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RESEARCH ARTICLE

Docking Studies on Inhibitors of carcinogenic retinoic acid metabolizing enzyme CYP26A1

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Abstract

Vitamin A deficiency (VAD) is associated with increased susceptibility to carcinogenesis in animal models and elevated risk for a number of human cancers. The gene encoding a Cytochrome P450 enzyme specifically involved in metabolic inactivation of retinoic acid (RA). Enhanced expression of CYP26A1 suppress cellular responses to anoikis and consequently promotes anchorage independent growth. This transformed phenotype was sufficient to markedly increase tumorigenic and metastatic potential. The protein – ligand interaction plays a significant role in structural based drug designing. In the present work the predicted model of CYP26A1 was used as target receptor protein. The potential chemical compound which could be used for the treatment were screened on the basis of drug likeliness, toxicity and ambiguity. The binding affinity between the receptor protein and final screened chemical ligands were analyzed by performing docking studies. Based on the binding affinity it was shown that the chemical compound named ZINC01607786 was to be potent drug (inhibitor) ligand molecule acting against the target receptor CYP26A1.

Introduction

Vitamin A is important for health. The vitamin A metabolite, all-trans retinoic acid (atRA), has essential roles in the formation and maintenance of tissues, stemming from its potency as a regulator of cell proliferation, differentiation and apoptosis. Although essentially all cell types express nuclear RA receptors (retinoic nuclear receptors and retinoid-X-receptors) and can potentially respond to RA to positively or negatively regulate expression of RA target genes [2], cellular responsiveness is determined by the bioavailability of RA. This is regulated by the vitamin A nutritional status and the coordinated balance between RA biosynthesis and catabolism. RA-catabolizing cytochrome P450 enzymes (CYP26A1, B1 and C1) are specifically involved in the metabolic inactivation of RA, and thus limit RA bioavailability of the cell by restricting RA access to transcriptional machinery by converting RA to biologically inactive metabolites [1,9,10,13].

Early studies of retinal deficiency on animals have shown a plausible association between vitamin A deficiency (VAD) and carcinogenesis [14]. However, the underlying mechanisms explaining how VAD might contribute to the high incidence of cancer and increased susceptibility to carcinogenic result remain to be defined. Despite the fact that the CYP26 enzymes may have a similar but separate role in limiting the consequences of fluctuations in nutritional vitamin A, the possibility that pathological conditions such as cancer might involve aberrant expression of CYP26A1 has recently emerged from several studies. Accumulating evidence has shown that enhanced RA catabolic activity has been observed in various types of cancer and elevated CYP26A1 expression has been detected in a number of cancer cell types [3,11,12]. The CYP26A1-

mediated catabolism of RA results in a state of functional VAD that can contribute to carcinogenic processes. In our research study we try to find the suitable analogues with high binding affinity, which could be a possible lead molecule.

Computational Biology and bioinformatics have the potential not only of speeding up the drug discovery process thus reducing the costs, but also of changing the way of drugs are designed. Rational Drug Design (RDD) helps to facilitate and speedup the drug designing process, which involves variety of methods to identify novel compounds. One such method is the docking of the drug molecule with the receptor (target). The site of drug action, which is ultimately responsible for the pharmaceutical effect, is a receptor [6]. Docking is the process by which two molecules fit together in 3D space.

Materials and Methods

For our present study we used bioinformatics tools, biological databases like ZINC collection of commercially available chemical compounds, PDB (Protein Data bank), ADME/T tools and softwares Hex and Vina.

Hex is an Interactive Molecular Graphics Program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules. Hex can also calculate Protein-Ligand Docking, assuming the ligand is rigid, and it can superpose pairs of molecules using only knowledge of their 3D shapes [7]. It uses Spherical Polar Fourier (SPF) correlations to accelerate the calculations and its one of the few docking programs which has built in graphics to view the result [4,5].

RASMOL [Raster Display of Molecules] is a molecular graphics program intended for the structural visualization of proteins, nucleic acids and small biomolecules. The program reads in molecular coordinate files and interactively displays the molecule on the screen in variety of representations and color schemes.

