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Virtual Screening And Docking Study to Identify Aspartate Semi Aldehyde Dehydrogenase (ASADH) Inhibitors

Mohammed Zuhaib, Anju Sharma*
Amity Institute of Biotechnology, Amity University Uttar Prades, Lucknow Campus, Uttar Pradesh

Abstract

Aspartate-semi aldehyde dehydrogenase (ASADH) is the enzyme that occurs in the biosynthetic pathway at a very first branch point. It lies in the biosynthetic pathway of important amino acids including methionine and lysine and the cell-wall component diaminopimelate (DAP). The enzymatic reaction of ASADH is the reductive de phosphorylation of aspartyl- β -phosphate (ABP) to aspartate β -semi aldehyde (ASA). Aspartate pathway is very essential for the survival of many microbes and is absent in humans, the enzymes involved in this pathway can be considered to be potential antibacterial drug targets. In this work, the structure of ASADH from Mycobacterium tuberculosis H37Rv (Mtb-ASADH) has been determined in complex with S-methyl-L-cysteine sulfoxide (SMCS) and sulfate at 1.95 Å resolution. The overall structure of Mtb-ASADH is similar to those of its orthologues. S-methyl cysteine sulfoxide is the known covalent bond forming inhibitor of ASADH enzyme. By this virtual screening and docking study an attempt has been made to identify unknown inhibitors of ASADH enzyme. Ligands (unknown inhibitors) with the least binding energy were selected. Selected ligands were then compared to the known inhibitor of ASADH enzyme on the basis of binding energy. ADMET properties of the screened inhibitor (unknown ligand) were calculated. These ADMET properties were analyzed to check drug likeliness. Based on the mentioned analysis it has been suggested that the screened potent compound is capable to inhibit the role ASADH enzyme in biosynthetic pathway.

Key Words: ASADH, Docking, Inhibitors, Virtual Screening

1. Introduction

1.1 Aspartate Semi aldehyde Dehydrogenase Enzyme

The reactions catalyzed by enzymes are organized into sequential pathways and are responsible for producing the molecules needed to sustain life.[1] There are many important metabolic pathways which are present in all known life forms , there is also significant differences between mammalian and microbial metabolism.[2] All-important metabolic building block molecules are synthesized by most of the microbial species, while mammals get them from dietary sources.[3]These metabolic differences provide a large number of potential protein targets to be examined for the development of selective antimicrobial agents. There is a demanding need to identify new antibiotics that are effective against new targets to fight the growing threat from pathogenic species that have become resistant to existing antibiotics .[4] There are, however, many problems that are needed to be resolved for biocides which are effective in developing against metabolic enzyme targets.[5] Microorganisms which are infectious often use their host as a source of essential metabolites, thus bypassing inhibitors designed to block key steps in microbial metabolism. In many instances microorganisms have developed alternative routes for the production of important metabolites that can readily bypass inhibited enzyme reactions .[6] Essential enzymatic pathways can also be altered in response to an antibiotic threat. Although these microbial pathways may not exist in mammals, related enzymes with similar active site geometries or substrate binding motifs can potentially interact with even the most carefully designed inhibitors.[7] The structure and mechanism of a bacterial metabolic enzyme must be thoroughly characterized before a potential drug target can be fully evaluated. Aspartate β-semi aldehyde dehydrogenase (ASADH) is a key enzyme in an essential amino acid biosynthetic pathway that is not present in mammals.[8] This enzyme has been thoroughly investigated and is now being examined as a target for the development of new antimicrobial agents.[9]

The aspartate biosynthetic pathway is present only in plants and microbes. The commitment step to this pathway is the phosphorylation of aspartic acid catalyzed by a family of aspartokinases (AK). The next enzyme in the pathway, ASADH, catalyzes the production of aspartate semi aldehyde (ASA) that is located at a critical junction in this pathway.[10]

1.2 Molecular Docking

Molecular docking is the process that involves placing molecules in appropriate configurations to interact with a receptor. Molecular docking is a natural process which occurs within seconds in a cell.[13]

