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Research Article

HOMOLOGY MODELLING, VALIDATION AND DOCKING OF DFHR WITH BREAST CANCER INHIBITORS

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ABSTRACT
Proteins are the biomolecules which have a vital role in the human beings. The biological activity of the proteins is based on the structures of the proteins. One of the structural importance of the proteins is making the proteins a validate drug targets. In the present article DHFR enzyme structure was predicted and validated. Further the experiment proceeds by performing the docking studies of this modeled structure with Breast cancer inhibitors giving a scope for one drug many cancer concept. The docking results were very promising with the H- bond interactions between Gly118, Gly21, Lys 56, Thr 147 and Asp 22.
Keywords: Homology Modelling, Molegro docking, Cancer, Breast Cancer, DHFR.

INTRODUCTION

In the present day the designing of the drugs has gained attention and attracted many researchers in the pharmaceutical research[1]. In this process the structure of the protein plays a key role[2]. Not only in this specifically, the protein structures at large determines the functions of the proteins[3]. It is the amino acid sequences which determine the structure of the protein[4]. The relationship between the similar structures and the identical structures during the evolution were first identified by Chothia and Lesk[5] in the year 1986 and later by Sander and Schneider in 1999[6]. Hence, the proteins with dissimilar structure perform the similar function[7] and the proteins with the same structures perform different functions[8]. For the protein whose structures are not available the homology modelling is an ideal method to predict its structure[9]. This is accomplished with the structure of the known proteins as templates, knowing by the fact that similar sequences adopt similar folds[10]. Homology modelling has reportedly by exhibits a host of applications[11]. There are several reports in which the 3 D structures of the proteins are used to design new drugs[11-13]. The objective of the present investigation is to predict the protein structure of Dihydrofolate reductase DHFR and to dock it with the breast cancer inhibitors. The experiment aims at achieving the common drug for cancers. The enzyme dihydrofolate reductase catalyzes the reduction of NADPH to 5, 6, 7, 8-tetra hydrofolate[14]. DHFR is a very important enzyme in the biosynthesis of purines and amino acids of the cells and makes it an Antifolate drug target[15]. It works as an anticancer drug and is the first enzyme to be known as an chemotherapeutic agent. The drug acts by blocking the action of this enzyme.

MATERIALS AND METHODS

Protein Selection

The protein for the present study was selected from Swissprot database which contains the details of the protein along with their sequences. Q86XFO, with the generic name Dihydrofolate reductase mitochondrial of the Homo sapiens was selected. The chain length is 187 residues.

Identification of the Template

The template identification was done by using BLAST (Protein-Protein blast) algorithm from Protein Data Bank.

Selection of the Template Chain

From the blast results, the template chain was selected on SPDBV. The FASTA sequence and the current layer are to be saved.

Alignment

The alignment of the sequence, both the target and the template was performed on CLUSTAL X.

Generation of the Structure and Validation

A Python based Modeller software was used to predict the python structure. The generated structure was validated by RAMPAGE and SAVS after the active site identification on Cast P.

Docking

The protein generated was then validated for its ability as a drug target. Molegro was used for docking the protein with the inhibitors. In the present experiment the inhibitors used are the Breast cancer inhibitors[16] giving a scope for one drug for multiple cancers.

RESULTS

Template identification

From the protein selected from the uniprot database, the template was identified upon the performance of BLAST. Based on the E- Value and identity (93 %) the protein with the accession no. 1MVS whose resolution is 1.90 and R-value, 0.186 was chosen. The results showed chain "A" to be identical.

Selection of the Chain From Template And Alignment

Using the SPDBV, the "A" chain was selected deleting the remaining chains and was saved. The target and the template were aligned and were saved in pir and DND file formats.

Generation of the Structure
Using the Modellar software the protein structure was generated. (Figure 1 & 2)

The validation of the structure was done on RAMPAGE[17] and SAVS[18] which analysis the structure depending on the Ramachandran plot. (Figure 3)

Evaluation of residues
Residue [3: LEU] (-97.67,-141.30) in Allowed region
Residue [111: ASP] (-78.13, -73.48) in Allowed region
Residue [127: ASN] (-118.52, 72.08) in Allowed region
Residue [186: ASP] (-159.43, 98.00) in Allowed region

Number of residues in favoured region (~98.0 % expected): 181 (97.8 %)
Number of residues in allowed region (~2.0 % expected): 4 (2.2 %)
Number of residues in outlier region: 0 (0.0 %)

SAVS (Figure 4)
Plot statistics

Residues in most favoured regions [A,B,L]	155	94.5 %
Residues in additional allowed regions [a,b,l,p]	9	5.5 %
Residues in generously allowed regions [~a,~b,~l,~p]	0	0.0 %
Residues in disallowed regions	0	0.0 %
Number of non-glycine and non-proline residues	164	100.0 %
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	11	
Number of proline residues	10	
Total number of residues	187	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20 %, a good quality model would be expected to have over 90 % in the most favoured regions.