Bioinformatics is seen as an emerging field with the potential to significantly improve how drugs are found, brought to the clinical trials and eventually released to the marketplace. Computer – Aided Drug Design (CADD) is a specialized discipline that uses computational methods to simulate drug – receptor interactions. CADD methods are heavily dependent on bioinformatics tools, applications and databases [6].

The structure of CYP26A1 generated from modeller9v9 and the structure optimization and energy minimization was performed by using the Gromacs. The active site was predicted in the modeled structure by using Q-siteFinder (http://www.bioinformatics.leeds.ac.uk/qsitefinder). It works by binding hydrophobic (CH3) probes to the protein, and finding clusters of probes with the most favourable binding energy. These clusters are placed in rank order of the likelihood of being a binding site according to the sum total of binding energies for each cluster. The chemicals short listed from NCI diversity dataset III through virtual screening by using Vina. These chemical screened on the basis of druglikness, toxicity and ambiguity.

Docking allows the scientist to virtualy screen a database of compounds and predict the strongest binders based on various scoring functions. It explores ways in which two molecules, such as drugs and human retinoic acid metabolizing enzyme CYP26A1 receptor fit together and dock to each other well, like pieces of a three-dimensional jigsaw puzzle. The molecules binding to a receptor, inhibit its function, and thus act as drug. The collection of top 10 screened molecules receptor complexes was identified via docking and their relative stabilities were evaluated using molecular dynamics and their binding affinities, using free energy simulations. The parameters used in the docking process were following:

- Correlation type – Shape only
- FFT Mode – 3D fast lite.
- Grid Dimension – 0.6
- Receptor range – 180
- Ligand Range – 180
- Twist range – 360
- Distance Range – 40

The drug were docked with the receptor using the above parameters.

The top ranked ligand receptor complexs were further used for study of protein ligand interactions using LigPlot+ program. LigPlot+ is a successor to the original LIGPLOT program for automatic generation of 2D ligand-protein interaction diagrams (http://www.ebi.ac.uk/thornton-srv/software/LigPlus) **[8]**. Using this program the hydrogen and hydrophobic interactions between the ligand and amino acid residues within the active site of the CYP26A1 enzyme were analyzed.

Result and Discussion

According to the Lipinski rule of five, the chemicals found to have a molecular weight less

than 500, logP not greater than 5, not more than 5 hydrogen bond donors and less than 10 hydrogen bond acceptors. Based on above rule 10 compounds were selected. From the primary (drug likeliness), secondary (toxicity) and tertiary (amiguity) screening processed, screened molecules was further validated through docking studies. The Docking results tabulated in the below table1. The comparative analysis was undertaken based on the best binding affinity of the drug candidate was predicted.From the overall analysis ZINC01607786 inhibitor was found to have best binding affinity with the receptor CYP26A1 owing to its lowest docking energy -418.2 in figure 1. This can be used used in the treatment of cancer after a considerable clinical trail.

ZINC01607786 in figure 2 was having four hydrogen bond and four hydrophobic interactions. The hydrogen bond interactions are with Lys 137, His 133, Arg 440, His 129 at the distance of 2.79, 2.75, 2.87,3.25 Å respectively and the hydrophobic interactions are shown at position Lys 134, Leu 439, Leu 378, Ser 109. Intrestingly it was found that His 129, His 133, Lys137 amino acid residue was involved in hydrogen bond interactions within the active site of CYP26A1 in all top ranked ligand. These residue might play crucial role in ligand interaction with the active site of retinoic acid metabolizing enzyme receptor CYP26A1. Top four ligands i.e. ZINC01607786, ZINC01568793, ZINC05462666 and ZINC03916235 are having lower lower docking energy scores which shows higher binding affinity towards retinoic acid metabolizing inactivation. These ligand might be a potent inhibitor of retinoic acid catabolism.

Table 1: Docking results of Cytochrome P450 26A1 receptor with top 10 screened ligand molecules.

