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Structure prediction and *in-silico* designing of drugs against homeobox C8 protein

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ABSTRACT

The Homeobox C8 (HOXC8) gene is a part of the homeobox family of genes. The homeobox genes encrypt a highly preserved family of transcription factors that perform a crucial act in morphogenesis in all multicellular organisms. Objective: HOXC8 is involved in the progression of epithelial ovarian cancer (EOC) and it could prove to be a potential target for prevention and treatment of EOC. Methods: In this work, an in-silico model of HOXC8 protein was generated using the approach of homology modeling and loop modeling. The model was validated with Ramachandran plot analysis. The ligands were generated with the help of Drug bank and ZINC data base and were docked against HOXC8 protein using online server Patchdock. The structure of ligand ZINC 64858686 with the maximum score was varied by using ACD/ChemSketch 8.0 and the docking was done for the resulting 09 new ligands. Results and Conclusion: The results indicated that the ligand ZINC 64858686 shows the maximum score on binding with HOXC8 protein and thus justifies further studies needed for the development of potent inhibitors for the over expression of HOXC8 protein making the management of EOC more efficient.

Keywords: Epithelial ovarian cancer; HOXC8 protein; docking; homology modeling

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INTRODUCTION

Epithelial ovarian cancer (EOC) is the prominent cause of death in women among all gynaecological malignancies, accounting for about 5% of all cancers and 4.2% of all cancer deaths in women worldwide. It is the most frequent type of ovarian cancer [1]. Epithelial ovarian cancer is composed of a heterogeneous group of tumours. The four most prevalent subtypes are serous, endometrioid, clear cell, and mucinous carcinoma. Less common are transitional cell tumours, including transitional cell carcinoma and malignant Brenner tumour [2]. Inspite of amelioration in the detection and cytotoxic therapies, only a humble increase in the expectancy rate exceeding five years after initial diagnosis of ovarian cancer has been accomplished. There are various factors responsible for the high casualty rate, including the inadequacy of any distinct manifestations in initial stages of ovarian cancer; late diagnosis, which becomes an obstacle in designing an intervention; and the development of chemoresistance in cancer cells. Thus, it becomes imperative to develop enhanced screening methods for EOC detection at early stage, as well as effective treatment for advanced stages of ovarian cancer patients [1]. The first line treatment comprising of platinum/taxane shows a decent response rate, but the incidence of reappearance of the disease is common. In addition, the second-line treatments are not efficacious in treating this disorder [3]. In addition, development of avant-garde biomarkers for each subtype of ovarian cancer has become a demand for designing a better and more diligent treatment approach for ovarian cancer [1].

The Homeobox C8 (HOXC8) gene is a part of the homeobox family of genes. The homeobox genes encrypt a highly preserved family of transcription factors that perform a crucial act in morphogenesis in all multicellular organisms. Mammals carry four analogous homeobox gene clusters, HOXA, HOXB, HOXC and HOXD, which are positioned on different chromosomes and consist of 9 to 11 genes aligned in tandem. This gene is one of the various homeobox HOXC genes positioned in a cluster on chromosome 12 [4]. Homeobox C8 protein (HOXC8) is one of the 39-member HOX family proteins. Moreover, the findings that both high HOXC8 and CDH11 expression correlate with poor recurrence-free survival of breast cancer patients further support the notion that the HOXC8-CDH11 functional axis plays a critical role in breast tumor progression and metastasis. One study indicated that innovative and productive therapeutic avenues might be matured by focusing on HOXC8-CDH11 functional axis [5]. Another study demonstrated that embigin is transcriptionally regulated by HOXC8 protein and its low/loss expression may play a critical role in the amelioration of breast cancers [6]. According to one study, HOXC8 expression is inversely related to pancreatic ductal adenocarcinoma (PDAC) progression and metastases and might thus serve as marker for PDAC progression [7]. Another study suggested that HOXC8 might play a part in the recovery of the invasive and metastatic phenotype of human prostate cancer [8]. One more study concluded that HOXC plays a crucial role in the pathogenesis of androgen-resistant prostate cancer [9]. In one study, it was seen that HOXC8 promoted hepatocellular carcinoma (HCC) proliferation and predicted poor prognosis. Moreover, HOXC8 overexpression is related with oxaliplatin resistance in HCC [10]. One study investigated the expression and role of HOXC8 in ovarian cancer. Western blot and immunohistochemistry analyses were executed to recognize the expression of HOXC8. Kaplan-Meier curve demonstrated that elevated expression of HOXC8 is linked to poor prognosis of patients with epithelian ovarian cancer (EOC). Starvation and refeeding assay were used to evaluate cell cycle, suggesting that HOXC8 plays an important role in EOC cell proliferation. HOXC8 reduction by small interfering RNA constrained cell proliferation, migration, and induced apoptosis in EOC cells. The study suggested that HOXC8 is involved in the progression of EOC and could be a potential therapeutic approach of EOC [11].

