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In silico Modelling and Docking Studies of Camptothecin Derivatives

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Abstract : The availability of experimental (X-ray, NMR) structures is very less. To overcome this problem the structures have been determined theoretically, especially those determined by homology modeling techniques. Protein-Ligand docking is increasingly used in Drug Discovery. The present study explains computational methods to design Polio virus related protein 2, alpha isoform of human enzyme using the crystal structure available from Protein Data Bank (PDB ID: 4FMK). Model was generated by using Modeler9.16. After designing the model, functional effect was confirmed in terms of protein ligand binding by molecular docking using Autodock4.2. The docking investigation of modelled Q9UEI6 protein with camptothecin derivatives using Autodock4.2 software was performed. Out of 30 compounds nine compounds are involved in interacting with ARG72, SER77 and THR86 with good binding energy. Rest of the compounds shows two interactions with good binding energy. More importantly, it provides insight into understanding and properly interpreting the data produced by these methods.
Key words: Homology modeling, Camptothecin derivatives, Molecular Docking.

Introduction:

Poliovirus, a member of the picornavirus group. Poliovirus is the etiologic agent of poliomyelitis. It is a human disease of the central nervous system. Invasion of PV into the CNS results in the destruction predominantly of motor neurons, leading to paralysis. Poliomyelitis, however, is a rare complication of PV infections of humans[2]. Vaccines against poliovirus have been developed against the three known poliovirus serotypes: poliovirus1, poliovirus2, and poliovirus3. Poliovirus is structurally related, non-enveloped RNA virus[1]. Polioviruses are the most studied members of the picornavirus family. Proteins are homologous to the poliovirus receptor. The structure of poliovirus consists of an icosahedral protein shell. All three poliovirus serotypes recognize CD155 cellular receptor molecule[3,4]. CD155-α and CD155-δ are membrane-bound variants that differ only in the sequence of their cell-internal C-terminal domain, while CD155 band –γ are secreted isoforms lacking the transmembrane domain.

In order to understand the possible binding modes of camptothecin derivatives at the human poliovirus related protein 2, alpha isoform receptor,[5] we have conducted a docking study. Since the crystal structure of the Q9UEI6 receptor has not been solved. Hence, our approach necessitated the use of a homology modeling paradigm. We decided to build and homology model using in silico tools in order to determine which model was best in line with our in vitro data. A model was built based on Crystal structure of mouse nectin-2 extracellular fragment D1-D2 (PDB code 4FMK) as template using molecular modeling – MODELLER9.16 program. We then performed docking/scoring experiments, using three docking programs: Autodock4.2.

In the present study, we determined that the homology model built by MODELLER9.16 and based on a bovine rhodopsin template together with an Autodock4.2 docking algorithm is in the best agreement with our in vitro results. These modelling and docking studies have provided useful insights into the possible binding modes of camptothecin derivatives at the Q9UEI6 receptor.

Methodology:

Homology modelling

The amino acid sequence of Polio virus related protein 2, alpha isoform of human was retrieved from uniprot.com. A sequence similarity search was performed using Protein BLAST[6] tool for identifying templates for homology model building. The sequence was searched for their structural similarity with the query mutant protein by running NCBI protein BLAST against Protein Data Bank (PDB). The template was identified on the basis of maximum score, smaller the e-value, >30% identity. 4FMK protein was selected as a template for modelled protein with 65% identity. A comparative sequence alignment was performed with the template structures using ClustalX and ClustalW tools.[7]

MODELLER 9.16 was used along with an automated approach to comparative modeling by satisfaction of spatial restrains to develop models. After manually modifying the alignment input file in MODELLER 9.16[8] to match template and query sequence, 20 models were generated and all were thermodynamically minimized using molecular dynamics and simulation approach. By using MODELLER9.16 automodel class, calculated three dimensional models of the target automatically. The Lowest Objective Function is used to select the best model by the smallest value of normalized Discrete Optimized Molecule Energy (DOPE) score. These models were then checked in detail for protein structure stereochemistry including Ramachandran plot and Psi/Phi angles using PROCHECK.[9]

Molecular docking studies

The structures of camptothechin derivatives shown in **(tables 1,2,3)**. Polio virus related protein 2, alpha isoform was retrieved from scientific literature. Later all the 30 inhibitors were sketched in sybyl6.7 software[10] and was energetically minimized by adding Gasteiger-Huckel charges. The molecule was then saved in .mol2 format for molecular docking purpose.

The modelled protein structure was imported to Autodock 4.2, a protein-ligand docking tool[11] and structurally optimized by adding polar hydrogens. The model was saved in PDBQT format. Later all the ligands were docking individually. After loading the molecule ligands were prepared by optimizing the torsion angles and saving them in PDBQT format. Potential binding site for the model was identified using 3Dligand site[12]. A grid was generated around to identify xyz coordinates (X=-0.684, Y=3.920 and Z=13.819) around binding site of modelled Polio virus related protein 2, alpha isoform of human. Lamarckian genetic algorithm (LGA)[13] was selected for freezing, docking and default parameters used in autodock4.2.

Results and discussion:

Polio virus related protein 2, alpha isoform (Q9UEI6) contains sequence length of 449aa was modelled by taking the template protein of *Crystal structure of mouse nectin-2 extracellular fragment D1-D2* (PDB entry: 4FMK). The most homologous template for building a homology model for Polio virus related protein 2, alpha isoform was identified through protein blast. Based upon the homology search, the template 4FMK was selected on the basis of E-value, % identify etc., initial alignment was performed by using clustaX. Twenty models were generated using Modeler 9.16 program. The alignment file was tweaked manually to excellent fit in the sequences. After the generated models for all the primary sequences, the model with the least object function was selected for further protein stereochemistry evaluation (phi and psi angles) with Procheck software. The cartoon of homology derived protein of Polio virus related protein 2, alpha isoform was shown in fig1.