S. No	LIGAND (ZINC IDs)	CHEMICAL NAME	E-VALUE
1.	ZINC03916235	1-(2-Cyano-3,12,28-trioxooleana-1,9(11)-dien-28-yl)-1H-imidazole (popular TP 235)	-321.7
2.	ZINC01855333	2, 2'-Spirobi[2H-benz[f]indene]-5,5'-dione	-164.4
3.	ZINC03830627	1-ethynyl-10a,12a-dimethyl-2,3,3a,3b,4,5,10,10a,10b,11,12,12a-dodecahydro-1H-cyclopenta[7,8]phenanthro[3,2-d][1,2]oxazol-1-ol (Danazol)	-251.8
4.	ZINC01629569	MLS000756825	-105.9
5.	ZINC01568793	8-[4-[4-(7,9-dioxo-8-azaspiro[4.4]nonan-8-yl)-3-methylphenyl]-2-methylphenyl]-8-azaspiro[4.4]nonane-7,9-dione	-339.6
6.	ZINC05462666	Chaetochromin B	-330.5
7.	ZINC01625092	ethyl 2-(biphenyl-4-ylmethylidene)-3-oxobutanoate	-253.9
8.	ZINC013208019	N-(2,3-dimethylphenyl)-N'-(4-fluorophenyl)-5-keto-7-propyl-thiazolo[3,2-a]pyrimidine-2,3-dicarboxami	-275.1
9.	ZINC01607786	N-(4-bromo-1-naphthyl)-1-hydroxy-2-naphthamide	-418.2
10.	ZINC05410104	MLS003115309	-267.5

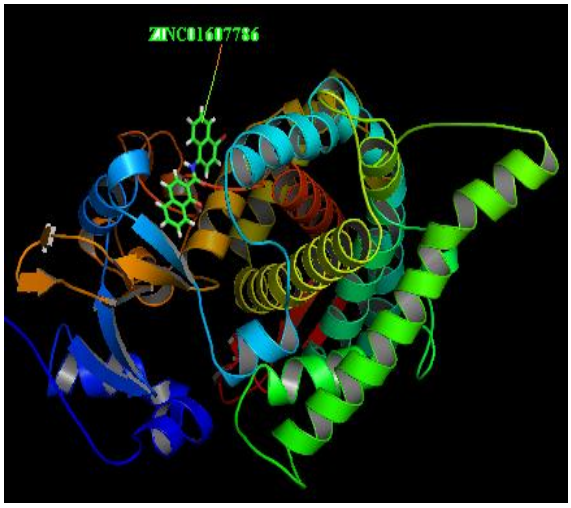

Figure 1: Docked structure of receptor CYP26A1 and ligand ZINC016077886.

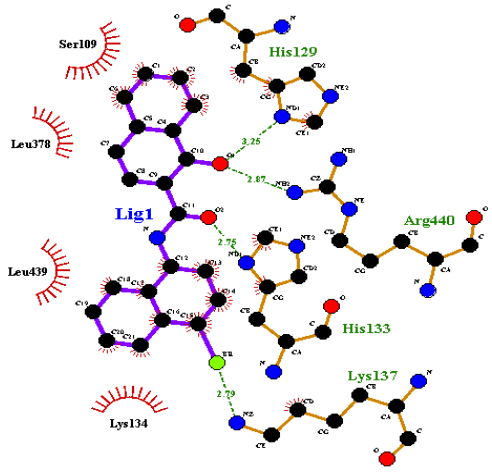

Figure 2: LigPlot representation of CYP26A1 (receptor)–ZINC016077886 (ligand) in human retinoic acid metabolism.

Conclusion

The Protein-Ligand interaction plays a significant role in structural based drug designing. In the present work we have taken the receptor human retinoic acid metabolizing enzyme CYP26A1 and identified the drugs that were used against carcinogenic retinoic acid metabolism. When the receptor CYP26A1 was docked with drugs minimum energy value was obtained. These ligands were seems as therapeutic target in cancer. When CYP26A1 is docked, it will cease the act as retinoic acid metabolism inactivation. Out of the ten compounds inhibitor ZINC016077886 (N-(4-bromo-1-naphthyl)-1-hydroxy-2-naphthamide) showed the least docking energy and thus can be considered as the most acceptable drug candidate. The study can be if followed by clinical study.

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