A protein structure is consistently proving to be a big help in the study of protein function, dynamics, interactions with ligands and other proteins. There are some proteins, which are too big for NMR analysis, and their structure cannot be anticipated by X-ray diffraction. Homology modeling evaluate the 3-D structure of a given protein sequence (target) based chiefly on its alignment to one or more proteins of known structures (templates). The estimation process consists of fold assignments, model building and model evaluation. The homology modeling has been widely used to predict the protein structure. Computer Aided Drug Designing is rapidly becoming a crucial tool in drug discovery, the in-silico study has provided the awareness of the interaction between receptor and ligands [12-14].

In this study, the structure of Homeobox C8 protein (HOXC8) was designed by using homology modeling. The docking of the ligands was done to anticipate the binding orientation of small drug molecules with their protein target (HOXC8) in order to prognosticate the affinity and activity of the small molecules in inhibiting HOXC8 so that it may lead to attenuated proliferation and migratory ability of epithelian ovarian cancer (EOC) cells.

EXPERIMENTAL SECTION

The hardware used for calculating molecular modeling includes a personal computer with Intel (R) Core (TM) i3 CPU processor, Windows 7 Home Premium 32-bit operating system having RAM of 2.00 GB.

Sequence alignment

Fast alignment (FASTA)

The FASTA format is a text based format for describing either nucleotide sequences or peptide sequences, in which nucleotides or amino acids are illustrated using single letter codes. A sequence in FASTA format begins with a single line narration, followed by lines of sequence data. The description line is demarcated from the sequence data by a greater-than (“>”) symbol in the first column [15]. The FASTA sequence of HOXC8 was attained from the website of National Centre for Biotechnology Information [16].

Basic Local Alignment Search Tool (*BLAST)*

The BLAST is an algorithm for analyzing primary biological sequence information, such as the amino acid sequence of different proteins or the nucleotides of DNA sequences [17]. The FASTA was utilized and standard protein BLAST was executed on the NCBI. The BLAST-P was accomplished using protein data bank proteins data base [18].

Three Dimensional Position-Specific Scoring Matrix (3D-PSSM)
The 3D-PSSM is a swift web based technique for protein fold identification using 1D and 3D sequence profiles paired with secondary structure and solvation potential information. The FASTA sequence was submitted to 3D-PSSM for fold recognition [19, 20].

Protein Homology/Analogy Recognition Engine (Phyre)
Phyre2 is a suite of tools available on the web to predict and analyze protein structure, function and mutations [21]. The FASTA sequence was submitted to Phyre for amino acid sequence prediction [22].

Templates Preparation
The data obtained from BLAST, 3D-PSSM and Phyre was analyzed at the RCSB protein data bank. The Protein Data Bank (PDB) archive is the single worldwide archive of the 3D structures of hefty biological molecules, including proteins and nucleic acids [23]. The templates were preferred on the ground of their resolution (Å) and R-value. All the above templates were introduced by X-ray crystallography method in PDB.

