumar R, Garg P, Bharatam PV. Shape-based virtual screening, docking, and molecular dynamics simulations to identify Mtb-ASADH inhibitors. *J Biomol Struct Dyn*. 2015;33:1082–93.
15. Wei Y, Li J, Chen Z, et al. Multistage virtual screening and identification of novel HIV-1 protease inhibitors by integrating SVM, shape, pharmacophore and docking methods. *Eur J Med Chem*. 2015;28:409–18.
16. Kirchmair J, Distinto S, Markt P, et al. How to optimize shape-based virtual screening: choosing the right query and including chemical information. *J Chem Inf Model*. 2009;49:678–92.
17. Kar RK, Ansari MY, Suryadevara P, et al. Computational elucidation of structural basis for ligand binding with Leishmania donovani adenosine kinase. *Biomed Res Int*. 2013;2013:609289.
18. Bhutoria S, Ghoshal N. Deciphering ligand dependent degree of binding site closure and its implication in inhibitor design: a modeling study on human adenosine kinase. *J Mol Graph Model*. 2010;28:577–91.
19. Ugarkar BG, Castellino AJ, DaRe JS, et al. Adenosine kinase inhibitors. 3. Synthesis, SAR, and antiinflammatory activity of a series of L-lyxofuranosyl nucleosides. *J Med Chem*. 2003;46:4750–60.
20. Ugarkar BG, DaRe JM, Kopcho JJ, et al. Adenosine kinase inhibitors. 1. Synthesis, enzyme inhibition and antiseizure activity of 5-iodotubercidin analogues. *J Med Chem*. 2000;43:2883–93.
21. Boyer SH, Ugarkar BG, Solbach J, et al. Adenosine kinase inhibitors. 5. Synthesis, enzyme inhibition, and analgesic activity of diaryl-erythro-furanosyltubercidin analogues. *J Med Chem*. 2005;48:6430–41.
22. Lee CH, Daanen JF, Jiang M, et al. Synthesis and biological evaluation of clitocine analogues as adenosine kinase inhibitors. *Bioorg Med Chem Lett*. 2001;11:2419–22.
23. Cerius^2, version 4.10; Accelrys Inc., San Diego, CA,USA, 2006. Available at: http://www.accelrys.com.
24. Minitab 17, State College PA, USA, 2014. Available at: http://www.minitab.com.
25. Jones G, Willett P, Glen RC, Leach AR, Taylor R. Development and validation of a genetic algorithm for flexible docking. *J Mol Biol*. 1997;267:727–48.
26. Hopfinger AJ, Tokarsi JS. *3D-QSAR. Practical Applications of Computer-Aided Drug Design*. New York: Marcel Dekker Inc. ; 1997:105–63.
27. Sharma P, Chhabra S, Rai N, Ghoshal N. Exploration of rate-limiting conformational state for 5-[(7-chloro-4 quinolinyl)amino]-3 [(alkylamino)methyl] [1,1'-biphenyl]-2-ols and Nω-oxides (tebuquine analogues) for antimalarial activity using molecular shape analysis and molecular field analysis studies. *J Chem Inf Model*. 2007;47:1087–96.
28. Sharma P, Ghoshal N. Exploration of a binding mode of benzothiazol-2-yl acetonitrile pyrimidine core based derivatives as potent c-Jun N-terminal kinase-3 inhibitors and 3D-QSAR analyses. *J Chem Inf Model*. 2006;46:1763–74.
29. MOE, version 2008.10; Chemical Computing Group Inc., Montreal, Canada, 2008. Available at: http://www.chemcomp.com.
30. Catalyst, version 4.1; Accelrys Inc., San Diego, CA, USA, 2006. Available at: http://www.accelrys.com.
31. Zhang YE, Kouni MH, Ealick SE. Structure of *Toxoplasma gondii* adenosine kinase in complex with an ATP analog at 1.1 angstroms resolution. *Acta Crystallogr D Biol Crystallogr*. 2006;62(pt 2):140–5.
32. Kuettel S, Greenwald J, Kostrewa D, Ahmed S, Scapozza L, Perozzo R. Crystal structures of T. b. rhodesiense adenosine kinase complexed with inhibitor and activator: implications for catalysis and hyperactivation. *PLoS Negl Trop Dis*. 2011;5(5):e1164.