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Molecular docking and *in silico* analysis of cancer using Survivin as drug target

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Abstract

Survivin is a member of inhibitor of apoptosis protein family and plays an important role in cell division. This protein is undetectable in normal cells but is highly expressed in variety of human cancers including that of colon, breast, lung, pancreas and stomach that makes it a potential target for cancer treatment. Survivin, 142 amino acid proteins, is encoded by gene located on human chromosome 17 at band q25. It contains a baculovirus inhibitor of apoptosis repeat (BIR) protein domain that consists of 4 a-helices and 3-beta sheets. This BIR domain is essential for anti- apoptotic activity. Apoptosis is programmed cell death which maintains equivalence between the cell division and cell death. Survivin mainly inhibit caspases (cystein dependent aspartate directed proteases) that are involved in apoptosis and thus suppresses the apoptosis. It also acts as a subunit of chromosomal passenger complex that is required for proper chromosomal segregation and cytokinesis. Thus, Survivin plays dual role in spindle monitoring at mitosis and ability to inhibit apoptosis through the inhibition of caspases. In silico analysis was performed for homology search, motif and domain prediction and validation of model to the level of drug designing. Here we choose a ligand from zinc database and its drug properties like drug likeliness, solubility, clogP, drug score etc. were checked by Osiris property explorer and via Mol Inspiration. Docking was performed by using Hex software and Autodock Vina tool.

Introduction

Cancer appears due to uncontrollable growth of cells. Normal cells follow an orderly way to grow, divide and die. There are various mechanisms by which cell death occurs. Apoptosis is a programmed cell death. Cancer cells do not undergo programmed cell death and divide continuously results in formation of abnormal cells mass that grow out of control and have ability to migrate from one part to other part of the body. There are different types of cancers depending upon from where they originate. If the cells lose the ability to self destruction, but has ability to proliferate, it may result in carcinogenesis.

There are various mechanisms by which cell death occurs. One of the mechanisms is apoptosis. Apoptosis, also known as programmed cell death, makes a balance between the cell division and cell death. Apoptotic cell death is triggered by extrinsic, receptor-mediated, or intrinsic, mitochondria-mediated, signaling pathways that induce death-associated proteolytic and/or nucleolytic activities. Activation of cystein dependent proteases called caspases leads to apoptosis. These caspases plays an important role in cell death. Upon activation of caspases, various cellular functions are affected that results in cell death. The apoptotic caspases are divided into two main groups (A) **Initiator caspases** (2, 8,9,10,

require binding to specific oligomeric adaptor protein) (B) **Effector caspases** (3, 6 and 7, are activated by the active initiator caspases through proteolytic cleavage) these active effector caspases degrade a host of intracellular proteins to carry out cell death process [4].

Further two major pathways are involved in activation of caspases: **1. Extrinsic pathway**: An extrinsic pathway involves death receptors that belong to tumor necrosis factor (TNF).The ligand binds to these receptors and ultimately results in cell destruction. Ligands for these receptors are FasL, TNFα and their corresponding receptors are FasR, TNFR1. This binding leads to caspase cascade initiation through caspase 8. Then by the activation and dimerization of caspase 8 directly activate caspase 3 and caspase 7 leads to apoptosis. **2. Intrinsic pathway**: An intrinsic pathway is mitochondria-dependent pathway that responds to variety of stimuli including DNA damage, chemotherapeutic agents etc. Due to these stimuli, mitochondrial membrane permeability increases and various proteins are released into cytoplasm such as cytochrome c, which in turn binds to and activates apoptotic protease activating factor (Apaf1) forms a complex known as apoptosome leads to activation of caspase 9 and protease cascade leading to apoptosis [7].

There are various anti-apoptotic proteins that inhibit the apoptosis. These are known as inhibitors of apoptosis proteins (IAPs). IAPs are structurally related proteins that act as inhibitor of programmed cell death by regulating caspase activity. IAPs have eight family members including X-linked inhibitor of apoptosis protein (X-IAP) cIAP1, cIAP2, Niap, ILP2, Livin, Apollon, and Survivin [7].