Molecular Modeling
Homology modeling of HOXC8 was done by using EasyModeller. EasyModeller is a front-end graphical interface to Modeller developed using Perl/Tk, which can be used as a standalone tool in windows platform with Modeller and Python preinstalled. EasyModeller can produce 3-D structural models of proteins from sequence and given template(s) information using Modeller in backend [24]. The Swiss-Pdb viewer, an application that provides a user friendly interface allowing analysing several proteins at the same time, was installed [25].

Structure Prediction
The chosen six templates were submitted to the EasyModeller. All the ten prepared models were evaluated based on DOPE, Molpdf and GA341 methods. The Discreet Optimized Protein Energy (DOPE) score is a statistical tool to evaluate homology models in protein structure prediction. In the Modeller objective function (molpdf), the EasyModeller minimizes the objective function F with respect to Cartesian coordinates of ~ 10,000 atoms (3D points) that form a system (one or more molecules). The GA341 method uses the percentage sequence identity between the template and the model as a parameter. The model with the minimum molpdf and DOPE score, and the GA341 value lying in between zero and one (the higher the better) can be chosen as the best feasible model [26].

Validation of Predicted Model
The validation of all the ten models was performed by submitting the PDB files to Rampage for Ramachandran plot assessment. Rampage is a program for visualising and assessing the Ramachandran plot of a protein structure. On the basis of a manually curated set of high-quality protein structures (from the Richardson's Group at Duke University) and a number of filters (such as B-factor cutoff and van der Waals clashes), reference phi/psi plots were derived for Gly, Pro, pre-Pro and general (other) residue types, and subdivided into "favoured", "allowed" and "outlier" regions [27]. The Ramachandran plot validated the result.

Loop Modeling
The protein function is decided by its shape and the physiochemical properties of its exposed surface, thus it is crucial to construct a precise model for protein/ligand interaction studies. The co-ordinate file was submitted for loop optimization to ModLoop, a web server for automated modeling of loops in protein structures. The server count on the loop modeling routine in MODELLER that anticipates the loop conformations by satisfaction of spatial restraints, without depending upon a database of known protein structures. This structure was evaluated by Ramachandran plot using Rampage. The process of loop modeling and successive validation was carried on until an optimized structured model of protein was accomplished [28, 29].

Ligand Generation
The DrugBank database is an exclusive bioinformatics and cheminformatics system that amalgamates detailed drug (i.e. chemical, pharmacological and pharmaceutical) data with comprehensive drug target (i.e. sequence, structure, and pathway) information. The FASTA sequence of the target protein was utilized to obtain the compounds that interact with the target. The ZINC database contains commercially available compounds for structure based virtual screening. It currently has about 90 million compounds that can simply be purchased. The ZINC database was utilized to obtain similar drugs [30, 31].

Molecular Docking

Molecular docking is a vital tool in structural molecular biology and computer-assisted drug design. The aim of ligand-protein docking is to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure. Successful docking methods search high-dimensional spaces efficiently and utilize a scoring function that justly ranks candidate dockings [32]. The macromolecule and the ligands were prepared for docking by using Pymol and ChemBio3D software [33, 34]. The molecular docking was done against Homeobox C8 protein using an online server Patchdock. The Patchdock is an algorithm for molecular docking. It is inspired by object recognition and image segmentation techniques used in computer vision. The algorithm has three main stages: a) Molecular shape representation; b) Surface patch matching; c) Filtering and scoring. The input is two molecules of any type: proteins, DNA, peptides, drugs. The output is a list of potential complexes sorted by shape complementarity criteria [35, 36]. The prominent compound was preferred based on scoring.

Ligand Designing and Docking

The chosen ligand was employed to design novel molecules with the help of ACD/ChemSketch 8.0 freeware. The Lipinski's rule of five was used to check the hypothetical effectiveness of the drugs. These structures were subjected to ChemBio3D for energy minimization. The molecular docking of these sketched molecules was done against the HOXC8 protein by using Patchdock.