Active Site Identification
Identification of the active sites was done using the online software Cast P[19] which also talks about the residues present in the active sites. (Figure 5, 6 & 7)

Docking
Molegro virtual docker -3.2, a molecular docking mechanism was used for docking and to know if the Modelled protein a validate drug target. The ligands (inhibitors) selected for this purpose are the breast cancer inhibitors[20]. Five inhibitors are selected and drawn on Marvin Sketch. (Table 1 & 2, Figure 8, 9 & 10)
The results generated 25 poses. The highest Moldock score, -104.667 is considered to be the best inhibitor. The H- bond interactions are observed between amino acids, Gly118, Gly21, Lys 56, Thr 147 and Asp 22.

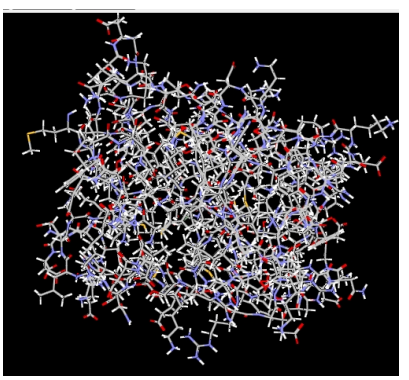


Figure 1: Modelled structure

Figure 2: Secondary structure

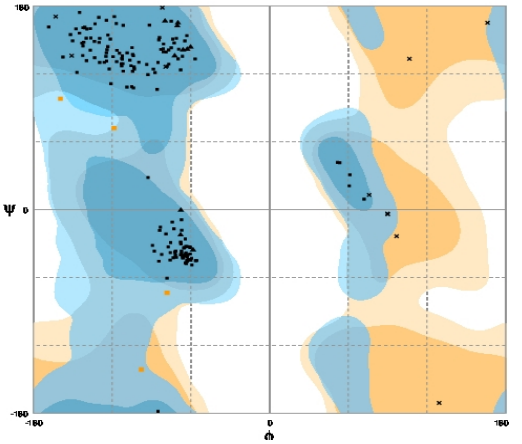


Figure 3: Rampage

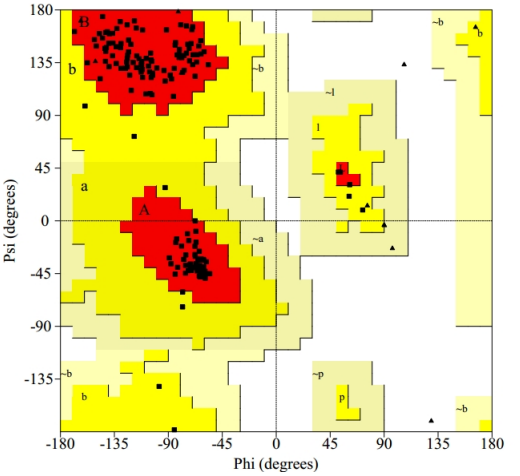


Figure 4: SAVS

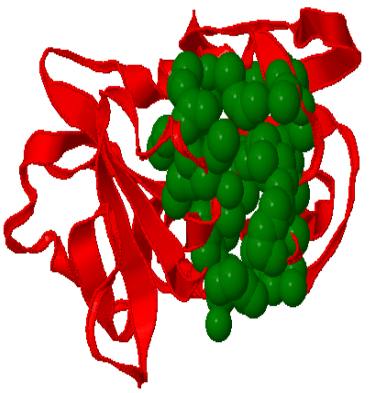


Figure 5: Active site prediction

Pocket Information

	ID	Area	Vol
☑	32	780.2	1155.2
☐	31	237.1	290.2
☐	30	206.5	315.2
☐	29	110.1	115.8
☐	28	105.2	121.9
☐	27	79.1	52.7
☐	26	84.9	71.9
☐	25	64.7	104.8
☐	24	34	35.3
☐	23	38.6	37.5
☐	22	45.6	47.6
☐	21	65.1	38.6
☐	20	48.7	41.7