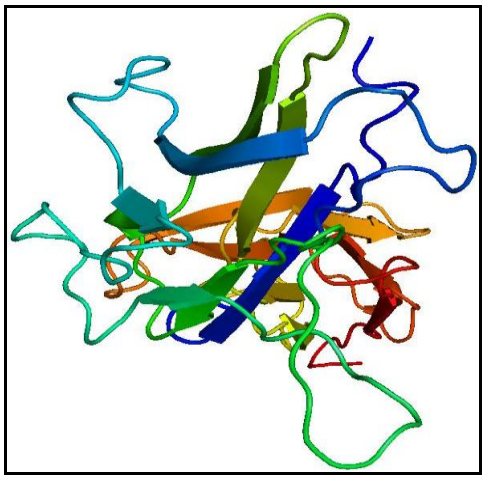


Fig1. The cartoon of homology derived protein of Polio virus related protein 2, alpha isoform

The PROCHECK software generates number of files which list complete residue by residue data and the assessment of the generally excellence of the producing structure as compared to well refined structures of the same resolution. The Ramachandran plot of the 4FMK shows 155 amino acid residues (83.8 %) in most favorable regions with 30 amino acid residues (16.2 %) falling into additionally allowed regions and there is no amino acid residues falling into the generously allowed and disallowed regions. whereas for the modelled protein shows, 177 amino acid residues (92.7 %) in the most favorable region, 14 amino acid residues (7.3 %) in the additionally allowed region, whereas there is no amino acid residue present in disallowed region. These results clearly indicate that the generated model is more conformationally superior to the template structure. Ramachandran plot of modelled protein was shown in figure 2

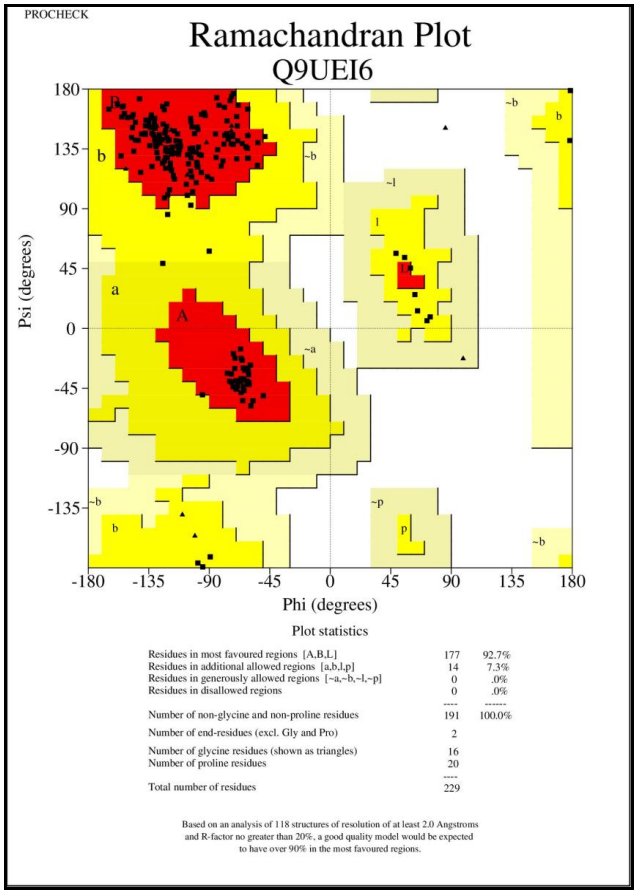
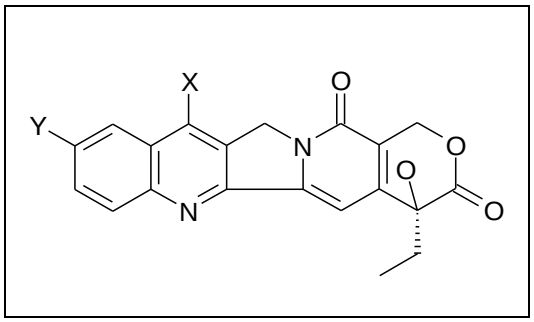


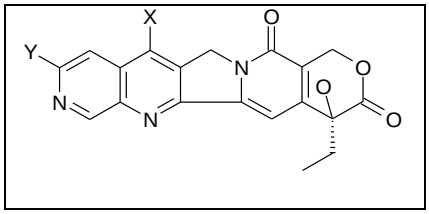
Fig 2: Ramachandran plot statistics of the modelled protein

Docking results:

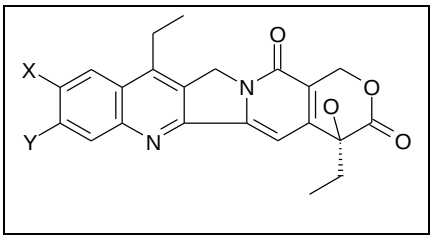
Molecular docking is the most widely used method for the calculation of protein–ligand interactions. Molecular docking studies was carried out thirty camptothecin derivatives against modelled Q9UEI6 protein. The binding energy, inhibition constant, hydrogen bond forming residues and interacting residues of all the compounds are shown in Table 1. The binding energy for all the molecules range from -5.91 to -8.20 kCal/mol. Compound six shows two interactions with Arg72, Thr86. Out of thirty compounds 10 compounds show three interactions, 16 compounds show two interactions and rest of the compounds shows only one interaction. The results of all the interacting amino acid residues with camptothecin derivatives are shown in table 1. All the 30 molecules interactions were shown in figure 3.