Molecular docking is a frequently used method in structure-based rational drug design. It is used for evaluating the complex formation of small ligands with large biomolecules, predicting the strength of the bonding forces and finding the best geometrical arrangements. [14]

1.3 Virtual Screening

Computational screening of databases has become very popular in the drug research. Virtual screening uses

computer based methods to discover new ligands on the basis of biological structures. Virtual screening is divided into screening using active compounds as templates (ligand based virtual screening) and structural based screening (docking) .[12] Ligand based screening techniques mainly focus on comparing molecular similarity analyses of compounds with known and unknown moiety, regardless of the methods of the used algorithm. Docking is a computational tool of structure based drug design to predict protein ligand interaction geometries and binding affinities.[15] In this review we provide an overview of the already used ligand based virtual screening and the docking with various databases, filters, scores and applications in the recent research in the pharmaceutical field.[11]

2. Material and Methods

The entire experiments were performed on Windows 10 and Linux operating systems with 8 GB RAM , NVIDIA graphics and 1 TB Hard disk. Hardware of the system is as follows Intel® Core™ i5-4210 CPU@1.40 GHz 2.40GHz.
Following soft wares were also used:
Auto dock 4.0, Adt tools, Python programming language, Biovia discovery studio visualizer, Cygwin command prompt, Also a Linux system with Schrödinger.

2.1 Receptor File Generation

The three dimensional structure of Aspartate Semi-aldehyde Dehydrogenase was downloaded from RCSB Protein Data Bank having PDB ID 3TZ6 in PDB file format. Refinement of the structure was done in Discovery Studio Visualizer.
Ligands and water molecules bound to the structure were deleted and the modified file was saved.
After that the receptor coordinate file with .pdbqt extension was created under Cygwin directory using Auto Dock tools.

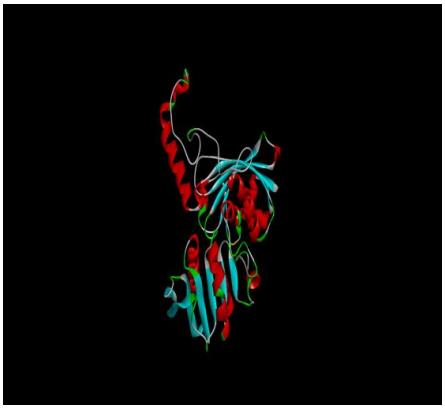

Figure 1: 3D Structure of Aspartate Semi-aldehyde Dehydrogenase represented in cartoon format

2.2 Dataset Generation And Ligand Coordinate File Preparation

A dataset of 100 natural compounds was downloaded from enhanced NCI databases in SDF format. ADME filter, drug likeliness and tanimoto index were considered as parameters while searching for the desired natural compounds. These files were converted in PDB format. Then ligand coordinate files were create having .pdbqt extension under Cygwin directory using Auto Dock tool. Each ligand was opened in Auto Dock Tools workspace and the atom in the ligand most suitable as its best root for the torsion tree is detected. Then the total number of active bonds or torsions is specified and the ligand coordinate file is saved under working directory.

2.3 Grid Parameter File Preparation

The Grid Parameter file is required file is required for computation of grid map for types of atom present in the ligand. A Grid box is made which consists of three-dimensional lattice centred on the macromolecule. The volume of grid box should be large enough to allow the ligand to rotate freely. The grid box should cover the active site of macromolecule allowing ligand molecule to move inside it without any constraints.

2.4 Docking Parameter File Preparation

Auto Dock requires the docking parameter file to specify map files ,the required ligand molecule ,the centre and number of torsions, to specify the docking algorithm for docking.

2.5 Virtual Screening And Docking Studies

Generated dataset was screened against the active site of the protein. A well-known inhibitor- S-methyl-L-cysteine sulfoxide was used as reference molecule .Molecular ligand docking was performed for Aspartate Semi-aldehyde Dehydrogenase with these molecules .