Survivin is a protein, also called baculoviral inhibitor of apoptosis repeat containing 5 or BIRC5, in human and is encoded by the gene BIRC5 [6]. Survivin is a smallest member of inhibitor of apoptosis family and plays an important role in apoptosis and in cell division. Survivin is 16.5Kd protein of 142 amino acids and is encoded by gene located on human chromosome 17 at band q25 [2]. The structure of survivin has been determined by NMR and by crystallographic techniques. Survivin contains a single baculovirus IAP domain that is essential for caspase inhibitory function. The N-terminal portion of survivin consists of three- stranded b-sheet and four α-helices and exists as dimer forming bow-tie shape [1]. Its C-terminal has an extended α-helix.

Survivin plays an important role in cancer by inhibiting apoptosis. It is over expressed in cancer cells but is undetectable in normal adult tissues. Due to its over expression in cancer cells it is used as a therapeutic drug target for cancer treatment. Survivin on association with cofactor including hepatitis b virus X-interacting protein (HBXIP) and X-IAP leads to the formation of complex that binds to and inhibits caspase-9 [6]. This results in the inhibition of caspase 3, 7 and thus suppresses the apoptosis. Viral HBX protein shows interaction with survivin-HBXIP complex that suppresses caspase activation in survivin dependent pathway [5]. Survivin also acts as a subunit of chromosome passenger complex which also includes aurora B, INCENP, Borealin. Survivin forms complex with INCENP and Borealin that are essential for localization of midbody [1]. It regulates the microtubule dynamics and is essential for proper chromosomal segregation.

Various methods are used in vivo for the suppression of survivin expression. Several preclinical studies have shown that disrupting survivin expression or function in cancer cells decreases their proliferation and enhances apoptosis. These include suppressing survivin expression by antisense oligonucleotides, ribozyme, siRNA or antagonizing survivin function by dominant negative survivin or by Cdk inhibitors [3]. Survivin antisense oligonucleotides binds to survivin mRNA; allow the specific inhibition of survivin mRNA and protein. Thus, reduces cell proliferation and induce caspase-dependent apoptosis. A small molecule YM155 (Astellas Pharma) inhibits survivin mRNA transcription and protein expression in several tumor cell lines and is under clinical development [6].

In silico analysis and molecular docking which is a computer-aided drug designing (CADD) approach used for drug target identification and drug discovery. Molinspiration, OSIRIS and various online tools are used to check the properties of putative drug molecule/ligand molecule such as drug likeliness, ADME, clogP, solubility, mutagenicity and overall drug score. Docking of ligand molecule with the target protein is done via Hex software.

Methodology

Chromosomal location of gene

Cytogenetic Location: 17q25.3
Location on human chromosome 17 at band q25

Survivin Sequence retrieval

Survivin is a member of IAP family. Amino acid sequence of Survivin was retrieved from SwissProt / Uniprot, database, which is an annotated database. It gives the description of a non redundant set of proteins, their function, motif and domain structure, posttranslational modifications,3D structure database, pathway databases, Gene Ontology(GO) and many other variants. There are total 142 amino acid residues in Human Anti-Apoptotic Protein Survivin sequence and molecular weight of the protein was 16388.71 as calculated from Emboss. Their Domain and Motif were observed through various tools: Pfam, CDD, SMART, Prosite and STRING database was used to predict protein interaction. The interaction included direct (physical) and indirect association; they were derived from source. STRING database was used to predict protein interaction. The interaction included direct (physical) and indirect association; they were derived from source.

Structure retrieval, Visualization and Energy minimization

Human anti-apoptotic protein surviving was retrieved from RCSB PDB database with pdb id 1F3H. Structure was then uploaded to Swiss PDB viewer; loop modeling and energy minimization was performed.

Swiss-PdbViewer

DeepView - Swiss-PdbViewer is an application that provides a user friendly interface allowing to analyze several proteins at the same time. The proteins can be superimposed in order to deduce structural alignments and compare their active sites or any other relevant parts. Amino acid mutations, H-bonds, angles and distances between atoms are easy to obtain thanks to the intuitive graphic and menu interface. Swiss-PdbViewer can also read electron density maps, and provides various tools to build into the density. In addition, various modeling tools are integrated and command files for popular energy minimization packages can be generated.