C.NO	X	Y	BINDING ENERGY(B.E)	kI (uM)	PROTEIN-LIGAND INTERACTIONS
1	H	H	-7.36	4.01	GLN84
2	CHO	H	-7.31	4.4	SER74,GLN84
3	CHO	OH	-7.89	1.64	ARG72,SER77,THR86
4	CHO	OCH_3	-7.65	2.48	ARG72,SER77,THR86
5	CH_2OCOCH_3	H	-7.50	3.16	ARG72,SER77,THR86
6	CH_2OCOCH_3	OH	-8.20	979.53 (nm)	ARG72,THR86
7	CH_2OCOCH_3	OCH_3	-8.08	1.19	ARG72,LUE73,VAL76
8	CN	H	-7.16	5.65	ARG72,SER77,THR86
9	CN	OH	-7.57	2.83	ARG72,SER77,THR86
10	CN	OCH_3	-7.28	4.63	GLY60,SER77,THR86
11	CH=CHCHO	H	-7.85	1.76	GLY60
12	$CH=CHCOOC_2H_5$	H	-6.19	29.04	ARG72,GLY60
13	CH=CHCN	H	-7.29	4.55	SER77,GLN84
14	CH=C(CN)CN	H	-6.49	17.52	THR86
15	$CH=C(CN)COOC_2H_5$	H	-6.52	16.61	SER77,GLN84
16	CH=C(Br)Br	H	-7.16	5.61	ARG72,SER74
17	$CH(OH)CH_2NO_2$	H	-7.63	2.55	GLN84,THR86
18	$CH_2CH_2COOC_2H_5$	H	-6.72	11.89	ARG72,SER77,THR86

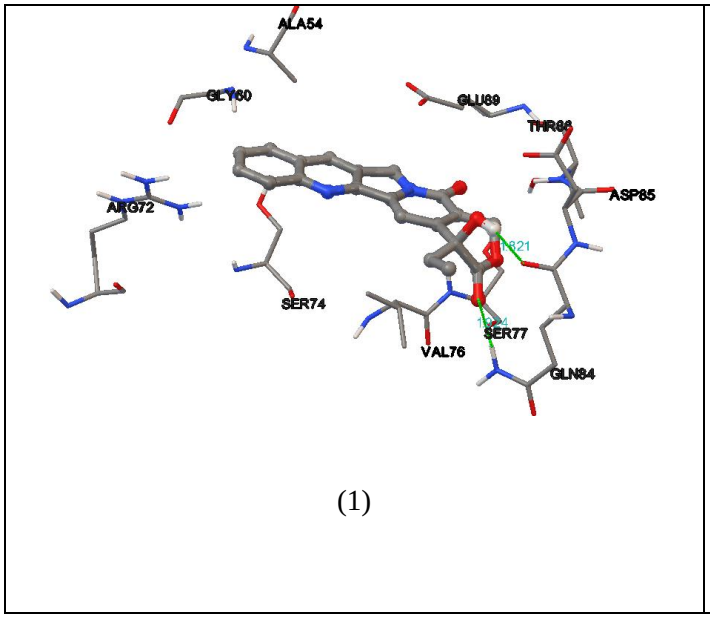


C.NO	X	Y	BINDING ENERGY(B.E)	kI (uM)	PROTIEN-LIGAND INTERACTIONS
19	CH_3	Br	-6.68	12.62	THR86
20	C_2H_5	Br	-7.00	7.43	ARG72
21	C_2H_5	CN	-7.64	2.49	GLY60,ARG72,SER74
22	CH_3	CH_2NH_2	-6.72	11.77	THR86
23	C_2H_5	CH_2NH_2	-6.79	10.53	GLY84
24	C_2H_5	$C(NH_2)NOH$	-5.91	46.32	GLN91,SER77
25	C_2H_5	$C(NH_2)NH$	-6.25	26.36	GLN84,ALA53
26	CH_3	$CC\text{-}CH_2NH_2$	-6.69	12.4	THR86,GLY60
27	C_2H_5	$CC\text{-}CH_2NH_2$	-6.32	23.33	SER77,THR86



C.NO	X	Y	BINDING ENERGY(B.E)	kI (uM)	PROTEIN-LIGAND INTERACTIONS
28	H	H	-7.74	2.13	GLN84
29	OH	H	-8.06	1.23	SER77,THR86,GLY60
30	OCH_3	F	-7.12	6.09	SER77,THR86

Table 1. Binding energy, protein-ligand interactions of camptothecin derivatives with modelled protein



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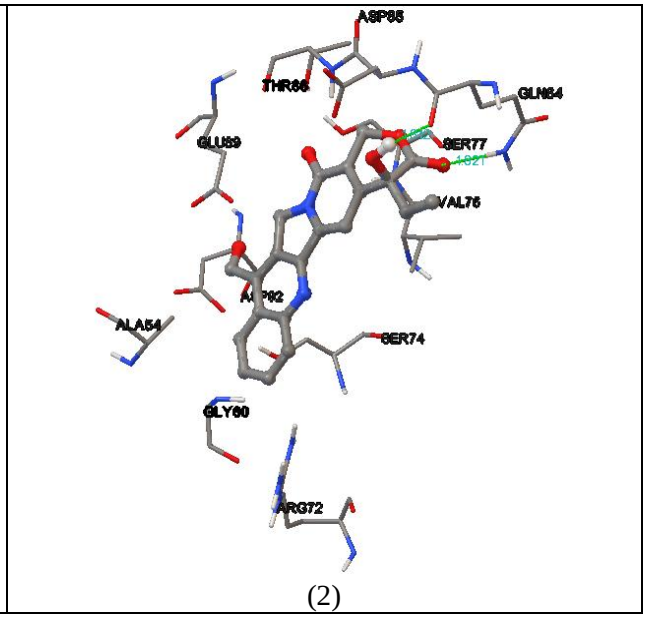