32	8	O	ILE
32	8	CG1	ILE
32	8	CD1	ILE
32	9	O	VAL
32	9	CG2	VAL
32	9	CA	VAL
32	9	C	VAL
32	10	O	ALA

Figure 6: Area and volume of Active sites

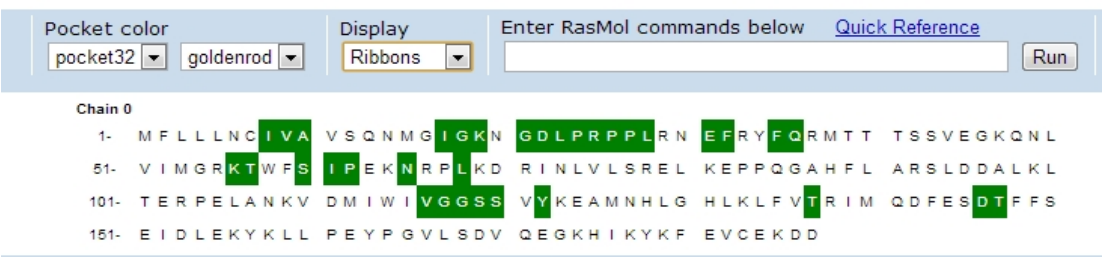
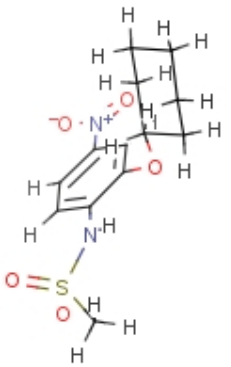
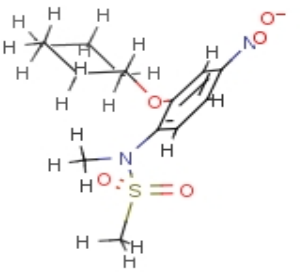
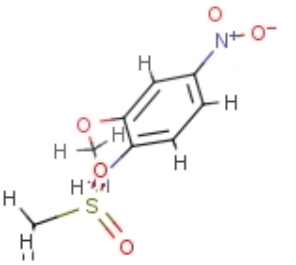
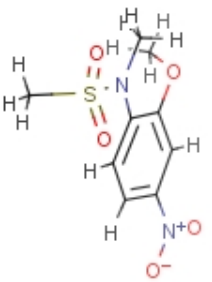
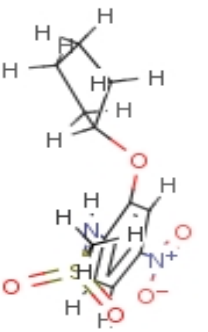