Energy minimization

Energy minimization methods can precisely locate minimum energy conformations by mathematically "homing in" on the energy function minima (one at a time). The goal of energy minimization is to find a route (consisting of variation of the intramolecular degrees of freedom) from an initial conformation to the nearest minimum energy conformation using the smallest number of calculations possible. The resulting structure was saved as Receptor structure after removing disallowed regions.

Procheck

The structure was then uploaded to SAVS server for PROCHECK of the protein structure.

The aim of PROCHECK is to assess how normal, or conversely how unusual, geometry of the residues in a given protein structure is compared with stereo chemical parameters derived from well-refined, high-resolution structures. The input to PROCHECK is a single file containing the coordinates of your protein structure. The outputs comprise a number of plots, together with a detailed residue-by-residue listing.The result from PROCHECK show the disallowed region, allowed region, Bad contact, and core values etc (Fig 4).The final receptor protein structure and ramachandaran plot was then visualized by using Swiss PDB Viewer (Fig 10). In a polypeptide the main chain N-Cα and Cα-C bonds relatively free to rotate. These rotations are represented by the torsion angles phi and psi, respectively.

The active site was predicted by literature reference and online tool AADS (Automated version of active site prediction) tool was used to find the active site for docking purpose.

Molecular docking with putative ligand molecule

The putative ligand molecule (2R)-5-oxo-N-[(1S)-1, 2, 3, 4-tetrahydronaphthalen-1-yl] pyrrolidine-2-carboxamide was used for docking study. The ligand molecule was sketched by using ACD/ChemSketch version 11.0. ACD/ChemSketch is an advanced chemical drawing tool and is the accepted interface into the industry's best NMR and molecular property predictions, nomenclature, and analytical data handling software. The ligand molecule was then tested among online validation servers Molinspiration Cheminformatics server and OSIRIS property explorer for SMILES notation, drug likeliness score, Toxicity risk, molecular weight, cLogP, Solubility prediction and drug score etc. The putative ligand molecule satisfies the Lipinski "Rule of 5".

Molecular Docking

The target protein molecule was then docked with the ligand via Hex software. 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 small-ligand/protein docking (provided the ligand is rigid), and it can superpose pairs of molecules using only knowledge of their 3D shapes. The main thing which distinguishes Hex from other macromolecular docking programs and molecular graphics packages is its use of spherical polar Fourier correlations to accelerate the docking and superposition calculations.

Result and Discussion

Molecular docking, motifs & domain prediction, orthologus and mutation study of target protein Survivin reported in different diseases was the main motive of the present study.

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The FASTA format amino acid sequence of Human Anti-Apoptotic Protein Survivin was retrieved from expasy server (www.expasy.org) .Protein structure was retrieved from PDB(1F3H). The domain and motif present in survivin were observed through Pfam, SMART (Simple Modular Architecture Research Tool) and PROSITE database (Fig 1).

Fig1: Domain result A. Pfam domain B. SMART domain

The X-ray analysis of survivin, reported in pfam, Prosite and SMART provides a model for the BIR domain that is shared by all survivins. This domain is found in inhibitor of apoptosis proteins (IAPs) and other proteins and also acts as a direct inhibitor of caspase enzymes. It also shows position of domain in target protein that starts from 13 and ends at 89, E-value 4.88e-36. (Fig 1). Survivin orthologs and their occurrence was observed from String database. This view summarizes the network of predicted associations for a particular group of proteins. The network nodes are groups of orthologus proteins. Each node will display its annotation, clicking on a node gives you the list of proteins. The network edges represent the predicted functional associations. An edge may be drawn with up to 3 differently coloured lines - these lines represent the existence of the three types of evidence used in predicting the associations. A red line indicates the presence of fusion evidence; a green line - neighbourhood evidence; a blue line – co occurrence evidence. Additionally, each line may be drawn bold to indicate an above-average confidence in the evidence. String results show the interaction of target protein BIRC5 with other proteins such as AURKB, INCENP, and CDK1 etc. (Fig 2).