RESULTS AND DISCUSSION

Template Generation

The NCBI was employed to secure FASTA sequence of HOXC8 protein. The GenBank No. is EAW96743.1 and gi no. is 119617149. It is a 242 amino acid protein. The BLAST was performed on the NCBI and 89 hits were recorded as shown in **Figure 1**. The 3D-PSSM and Phyre were utilized for prediction of protein structure. The information received from BLAST, 3D-PSSM and Phyre was evaluated at the RCSB protein data bank. The obtained results were arranged in the descending order of % ID followed by ascending order of Resolution as shown in **Table 1**. The six templates (9ANT, 4XIC, 4UUS, 4UUT, 1PUF and 4CYC) were preferred on the ground of their chains, ID %, resolution (≤ 3 Å) and the R-value (≤ 0.5). A total of ten models were generated with the help of EasyModeller (**Table 2**). Models with the lowest DOPE assessment score and Molpdf or with the highest GA341 assessment score have the most stable minimized energy. The model number 7 was selected on these bases for further analysis.

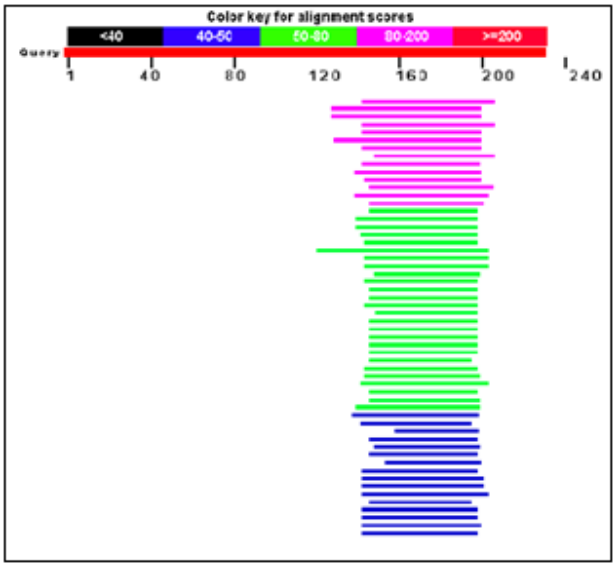


Figure 1 Distribution of 89 BLAST hits on the query sequence (query Id: ICI|Query_226592) in pdb protein database and the program is BLASTP 2.3.1 +

Table 1 Generation of templates using Blast, 3D-PSSM, Phyre and RCSB protein data bank

S.No	Template/Accession No	ID %	Resolution (Å)	R-Value (Obs/ Free)	S.No	Template/Accession No	ID %	Resolution (Å)	R-Value (Obs/ Free)
1	9ANT	83	2.40	0.239	15	4XRS	52	3.5	0.359
2	4XIC	83	2.69	0.272	16	2HDD	50	1.9	0.251
3	4UUS	81	2.55	0.240	17	2HOS	50	1.9	0.257
4	4UUT	76	2.8	0.237	18	1DU0	50	2.0	0.270
5	1PUF	69	1.9	0.268	19	1IG7	48	2.2	0.274
6	4CYC	68	2.36	0.227	20	1JGG	47	2.0	0.316
7	1B8I	68	2.4	0.304	21	1FJL	34	2.0	0.198
8	2H1K	67	2.42	0.277	22	1OCTC	30	3.0	0.237
9	1B72	61	2.35	0.277	23	1AU7A	28	2.3	0.302
10	2R5Y	60	2.6	0.299	24	1MNM	22	2.25	0.285
11	1P7I	52	2.1	0.240	25	1IFB	17	2.8	0.364
12	1P7J	52	2.1	0.241	26	1KIO	15	1.75	0.236
13	3HDD	52	2.2	0.232	27	1AOH	15	3.2	0.242
14	1HDD	52	2.8	0.225					

Table 2 DOPE score and Ramachandran plot analysis of the ten possible models of HOXC8 protein

