An Efficient Virtual Screening Protocol for the Search of Adenosine Kinase Inhibitors

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ABSTRACT

In the virtual screening approach, the basic idea is to incorporate structural information about pharmacophoric features derived from bioactive conformation of known ligands. A common feature pharmacophore represents a significant step towards the understanding of a receptor ligand binding interaction and therefore the pharmacophoric features will reflect the mode of interaction with the receptor. However, sometimes similar ligands do not bind in a similar fashion. Thus by developing a common feature hypothesis may mislead in virtual screening. This study exploits the structural information from protein, ligands and their binding mode for virtual screening.

Adenosine Kinase (AK) is an enzyme which converts adenosine to adenosine monophosphate in an ATP dependent manner. Recently, studies have been performed on analogues of tubercidin as potent adenosine kinase inhibitors possessing antiseizure activity [1, 2, 3]. So far, several highly potent AK inhibitors were identified but none of them suitable for further development. Here we took a set of active ligands (tubercidin analogues) which possess common core but differ in side chain substitution. This study combines the pharmacophore analysis and docking to derive binding mode of tubercidin analogues. The docking studies prove the existence of diverse (different clusters in the active site) binding modes of analogues as presumed by a workgroup based on the SAR of these molecules [1]. The hydrophobic interaction is likely to be a fundamental determinant of the difference in their binding modes. These docking based pharmacophores could be used for virtual screening of potential inhibitors.

INTRODUCTION

Adenosine kinase (AK) is a key enzyme in the regulation of extracellular adenosine and intracellular adenylate levels. Inhibitors of adenosine kinase elevate adenosine to levels that activate nearby adenosine receptors and produce a wide variety of therapeutically beneficial activities. Crystal structure of AK (PDB code 1BX4) is having adenosine molecule in the active site and tubercidin analogues are known to bind at the same site. Crystal structure (PDB code 2I6A) of AK bound to 5-iodotubrcidin shows the similar type of interaction with the AK as of adenosine. In the present study, docking strategies were applied to find the binding mode and was used for the generation of pharmacophore.

METHODS

All ligands (71 tubercidin analogues) and the receptor were prepared using Cerius2 4.10. The ligands were energy minimised using the universal force-field and the protein was minimized using CHARMM force field. Each ligand was docked using GOLD 3.1 in 10 genetic algorithm (GA) runs. The position of the active-site was introduced and the radius was set to 10 Å. Chemscore fitness function was used for selection of best posses. Two water molecules w1 and w2 were taken as off and on modes respectively (Fig1). Pharmacophore were generated using CATALYST based on the analysis of interactions seen in Discovery Studio 1.7.

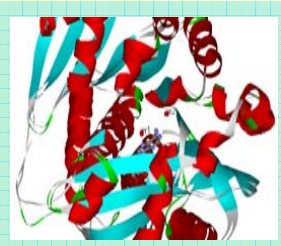


Fig:1 Position of water molecules. W1 replaceable, W2 present in active site

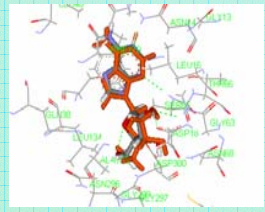


Fig: 2 docked adenosine in active site (crystal bound structure shown in brown)

RESULTS & DISCUSSION

Adenosine molecule was initially docked as a control, and the result compared to the crystal structure of the enzyme inhibitor complex, shown in fig.2. In 5- iodotubercidin, iodine replaces a highly conserved water molecule(4) . While comparing the PDB structures(1BX4 and 2I6A) it was found that one conserved water molecule is present (W2) and one molecule is replaceable(W1)(fig:1). The docking identifies different orientation of core structure within the binding site(fig3). The aromatic ring of tubercidin moeity in diaryl analogues were found to make amino pi interation with the Glu38 whereas the same forming stacking interaction with Phe 170 in case of nonaryl analogues. As seen from the graph (1), non aryl analogues (group 1) possess less rmsd with the substrate, while aryl analogues (group 2) possess large rmsd with the substrate.