Auto Dock uses Lamarckian genetic algorithm to perform docking of ligand molecules with the receptor. After several runs, analysis of predicted energy and consistency of results is combined to identify the best docked model. The obtained models are evaluated on the basis of binding energy.

2.6 Calculation of ADME and Toxicity

ADME properties like Molecular Weight , hydrophilicity , hydrophobicity , solvent accessible surface area , number of rotable bonds ,donor–hydrogen bonds, acceptor-hydrogen bonds etc. of natural inhibitors were calculated by QuikProp v3.9 module of Maestro.

OSIRIS property explorer was used for toxicity prediction of inhibitor of ASADH enzyme . (http://www.organic-chemistry.org/prog/peo/). It is an online prediction tool for the analysis of drug relevant property like mutagenic, tumorigenic, irritant, reproductive effective , cLogP, Solubility, Molecular Weight.

3. Result And Discussion

3.1 Protein Preparation

Preparation of ASADH enzyme included bond order refinement and their minimization. The cavity was selected and prepared by adding H-bonds, disulphide bonds and water molecules beyond 5 A were removed with the help of Discovery Studio Visualizer. At last the protein was reviewed and modified by checking the metal binding states , chains balance and pH maintenance of the system.

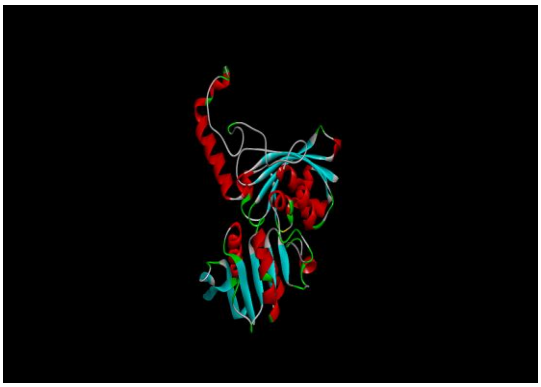

Figure 2 3D Structure of refined protein molecule (ASADH) without any water molecule

3.2 Analysis of Virtual Screening

The known inhibitor (S-methyl-L-cysteine sulfoxide) was docked with the refined structure of protein. Also the unknown inhibitors were docked wth the protein.

The analysis and the screening of the unknown inhibitors was done on the basis of least binding energy as compared to the naturally existing inhibitor (S-methyl-L-cysteine sulfoxide). The obtained models are evaluated on the basis of binding energy.

Detailed investigation of docking scores and binding affinity of natural screened inhibitors with both proteins was done. Out of which, four natural compounds have shown common inhibitory activity against both the receptors and these compounds had also good binding affinities with both receptors on the basis of their docking score (Table 1). 2D structures of screened molecules are shown in Fig. 3. In Table 1, reference molecule which is already known for its inhibitory effect against the protein were also listed and their scores were analyzed & compared with the screened inhibitors.

Various energies like van der Wall energy, and Electrostatic energy of ASADH with these screened six common ligands were also calculated by using Cygwin depicted in Table 2

The docking studies indicated that the screened top ranked compounds showed strong hydrogen bonding interactions with the receptor

Table 1 Common Natural inhibitor of protein with their Docking Score

S.No.		Compound Name	Receptor ASADH Docking Score/Binding Energy
1	Selected Ligands/Unknown inhibitors	NSC12806	-7.59
2		NSC12812	-7.57
3		NSC3084	-7.52
4		NSC11704	-7.46
5		NSC12819	-7.34
6		NSC12809	-7.33
7	Reference/Known inhibitors	S-methyl-L-cysteine Sulfoxide	-6.77

Table 2 Energy in Kcal/mol for ASADH complex with top scoring natural inhibitors

S.no.	Natural Compounds	ASADH V.D.W Electrostatic Energy

1	NSC12806	-5.94	-3.45
2	NSC12812	-6.61	-3.34
3	NSC3084	-5.45	-3.86
4	NSC11704	-5.05	-4.5
5	NSC12819	-6.77	-3.26
6	NSC12809	-6.46	-2.96

