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Design and Development of Potent Drug Inhibitor to MDM2 Protein in Cancer Through Molecular Docking Studies

KEYWORDS: cancer, MDM2 protein, GOLD Docking.

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ABSTRACT *Cancer is a class of diseases characterized by out-of-control cell growth. Cancer is a leading cause of death worldwide. The p53 tumor suppressor (MDM2) is one of the principal mediators of cell-cycle arrest and the activation of apoptosis in response to cellular injuries. The aim of present study is designing a small molecule(antagonist) having capability to bind with the over expressed MDM2 protein and blocking its path to bind with p53 tumor suppressor protein that is having sufficient absorption and free of hepatotoxicity and carcinogenicity. A series of new lead analogs were designed on the basis of structure activity relationship properties then minimized and docked against protein which has the template 1YCR using online tools and software's. Docking studies of lead molecule analogs designed by substituting different chemical groups shows good binding affinity towards active site of the protein. This studies may paves a new way for better treatment for cancer*

INTRODUCTION

Cancer is the second leading cause of death in economically developed countries and the third in emergent nations.1 Although survival rates have increased due to efficient anticancer drugs and prevention, many types of cancer still have no effective cure. Cancer is an abnormal growth and proliferation of cells. It is a frightful disease because the patient suffers pain, disfigurement and loss of many physiological processes. Cancer may be uncontrollable and incurable, and may occur at any time at any age in any part of the body. It is caused by a complex, poorly understood interplay of genetic and environmental factors. 2-8 all cancers occur due to activation or mutation of oncogenes, or inactivation of suppressor genes. More than 20 tumor suppressor genes and 50 oncogenes have been identified and characterized. Most of these genes are involved in activation and detoxification of polycyclic aromatic hydrocarbons (PAHs), suggesting a potential role of these compounds in carcinogenesis. 9

The human p53 tumor suppressor protein has been one of the most investigated proteins in cancer research due to the fact that loss of p53 function through mutation and/or deregulation is involved in more than 50% of all human cancers. The role of p53 in controlling the cell cycle and monitoring the integrity of the genome has made it known as the "guardian of the genome". P53 is tightly controlled in the non-stressed cell by its cellular antagonist MDM2 (murine double minute 2) through an auto regulatory feedback loop. Besides the functional loss of p53 through mutation, it can also be inactivated by the over expression or amplification of MDM2, which is the case in many tumors. Thus, disruption of the MDM2–p53 interaction is considered a novel therapeutic strategy for cancer cells that still are endowed with wild-type p53, and a variety of small molecule drug like compounds have been reported that bind to the p53 binding site of MDM2.

EXPERIMENTAL SECTION

Target Identification:

Exact protein targets are identified only for a small fraction of biologically active compounds. Moreover, most small molecule compounds have multiple targets differentially expressed and interconnected in tissue -, cell type - and disease - specific manner. In life sciences research and drug discovery, target identification is a daunting task, which requires expertise in multiple fields, sophisticated experimentation, powerful knowledge resources, computational tools – and a lot of luck.

The methods of Target identification extract useful knowledge from the raw data and help to focus on the relevant items of data. The most sophisticated aspect is the generation of new insights through the combination of information from different sources. Knowledge on the three- dimensional structure (fold) of a protein provides clues on its function and aids in the search for inhibitors and other drugs. To retrieve and validate the MDM2 protein sequence using computational tools such as NCBI, UniProtKB, GeneCards, etc. The X-ray structure of unliganded human MDM2 with the p53 trans activation domain was used in the present study (PDB code: 1YCR).

Active site Identification

Active site of p53 protein was identified using CASTP server. 10 A new program, CAST, for automatically locating and measuring protein binding pockets and cavities, is based on precise computational geometry methods, including alpha shape and discrete flow theory. CAST identifies and measures pockets and pocket mouth openings, as well as cavities. The program specifies the atoms lining pockets, pocket openings, and buried cavities; the volume and area of pockets and cavities; and the area and circumference of mouth openings. When the search is complete, the largest site is automatically displayed on the structure. The results can be used to guide the protein–ligand docking experiment.