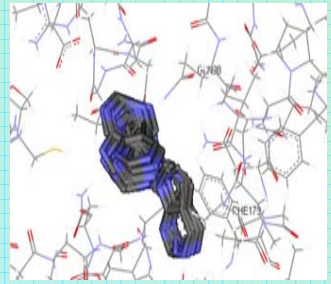
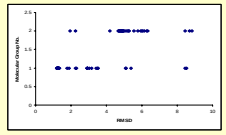
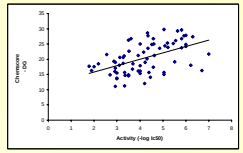


Fig:3 showing alignment of core moiety of nonaryl and aryl analogues within the active site



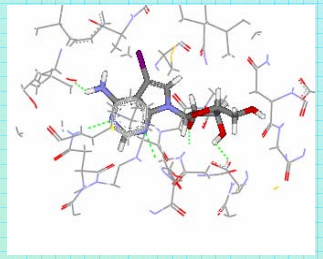
Graph:1 showing molecules(grouped in 1 as non aryl and 2 as aryl analogues) and their RMSD's with the bound ligand.



Graph::2 showing correlation (R=0.518) of chemscore function(- DG) and Activity

In the nonaryl ligands the aromatic ring of tubercidin moeity was found to make hydrogen bonding with water molecule and stacking interaction with Phe 170 (fig:4a).

None of the scores were correlated with the IC50 values. Binding affinity function of chemscore was having correlation of 0.518 (Graph:2) with the activity. This was inturn found to be correlated (R=0.90) with chemscore -lipo suggesting that for binding libophilic factor is important. It was also observed that the polarizability and activity of the aryl analogues are correlated (Graph:3), suggesting that electrostatic interaction may play important role in showing high activity. Aryl analogs possess high electron density over the rings and thus favour electrostatic interaction with the amino groups of amino acid side chains. The orientation of aryl analogues is stabilized by amino pi interactions between Asn and aromatic rings (fig:4b). Figures (5 and 6) are showing pharmacophoric features, and based on these pharmcophores we have searched Maybridge database and found few hits which binds in both type of binding modes.



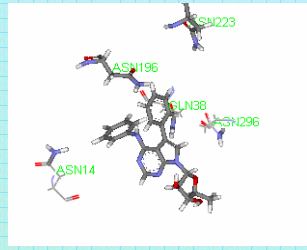
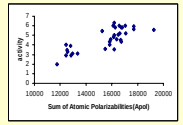


Fig: 4a: Interactions of a nonaryl analogue with protein side chains
4b: Interactions of a very active diaryl analogue with protein side chains



Graph:3 showing relationship between Sum of atomic polarizabilities (Apol) and activity (R=0.801)

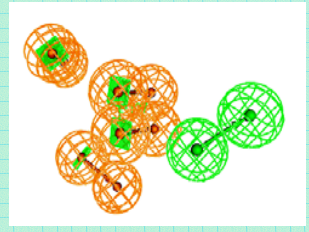


Fig:5 Pharmacophore model based on docked mode of an aryl tubercidin analogue. Brown spheres for aromatic feature and green for hydrogen bond acceptor feature.

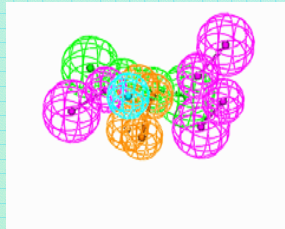


Fig:6 Pharmacophore model based on docked mode of nonaryl tubercidin analogue (5-Iodotubercidin). Brown spheres for aromatic feature and green for H bond acceptor feature, purple for H bond donor and blue for hydrophobic feature.

CONCLUSIONS

In this study pharmacophores have been developed based on docking and analysis of binding modes. These pharmacophores could be used efficiently for the identification of new hits by virtual screening.

ACKNOWLEDGMENTS

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