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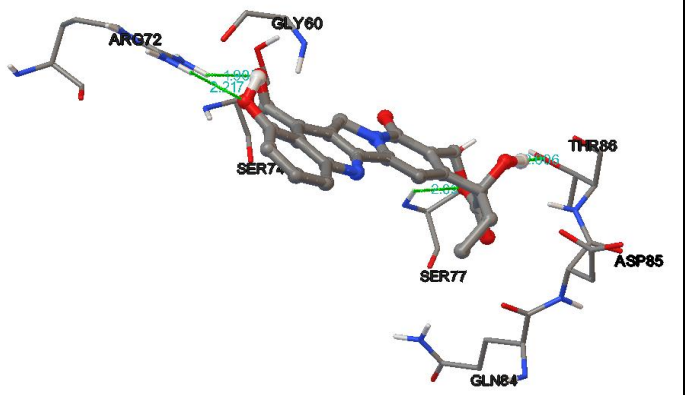
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THR86
SER77
ASP85
GLN84

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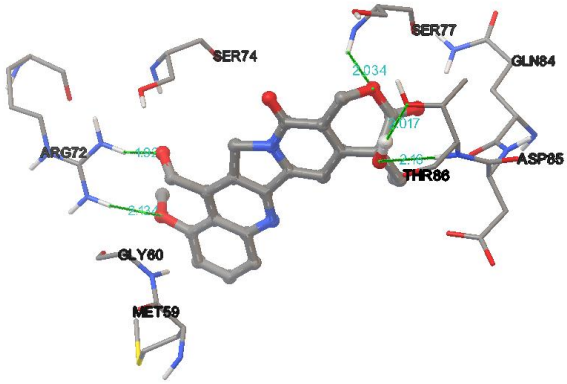
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ARG72
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GLY60
MET59

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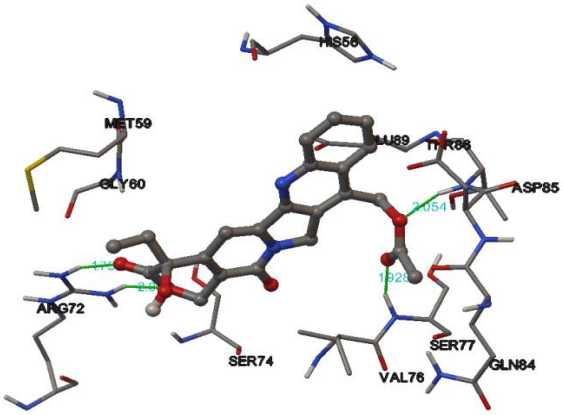
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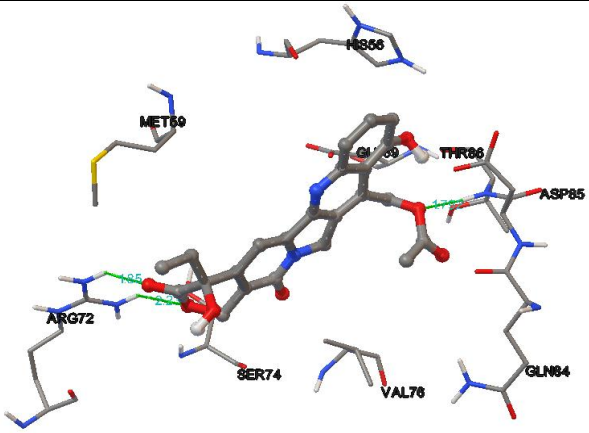
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GLN84

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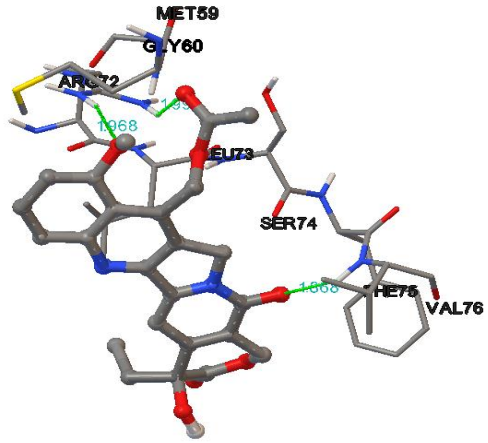
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GLU73
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VAL76

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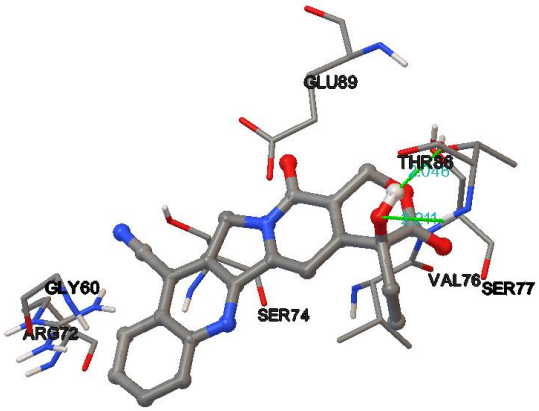
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VAL76
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SER74
ARG72