Chemical Library:

A chemical library or compound library is a collection of stored chemicals usually used ultimately in high throughput screening. The chemical library can consist in simple terms of a series of stored chemicals. Each chemical has associated information and its physiochemical properties with information such as the chemical structure, molecular formula, weight, logP, hydrogen bond donor, hydrogen bond acceptor, etc. characteristics of the compound. For this library of screening Accelyrs Discovery Studio, ChemSpider, PubChem, ChemBank, etc. databases were used. There are millions of compounds available in these databases. Through the help of these tools we can find a new compound against a melanoma cancer and tested for their ability to modify / inhibit the target protein. In compound screening the major part to test that compound is having druglkeness or must passed ADME properties. .We have used Accelyrs Discovery Studio for the present work.

Molecular Modeling:

If the structure of target or receptor protein is already available in protein structure database then no need to go for

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modeling. Molecular modeling is a powerful methodology for analyzing the three dimensional structure of biological macromolecule. Many methods are used for molecular modeling have been used to address the problem in structural biology. The aim of molecular modeling is to try to relate the biology activity to structure. Computational or Homology modeling or comparative modeling is an able to predict the 3D structure of protein using similar protein having structure is available called template. If three dimensional structure of protein has been derived from template, it may be usable for structure based drug design. The target protein has been modeled by using several tools such as Modeller, Swiss Model, and ESyPred3d etc. The three dimensional structures of receptor protein get by template. Once we get a structure then detect the receptor cavity/active site of the target.

Lead Optimization:
There are many tools available for designing of lead/drug such as Discovery Studio, HyperChem, ChemDraw, ChemSketch, etc. When a drug is a complex chemical mixture, this activity is exerted by the substance's active ingredient or pharmacophore but can be modified by the other constituents. Activity is generally dosage-dependent and it is not uncommon to have effects ranging from beneficial to adverse for one substance when going from low to high doses. Activity depends critically on fulfillment of the ADME criteria. To be an effective drug, a compound not only must be active against a target, but also possess the appropriate ADME (Absorption, Distribution, Metabolism, and Excretion) properties necessary to make it suitable for use as a drug. The drug must possess the ADME parameter for its novel properties. ADME is nothing but the properties prediction of that drug. The properties such as molecule's bioavailability, it is carcinogenic or not, lethal dose (LD50), value of developmental toxicity prediction etc. The all values are calculated by protocols of ACD/LAB.

Molecular Simulation and Docking:
High-throughput screening (HTS) of compound libraries is used to discover novel leads for drug development. When a structure is available for the target, computer-based screening using molecular docking may also be considered. Molecular docking is a computer simulation procedure to predict the conformation of a receptor-ligand complex, where the receptor is usually a target protein and the ligand is either a small designed molecule. It can also be defined as a simulation process where a ligand position is estimated in a predicted or pre-defined binding site. Molecular docking simulations may be used for reproducing experimental data through docking validations algorithms, where protein-ligand conformations are obtained in silico and compared to structures obtained from X-ray crystallography or nuclear magnetic resonance. Furthermore, docking is one of main tools for virtual screening procedures, where a library of several compounds is "docked" against one drug target and returns the best hit. Before docking study, we need to minimize the energy of both molecule (ligand) and receptor (target molecule).These all study carried out through Discovery studio. With the help of this tool we can see the proper intermolecular bonds between ligand-receptor complexes. There were three intermolecular hydrogen bonds seen in the complex of receptor and screened molecule.

Docking method
Docking was carried out using GOLD (Genetic Optimization of Ligand Docking) software which is based on genetic algorithm (GA). This method allows as partial flexibility of protein and full flexibility of ligand. The compounds are docked to the active site of the p53. The interaction of these compounds with the active site residues are thoroughly studied using molecular mechanics calculations. During docking, the default algorithm speed was selected and the ligand binding site was defined within a 10 A° radius. After docking, the individual binding poses of each ligand were observed and their interactions with the protein were studied.