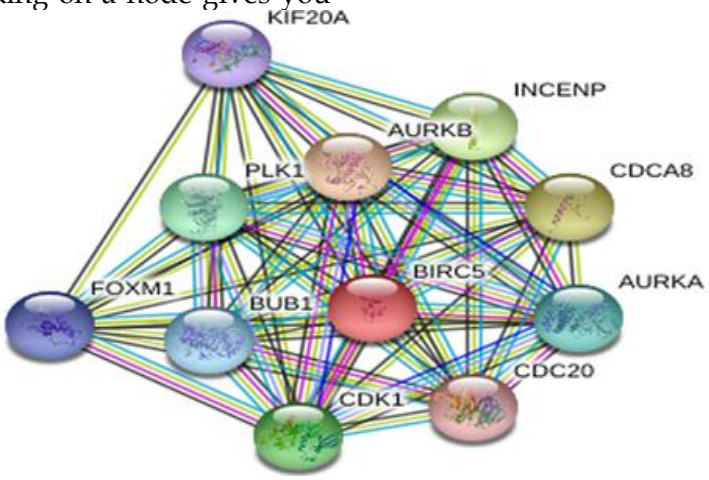

Fig 2: Showing Summary network of String result.

The secondary structures present in Survivin were predicted by using SOPM secondary structure prediction tool (Fig 3). It categorizes the sequence according to Helices, coils, sheets and turns present in the structure.

```
         10        20        30        40        50        60        70
          |         |         |         |         |         |         |
MGAPTLPPAWQPFLKDHRISTFKNWPFLEGCACTPERMAEAGFIHCPTENEPDMAQCFFCFKELEGWEPD
ccccccctt cchhctt ccccccccccc eett ccccc hhhhhtt eee ccccccccc hhhhhhhhhhhtt cccc
DDPIEEHKKHSSGCAFLSVKKQFEELTLGEFLKLDRERAKNKIAKETNNKKKEFEETAKKVRRAIEQLAA
cccchhhccccttceeeehhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhttchhhhhhhhhhhhhhhhhhhh
MD
ht

Sequence length :   142

SOPM :
   Alpha helix      (Hh) :    71 is  50.00%
   3(10) helix      (Gg) :     0 is   0.00%
   Pi helix         (Ii) :     0 is   0.00%
   Beta bridge      (Bb) :     0 is   0.00%
   Extended strand  (Ee) :     9 is   6.34%
   Beta turn        (Tt) :    15 is  10.56%
   Bend region      (Ss) :     0 is   0.00%
   Random coil      (Cc) :    47 is  33.10%
   Ambigous states  (?)  :     0 is   0.00%
   Other states          :     0 is   0.00%
```

Fig 3: Secondary structure prediction using SOPM result.

The predicted target structure was than uploaded in swiss-pdb viewer for loop modelling and energy minimization. The predicted structure was than validated by procheck tool. The result from procheck showed the disallowed region (0.0%), allowed region (2.4%), bad contact (0) and core value (88.3%) (Fig 4).

```
+----------<<<  P R O C H E C K    S U M M A R Y  >>>----------+
|                                                               |
| /var/www/html/Services/SAVES_3/jobs/1187125/em..pdb   2.0     136 residues |
|                                                               |
| Ramachandran plot:   88.3% core   11.7% allow    0.0% gener    0.0% disall |
|                                                               |
| All Ramachandrans:    4 labelled residues (out of 134)        |
| Chil-chi2 plots:      1 labelled residues (out of  93)        |
|                                                               |
| Main-chain params:    6 better     0 inside      0 worse      |
| Side-chain params:    5 better     0 inside      0 worse      |
|                                                               |
| Residue properties: Max.deviation:     4.1            Bad contacts:    0 |
|                     Bond len/angle:    3.9    Morris et al class:  1  1  2 |
|                                                               |
| G-factors           Dihedrals:   0.00  Covalent:   0.29    Overall:   0.12 |
|                                                               |
| M/c bond lengths: 99.9% within limits   0.1% highlighted      |
| M/c bond angles:  96.9% within limits   3.1% highlighted      |
| Planar groups:    81.0% within limits  19.0% highlighted       5 off graph |
|                                                               |
+---------------------------------------------------------------------------+
  + May be worth investigating further.  * Worth investigating further.
```

Fig 4: Procheck Result.