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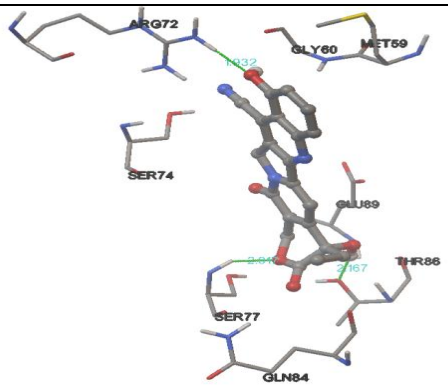
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GLN84

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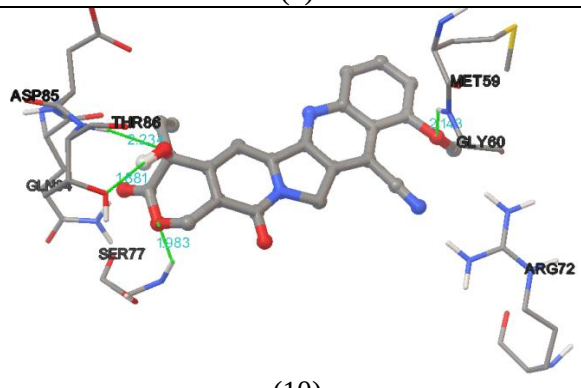
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GLY60
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SER77
ARG72

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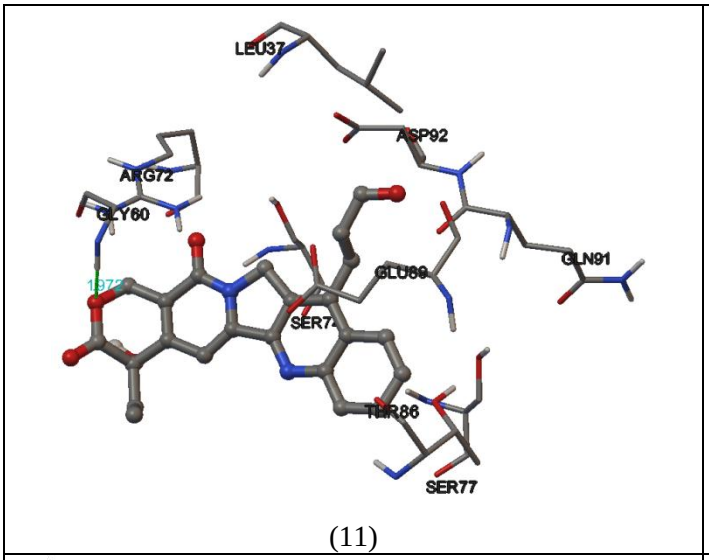

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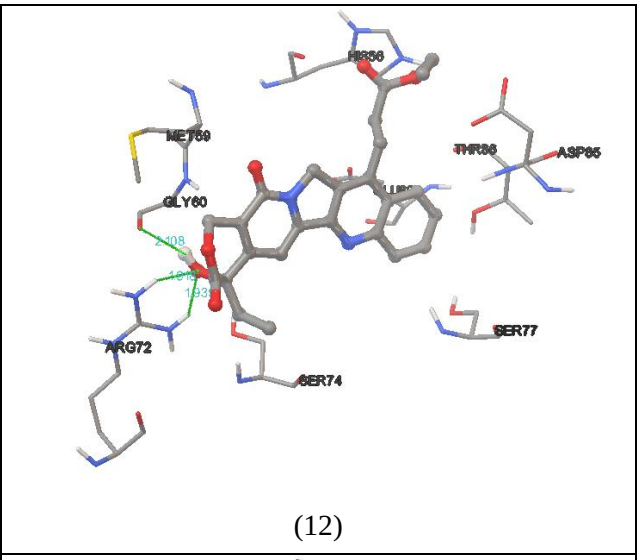

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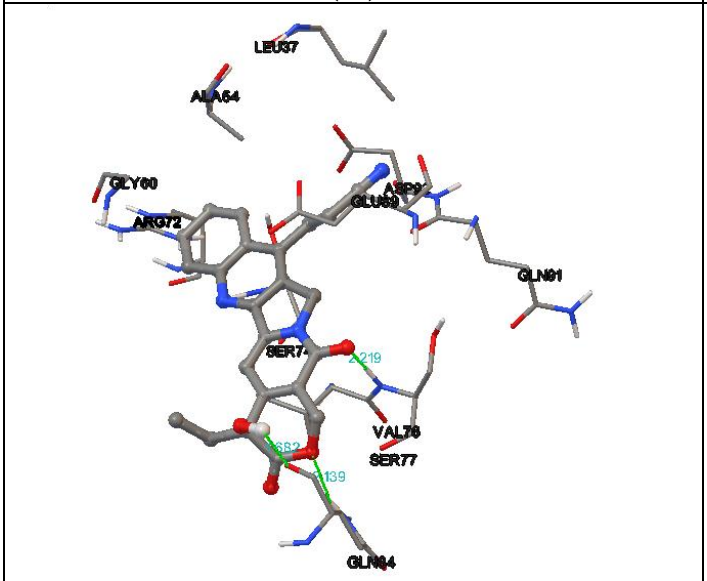