Figure 7: Active site residues

Table 1: Inhibitors

 Compound 1	 Compound 2	 Compound 3
 Compound 4	 Compound 5	

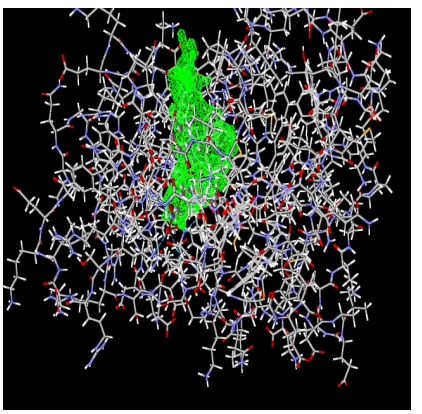


Figure 8: Protein- Ligand Docking

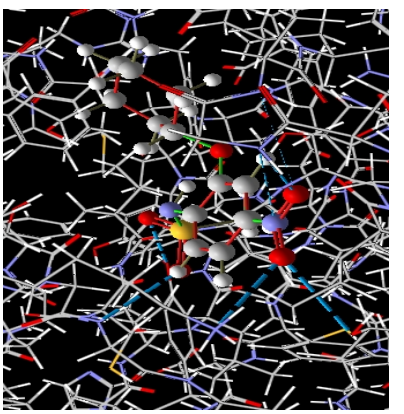


Figure 9: H-Bond Interactions

Table 2: Dock Table

Name	Ligand	MolDock Score	Rerank Score	HBond
[00]Unkn...	Unknown 1	-104.667	-85.3146	-7.12168
[04]Unkn...	Unknown 1	-101.501	-84.6454	-3.83392
[01]Unkn...	Unknown 1	-98.2787	-61.9701	-2.2123
[03]Unkn...	Unknown 1	-98.2158	-79.0449	-2.57363
[02]Unkn...	Unknown 1	-94.9387	-73.0431	-7.35536
[01]Unkn...	Unknown 1_1	-104.385	-86.099	-7.86885
[02]Unkn...	Unknown 1_1	-103.783	-86.2611	-2.5
[04]Unkn...	Unknown 1_1	-102.151	-84.741	-8.02277
[00]Unkn...	Unknown 1_1	-98.9941	-83.0596	-1.99761
[03]Unkn...	Unknown 1_1	-97.6198	-80.0998	-0.0829747
[00]Unkn...	Unknown 1_2	-92.7102	-78.669	-6.65667
[01]Unkn...	Unknown 1_2	-88.6894	-75.5133	-6.04825
[04]Unkn...	Unknown 1_2			-3.66905
[03]Unkn...	Unknown 1_2			-6.13739
[02]Unkn...	Unknown 1_2	-82.0397	-72.1073	-6.27358
[03]Unkn...	Unknown 1_3	-86.923	-78.3225	-4.80046
[02]Unkn...	Unknown 1_3	-86.6838	-78.5369	-6.04108
[00]Unkn...	Unknown 1_3	-85.9235	-78.2922	-6.83222
[01]Unkn...	Unknown 1_3	-85.4821	-76.1679	-5.54435
[04]Unkn...	Unknown 1_3	-82.121	-72.002	-6.58348
[01]Unkn...	Unknown 1_4	-109.163	-83.7865	-4.42208
[00]Unkn...	Unknown 1_4	-109.142	-83.2547	
[02]Unkn...	Unknown 1_4	-103.529	-77.5257	
[04]Unkn...	Unknown 1_4	-102.355	-77.4259	-4.31221
[03]Unkn...	Unknown 1_4	-100.507	-69.4456	-2.56423

Name: [00]Unknown 1_2
Rank: 11

Name: [04]Unknow
Rank: 20

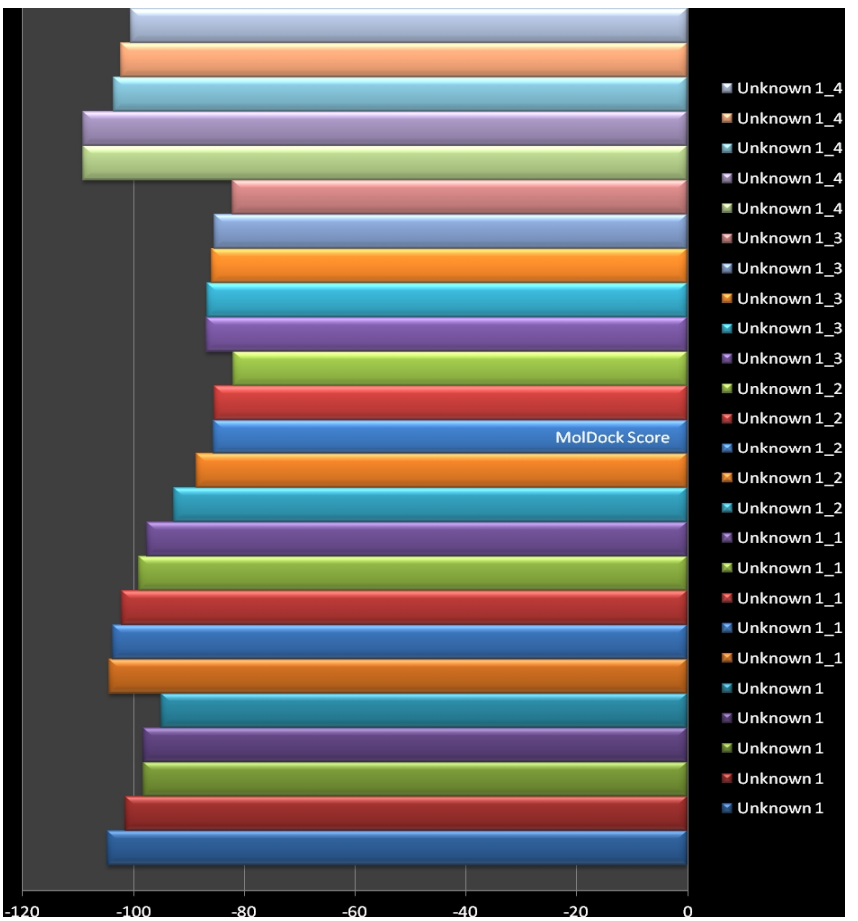


Figure 10: Plot Statistics

CONCLUSION

The proteins are the biomolecules which have a wide range of applications[21]. Besides their biological role, the proteins also act as drug targets. In the present investigation, the homology modelling of the protein which is considered as a potential cancer target was achieved. On analyzing the Ramachandran plots the modeled protein is considered as the good protein. Molegro docking results showed that the protein can be considered as an alternative drug target. The results also proved that the breast cancer inhibitors also act for DHFR.

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