Gold Score fitness function
Gold Score performs a force field based scoring function and is made up of four components: 1. Protein-ligand hydrogen bond energy (external H-bond); 2. Protein-ligand Vander Waals energy (external vdw); 3. Ligand internal Vander Waals energy (internal vdw); 4. Ligand intermolecular hydrogen bond energy (internal- H- bond). The external vdw score is multiplied by a factor of 1.375 when the total fitness score is computed. This is an empirical correction to encourage protein-ligand hydrophobic contact. The fitness function has been optimized for the prediction of ligand binding positions.

Gold Score = S (hb_ext) + S (vdw_ext) + S (hb_int) + S (vdw_int) Where S (hb_ext) is the protein-ligand hydrogen bond score, S (vdw_ext) is the protein-ligand van der Waals score, S (hb_int) is the score from intermolecular hydrogen bond in the ligand and S (vdw_int) is the score from intermolecular strain in the ligand.

RESULTS
From the designed library of molecule very few candidates screened out from the ADME parameter. The best candidate molecule has been selected for further analysis. By using molecular simulation and docking technique the best drug candidate were identified which satisfied the all rules and possess the inhibitor property. The inhibitor shows the highest binding affinity towards the receptor cavity is chosen for the best drug candidate molecule among synthesized library .The drug 2-(hydroxyl methyl) phenyl 6-O-phosphono-beta-D-glucopyranoside fig (1) passing all ADME parameter as shown in table (1) below.

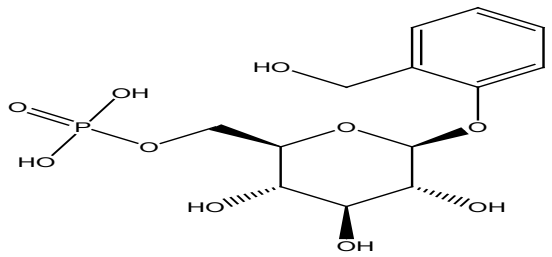

Figure: 1-(hydroxyl methyl) phenyl 6-O-phosphono-beta-D-glucopyranoside

TABLE 1: A set of designed compound displaying and their molecular properties and ADMET properties

Name	Mol wt.	LogP	H Donor	H Acceptor	ADMET Absorption Level	ADMET Solubility	ADMET Hepatoxicity	ADMET Hepatoxicity probability	ADMET CYP2D6 PROB.
2(hydroxyl methyl)phenyl 6-O-phosphono-beta-D-glucopyranoside	366	1.706	6	10	0	-0.66	1	0.58	0
4(hydroxy methyl)phenyl |A-d-glucopyranoside	286	0.991	5	7	0	-0.47	1	0.45	0
(4-Hydroxyphenyl-beta-D-glucopyranoside	272	0.808	5	7	0	-0.35	1	0.5	0

DOCKING RESULT
The selected drug candidate undergoes docking simulation with the protein, pdb id 1YCR and resulted in dock score -29.21,-27.54 and 25.59. The result has been proposed that a group of amino acid residues located on the binding cavity such as SER-40, TYR-60 and GLN-59 in target protein of

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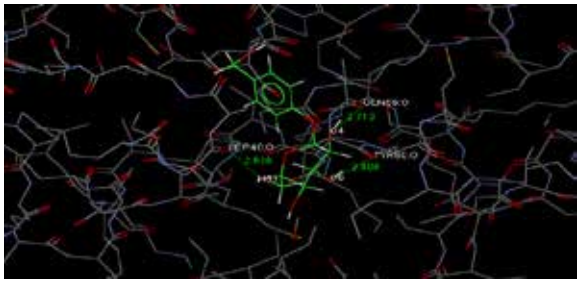
Mdm2. This interaction / affinity play an important role in ligand binding.