S.No	Model No.	Molpdf	DOPE	GA341	Residues in favoured regions (Number/ Percentage)	Residues in allowed regions (Number/ Percentage)	Residues in outlier regions (Number/ Percentage)
1	B99990001	3502.83179	-10851.24902	0.99937	225/ 93.8	13/5.4	2/ 0.8
2	B99990002	3373.75293	-10752.20313	0.99981	234/ 97.5	6/ 2.5	0/ 0.0
3	B99990003	3336.97437	-10974.70117	1.00000	234/ 97.5	5/ 2.1	1/ 0.4
4	B99990004	3486.35938	-10733.01367	1.00000	228/ 95.0	8/ 3.3	4/ 1.7
5	B99990005	3406.89722	-11141.40332	0.99999	232/ 96.7	7/ 2.9	1/ 0.4
6	B99990006	3397.70825	-10799.44336	1.00000	231/ 96.2	5/ 2.1	4/ 1.7
7	**B99990007**	**3311.55103**	**-11213.22949**	**1.00000**	**235/ 97.9**	**5/ 2.1**	**0/ 0.0**
8	B99990008	3459.72534	-10921.95996	1.00000	230/ 95.8	8/ 3.3	2/ 0.8
9	B99990009	3404.62891	-10763.97461	1.00000	235/ 97.9	2/ 0.8	3/ 1.2
10	B99990010	3427.75244	-11206.22754	0.99994	236/ 98.3	3/ 1.2	1/ 0.4

Validation

The models were further validated by Ramachandran plot, by submitting the files to PDBsum. The model number 7 was approved as the residues in favoured region, allowed region and outlier regions are 97.9 %, 2.1 % and 0.0 % respectively (**Table 2**).

Loop modeling

The PDB file format of model number 7 was endorsed for loop optimization to ModLoop and the output model was evaluated with the help of Ramachandran plot obtained using PDBsum. The protein model having maximum percentage (94.7 %) of residues in most favoured region and 5.3 % residues in additional allowed regions with no residues in generously allowed as well as disallowed regions (**Figure 2** and **Table 3**). The model of HOXC8 protein (**Figure 3**) was successfully submitted to Protein model data base (http://bioinformatics.cineca.it/PMDB/) bearing the PMDB ID: PM0080452.