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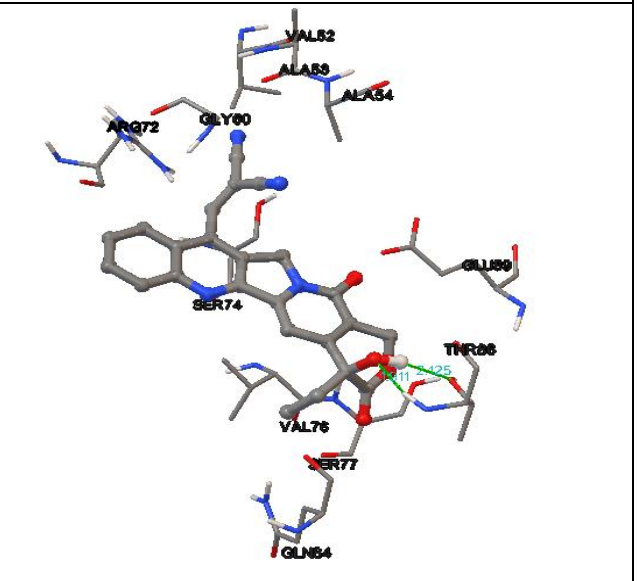

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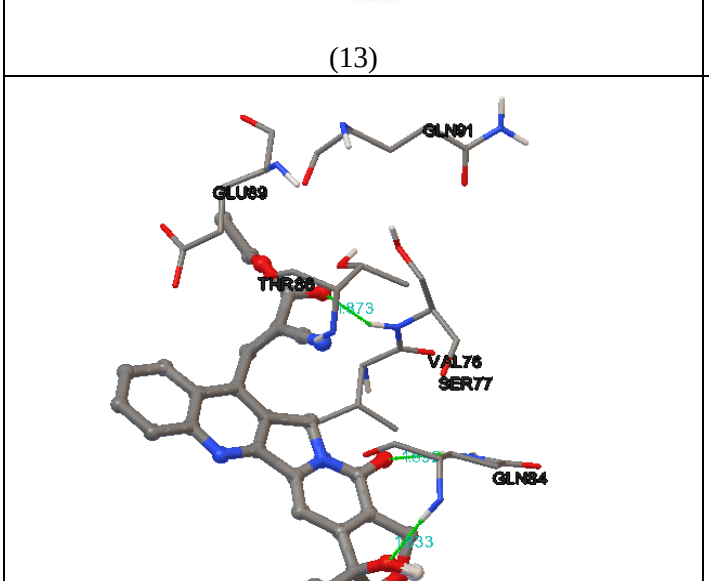

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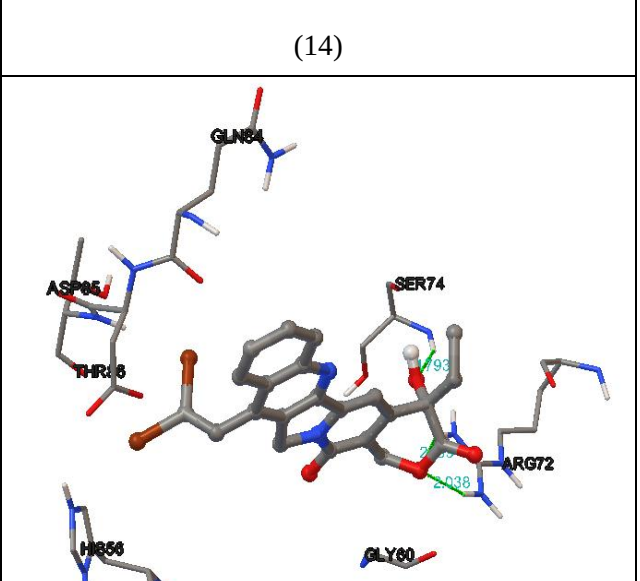

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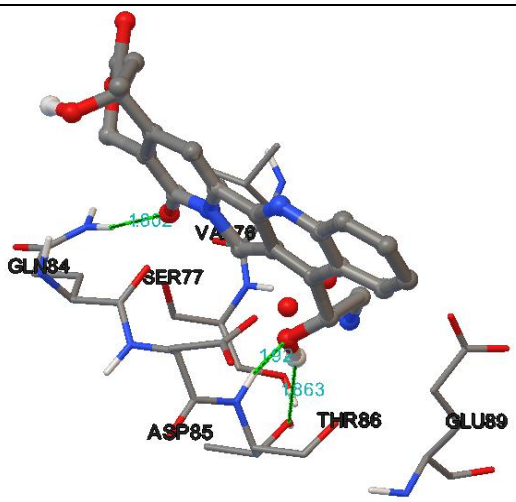

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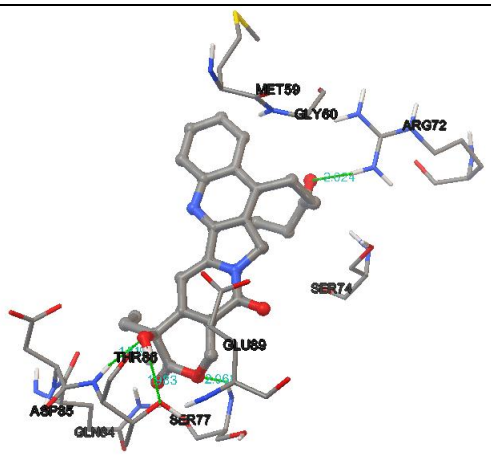