TABLE -2: The list of inhibitors with their gold docking interaction energy to active site of target receptor

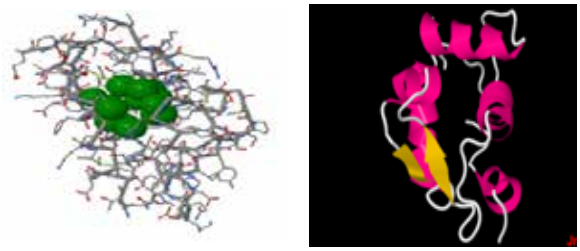
Complex	H –Bond Donor-Acceptor	Bond Length (Å)	Vander Waals interactions (Complex – DNA)	Bond Length (Å)	E (kcal/mol)
Comp1	O24-SER40: O	2.959	O8-LYS64:CG	2.711	--29.2156
	O12-SER40: OG	2.686	O8-LYS64:CD	2.321	
	O 6 -TYR60: O	2.749	O6-THR63:CB	2.199	
			O12-SER40:CB	2.612	
			C18-SER40: O	2.489	
			C17-SER40: O	2.313	
			C22-SER40: O	2.687	
Comp 2	H 31- SER40: O	2.628	O6 – LYS64:CD	2.444	-27.541
	O 4 – GLN 59: O	2.713	O6 – LYS64:CG	2.667	
	O 6-TYR 60: O	2.800	O6- TYR60:CG	2.703	
			O 4-TYR60:CA	2.501	
			C15- VAL41: O	2.561	
Comp3	O 8- TYR60: O	2.673	O7-LYS64:CG	2.473	--25.696
	O9- TYR63:OG1	2.987	O7-LYS64:CD	2.718	
			O8-THR63:CB	2.491	
			C11-SER40: O	2.649	
			C12-SER40: O	2.468	

Figure 2: Binding orientation of designed compound 2-(hydroxy methyl) phenyl 6-O-phosphono beta-D-glucopyranoside with the target associated protein 1YCR



Active site Identification of 1YCR domain

After the final model was modified, the possible binding site of 1YCR was searched with CASTP server (Fig. 3).



a b

Fig.2 Final improved 3D structure of 1YCR, a: Final 3D model of 1YCR; b: Possible binding site of 1YCR.

The structure is obtained by energy minimizing the average conformation over the last 1000 femto seconds of molecular dynamics simulation. The α- helix is represented by red cylinders and β-sheet by yellow arrows. It was evident that this protein has 4 helices and 6 sheets (Fig. 3a). It was found that the residues, SER-40, TYR-60 and GLN-59 are conserved with the active site of template (Fig 3b). Thus in this study SER-40, TYR-60 and GLN-59 were chosen as the more favorable sites to dock the substrate, and the other residues are not discussed further.

DISCUSSION

The interactions between designed potent inhibitor and receptor were studied by using various computational methods. Based on binding energy, and hydrogen bond formed, docking results were analyzed to find out the best ligand which can inhibit the target receptor 1YCR protein. Based on these observations, the ligand 2-(hydroxyl methyl) phenyl 6-O-phosphono-beta-D-glucopyranoside has high values to inhibit the target among the all best three ligands. Thus the in silico method adopted in the present study helped in identifying the ligands using the commercial software and online tools for the treatment of cancer. This method reduces the time and cost in designing a drug as well as in analyzing the drug likeliness before it enters the clinical trials. The further studies were carried out by preclinical trials.

CONCLUSION

The drug we developed that is 2-(hydroxyl methyl)phenyl 6-O-phosphono-beta-D-glucopyranoside .The above drug molecule is binding with MDM2 ,acting as Mdm2 antagonist , inhibiting its role to interact with P53 protein and there by P53 is freely available and can induce apoptosis and can regulate cell cycle progression in the case of damaged DNA and in the case of mutation. After the all research by using Insilco tools we can conclude that the above drug can be the probable drug for inhibiting Mdm2 protein.

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