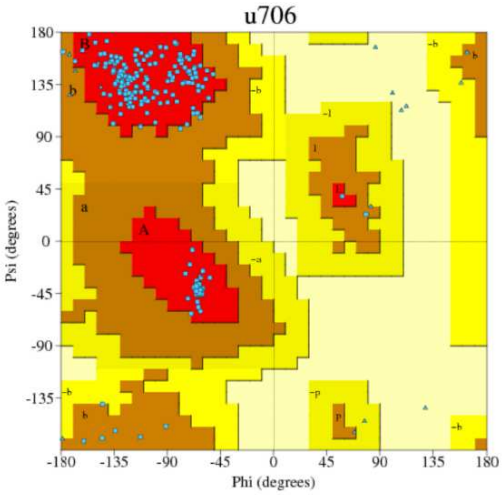


Figure 2 Ramachandran plot for optimized model of HOXC8 protein

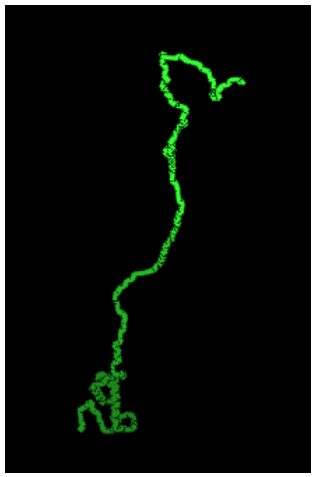


Figure 3 Optimized model of HOXC8 protein

Table 3 PROCHECK statistics

Ramachandran Plot Statistics*			G-Factors**		
Regions	**No. of Residues**	**Percentage (%)**	**Parameters**	**Score**	**Average Score**
Most favoured regions [A, B, L]	196	94.7	Dihedral angles		
Additional allowed regions [a, b, l, p]	11	5.3	Phi-psi distribution	-0.23	
Generously allowed regions [~a, ~b, ~l, ~p]	0	0.0	chil-chi2 distribution	-0.06	
Disallowed regions [XX]	0	0.0	chil only	0.16	
Non-glycine and non-proline residues	207	100	chi3 and chi4	0.42	
End residues (excl. Gly and Pro)	2	-	Omega	0.24	
Glycine residues	19	-	Average Score	0.06	
Proline residues	14	-	Main-chain covalent forces		
Total no. of residues	242	-	Main-chain bond lengths	-0.24	
			Main-chain bond angles	-0.34	
			Average score	-0.30	
			Overall average	-0.07	

**Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20.0 a good quality model would be expected to have over 90% in the most favoured regions [A,B,L]. **G-factors provide a measure of how unusual, or out-of-the-ordinary, a property is.*
Values below -0.5 – unusual; Values below -1.0 - highly unusual.

Ligand Generation and Docking

A total of 21 drugs like compounds were downloaded from The Drug Bank and ZINC data base. These were docked against HOXC8 protein using Patchdock. The results (**Table 4**) indicated that the best score (5460) is given to the ligand ZINC 64858686 (**Figure 4, 5**)**.** The result suggested that the compound could be a promising ligand for the target HOXC8 protein.

Table 4 The docking results of ligands generated using Drug Bank and ZINC data base against HOXC8 protein as target

S.No	Ligands	PatchDock Score	S.No	Ligands	PatchDock Score
1	**ZINC 64858686**	**5460**	12	ZINC 68986232	3404
2	ZINC 37261877	4138	13	ZINC 36745104	3238
3	ZINC 37261847	3722	14	ZINC 1710230	3196
4	ZINC 36744921	3718	15	DB02219	3176
5	ZINC 36437300	3674	16	ZINC 33637353	3172
6	ZINC 36744840	3596	17	DB02317	3078
7	ZINC 33637354	3594	18	DB03309	3028
8	ZINC 36744923	3484	19	ZINC 72194499	2876
9	ZINC 68986231	3470	20	ZINC 2913677	2846
10	ZINC 37261848	3454	21	ZINC 72194498	2802
11	ZINC 3674838	3406			