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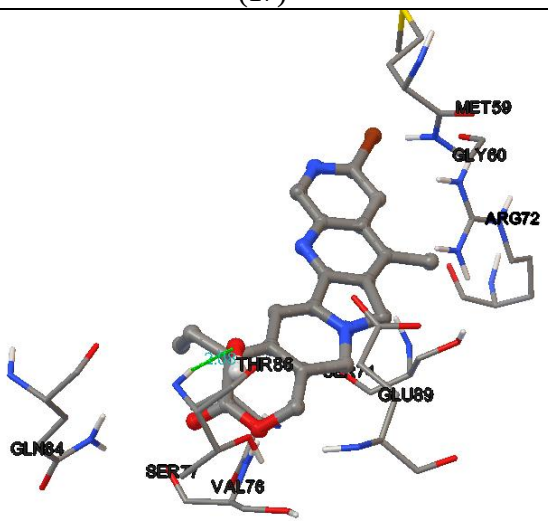

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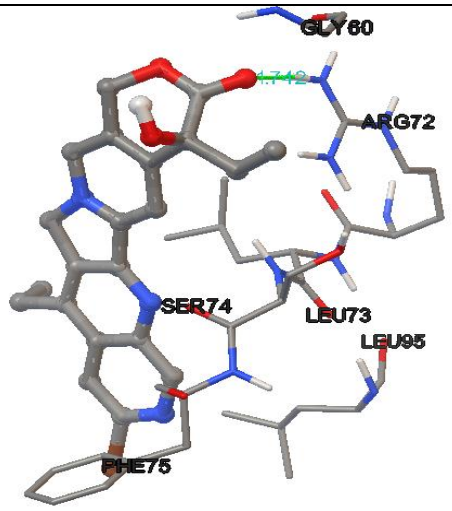

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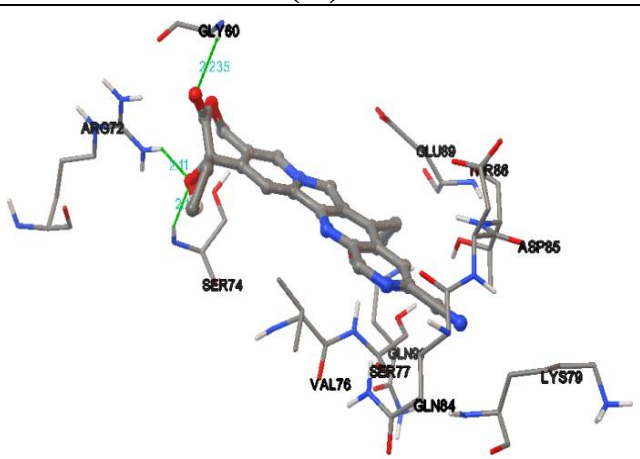

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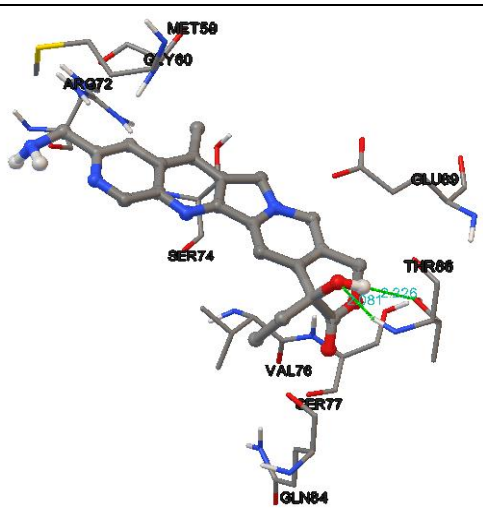

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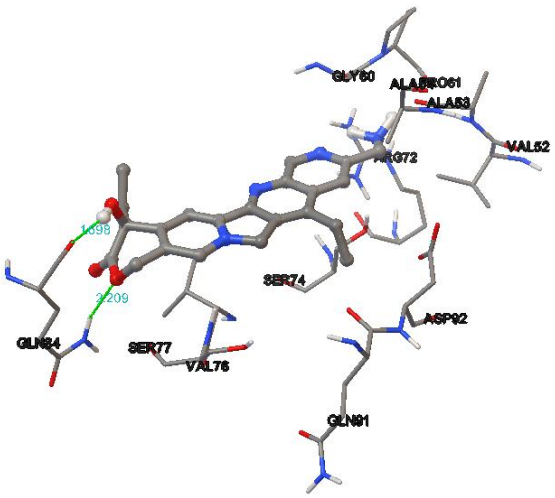

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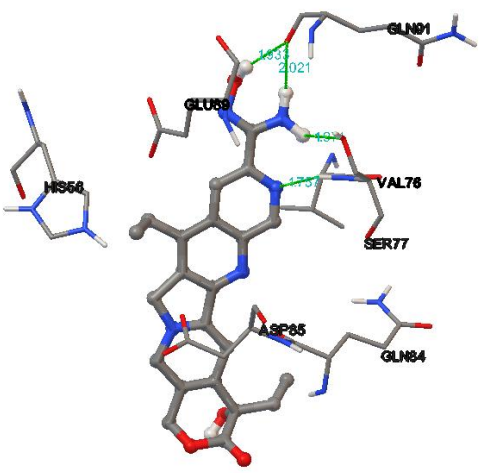

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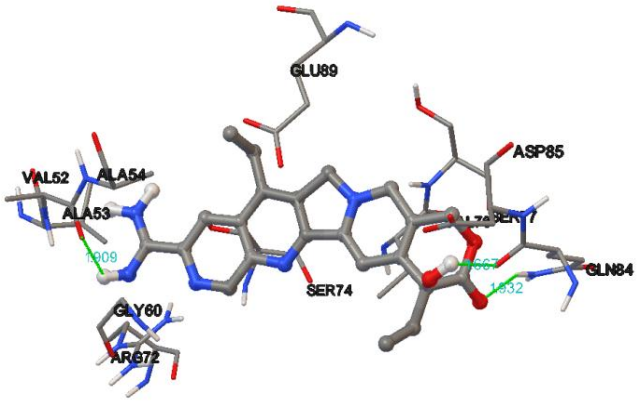