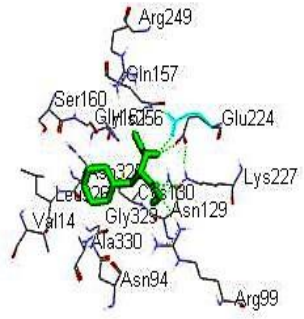

Figure 3 Docked complex of ASADH with natural ligand/inhibitor in ribbon representation and their interacting residues NSC12806; dotted lines shows the hydrogen bond interaction.

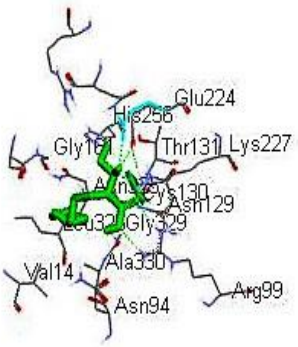

Figure 4 Docked complex of ASADH with natural ligand/inhibitor in ribbon representation and their interacting residues NSC12812; dotted lines shows the hydrogen bond interaction.

3.3 ADME descriptors analysis

Fifteen principle descriptors as shown in table 3 were calculated for selected natural inhibitors by QuikProp module of the Schrodinger software.

ADMET properties are significant measures for a molecule which can serve as a drug as shown in table 4 . ADMET properties like percentage age of human absorption of good drug should be more than 25%.

Table 3 ADME Principle descriptors of known and unknown inhibitory molecules

S.no	Principle Descriptors	NSC12806	S-methyl-L-cysteine sulfoxide	Range 95% of drugs
1	Molecular Weight	119.292	151.180	130/725
2	Dipole Moment	2.975	4.921	1/12.5
3	SASA	462.183	339.818	300/1000
4	FOSA	368.988	136.494	0/750
5	FISA	93.195	189.763	7/330
6	PISA	0	0	0/450
7	WPSA	0	13.560	0/175
8	Molecular volume	769.382	517.576	500/2000
9	PSA	56.036	93.75	7/200
10	Donor HB	2.00	2	0/6
11	Acceptor HB	3.5	6	2/20
12	Glob	0.879	0.917	0.75/0.95
13	RotBond	5	4	0/15
14	IP	9.55	9	7.9/10.5
15	EA	-0.751	0.494	-0.9/1.7

Notations: a Molecular Weight; b Dipole Moment; solvent Accessible Surface Area; d Hydrophobic SASA; e Hydrophilic SASA; f Carbon Pi SASA; g Weakly Polar SASA; h Molecular

Volume; I van der Waals Polar SA; j Donor-Hydrogen Bonds; k Acceptor-Hydrogen Bonds; l Globularity; m Rotable Bond; n Ionization Potential; o Electron Potential

4. Conclusion

As per the best of our knowledge, none of the reported work explored the in-silico identification of natural inhibitors for Aspartate Semi-aldehyde Dehydrogenase(ASADH) . In this work, we have identified four multi- targeted natural compounds by virtual screening, molecular docking. Drug likeness, bioavailability, and toxicity of the selected compounds were also calculated by ADMET analysis. The ADMET score of these screened ligands suggest an overall favorable pharmacokinetics to be accepted as lead molecule. Molecular docking studies showed that this screened natural compound is expected to bind the inactive form of ASADH as S-methyl-L-cysteine sulfoxide do, although requiring a lower conformational stringency, with the ability of binding more intermediate conformations than known inhibitors. The improved binding affinity score and number of hydrogen bonds suggest better interactions in active site of candidate receptors. Taken together, these observations raised the possibility that the small molecule inhibitors can act against ASADH, proving its effectiveness to become a drug. Thus, we can safely conclude this natural compound individually can be used to synthesize new drug molecules involved in biosynthesis pathway.

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