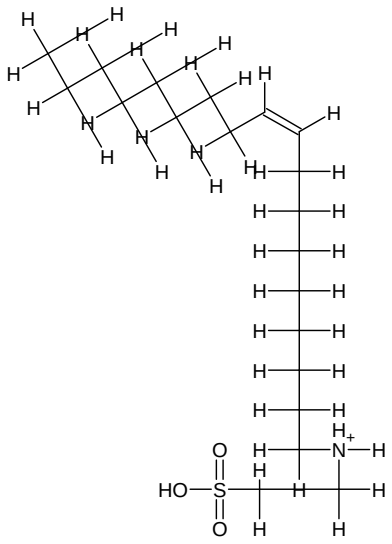


Figure 4 The structures of ligand ZINC 64858686

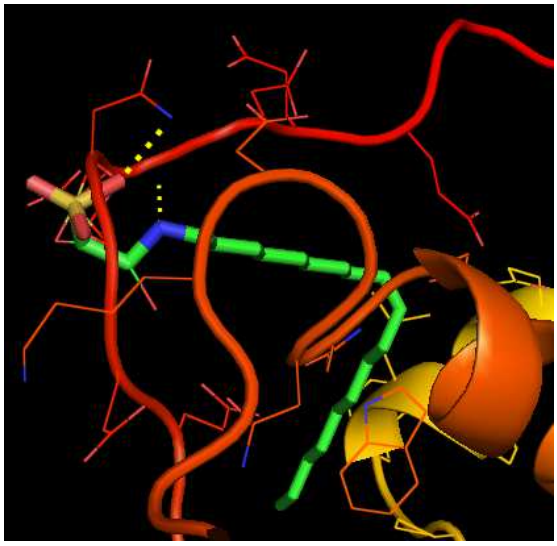


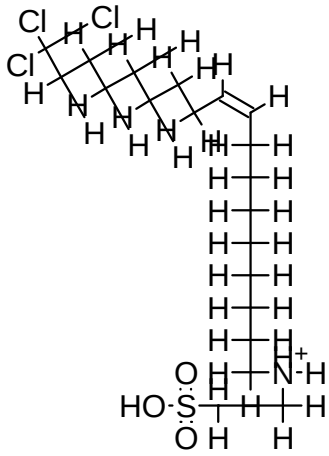
Figure 5 Docking of ligand ZINC 64858686 with HOXC8 protein

Ligand Designing and Docking

The structural variation was done in the molecule ZINC 64858686 and 09 new compounds were designed with the help of ACD/ChemSketch 8.0. The docking of these compounds was done against HOXC8 protein using Patchdock. The results (**Table 5**) indicated that out of all these compounds, **ligand ZINC 64858686** dock with the maximum score. The docking of the ligand 8 (**Figure 6**) scored 5308, which is near to the maximum. Thus, further structural variation in ligand 8 can be done in order to achieve better docking results.

Table 5 The docking results of ligands generated using chemSketch against HOXC8 as target

Ligands	Molecular Formula	Formula Weight	Patchdock Score
ZINC 64858686	**$C_{20}H_{42}NO_3S$**	**376.6168314**	**5460**
Ligand1	$C_{20}H_{40}NO_4S$	390.6003514	4706
Ligand2	$C_{20}H_{43}N_2O_3S$	391.6314714	4778
Ligand3	$C_{20}H_{45}N_4O_3S$	421.6607514	4692
Ligand4	$C_{20}H_{49}N_8O_3S$	481.7193114	4880
Ligand5	$C_{20}H_{47}N_6OS$	419.6912314	4626
Ligand6	$C_{20}H_{42}NO_6S$	424.6150314	4318
Ligand7	$C_{20}H_{42}NO_9S$	472.6132314	4570
Ligand8	**$C_{20}H_{39}Cl_3NO_3S$**	**479.9520114**	**5308**
Ligand9	$C_{20}H_{42}Cl_3N_4O_3S$	524.9959314	4982



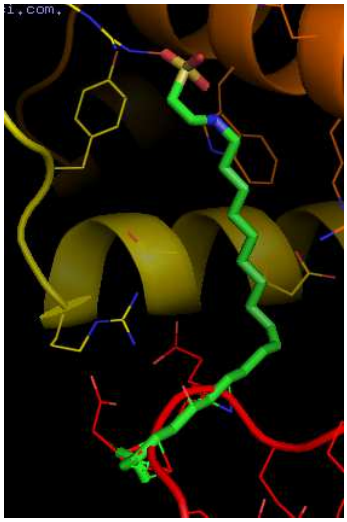


Figure 6 Ligand 8 a) Chemical structure b) Docking pattern of ligand 8 with HOXC8 protein

CONCLUSION

The homology modeling and loop modeling methodology were employed to design model of HOXC8 protein. The Ramachandran plot evaluated the models. The Drug bank and ZINC data base were used to identify various ligands. The molecular docking done against HOXC8 protein of these ligands using online server Patchdock, identified ZINC 64858686 ligand with maximum score. The structure of this compound was varied by using ACD/ChemSketch 8.0 and then docking was done against the target protein. The present study indicates that the *in silico* molecular docking studies of selected ligand, *i.e*., ZINC 64858686 with HOXC8 protein manifested favorable binding interactions and justifies further studies (*in vitro* as well as *in vivo*) required for the evolution of potent inhibitors for the over expression of HOXC8 protein so as to develop a novel compound for the prevention and treatment of epithelial ovarian cancer.

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