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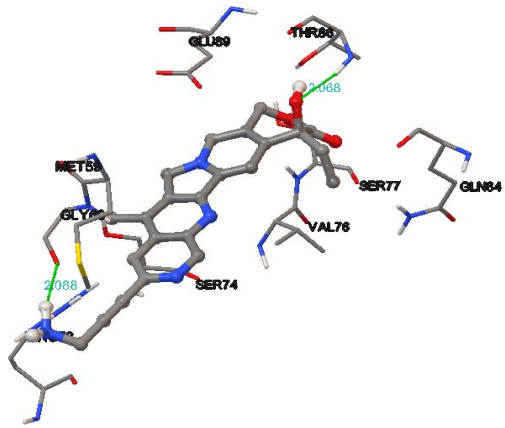

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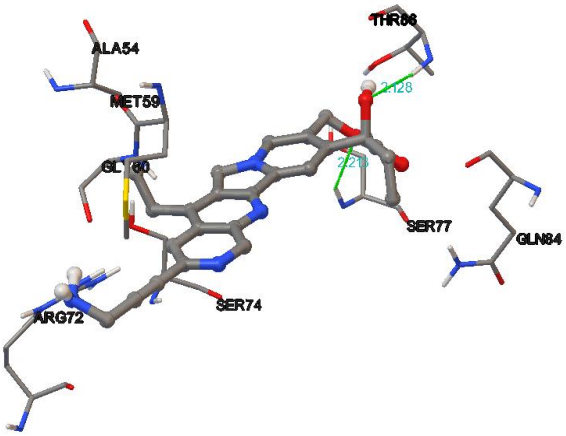

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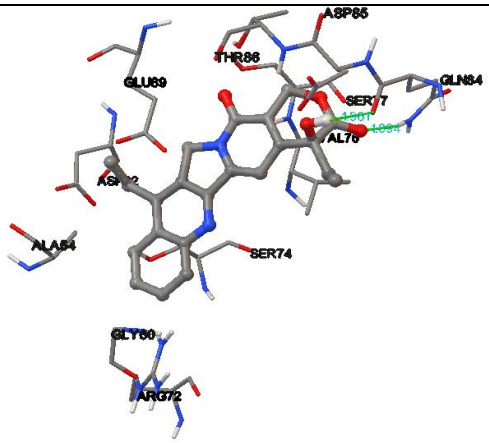
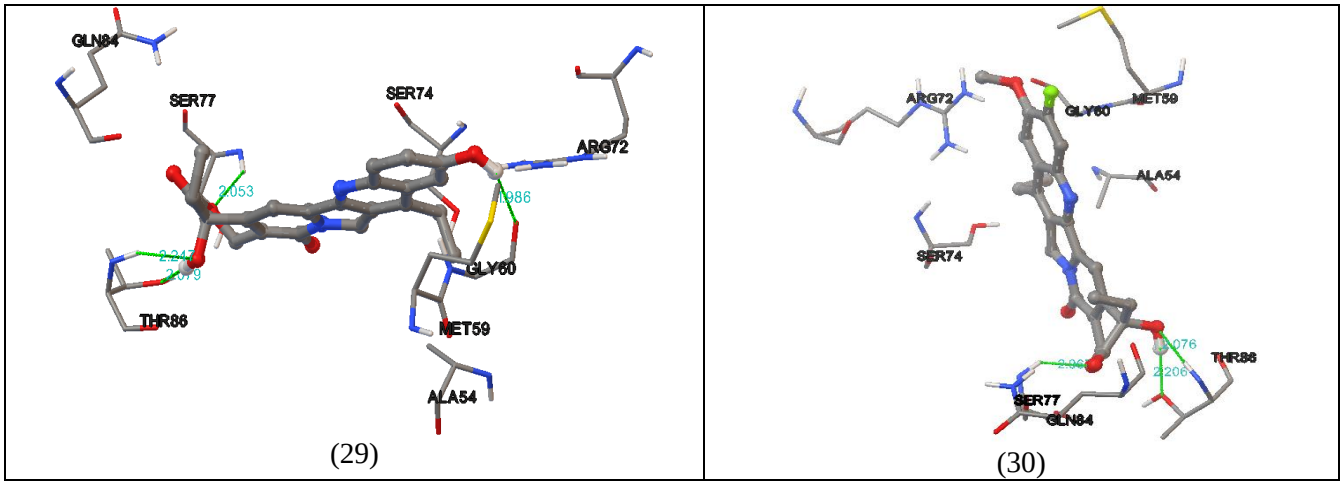


(28)



(29) (30)

Fig.3: Protein-ligand interactions of 30 camptothecin derivatives

Conclusion:

Structure-based drug design techniques were implemented in the past by the lack of a crystal structure for the target protein. Homology modeling is a powerful tool to suggest modeling of ligand-receptor interactions. The modelling studies are also useful for mutagenesis experiments, lead optimization etc., lack of crystal structure of human poliovirus related protein 2, alpha isoform motivated us to apply *in silico* techniques to initiate the drug discovery process for this protein. To understand the characteristics, structural features of Q9UEI6 and to execute the structure based drug discovery strategy we developed a model by using modeller. Several amino acid residues Arg72, Ser77, Thr86 are involved in direct interactions with camptothecin derivatives. These studies should help to improve our knowledge of understanding the role of homology modeling and docking studies in drug discovery process.

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