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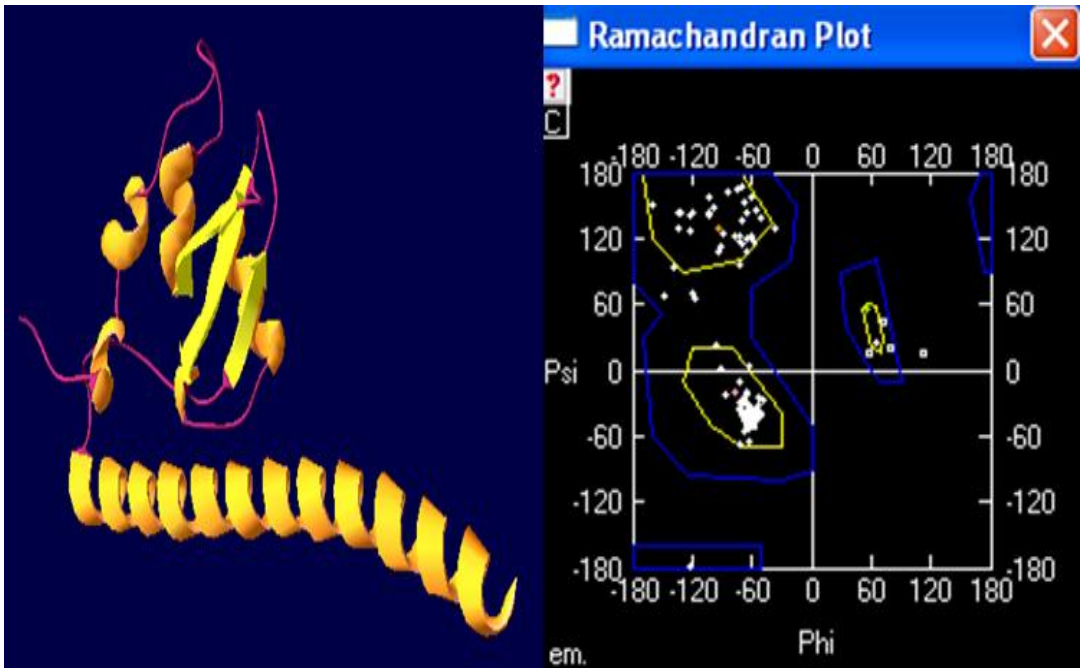
The final protein structure and ramachandran plot then visualized in Spdbv viewer (Fig 5).



A. Survivin Structure **B.** Ramachandran Plot

Fig 5: Survivin structure and Ramachandran plot.

Then AADS (Automated version of active site prediction) tool was used to find the active site for docking purpose [9]. The biggest cavity was selected for further docking study after calculating the nearest residue to active site by swisspdb. The ligand molecule was sketched using ACD/chemsketch version 11.0(Fig 6).

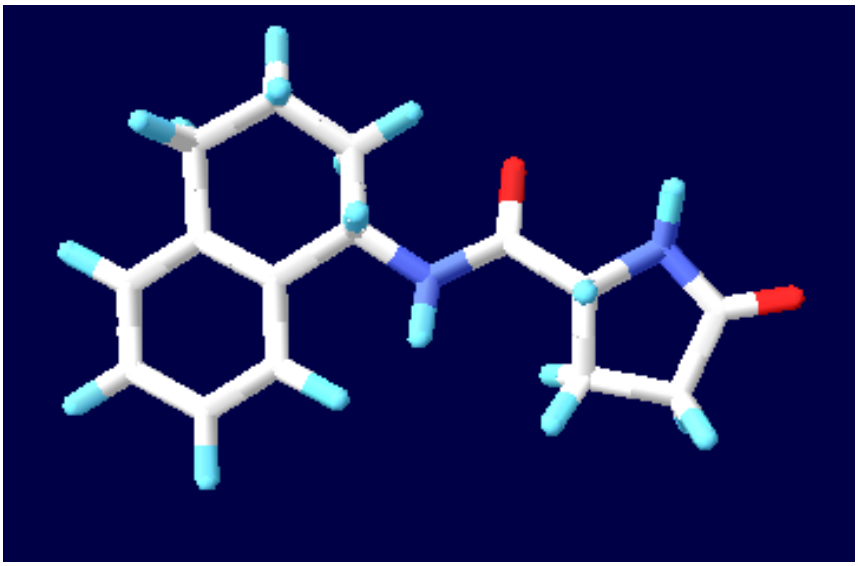


Fig6: Ligand molecule structure.

The drug molecule was tested among various online validation server (molinsipiration, Osiris) for various drug properties (Fig 7).

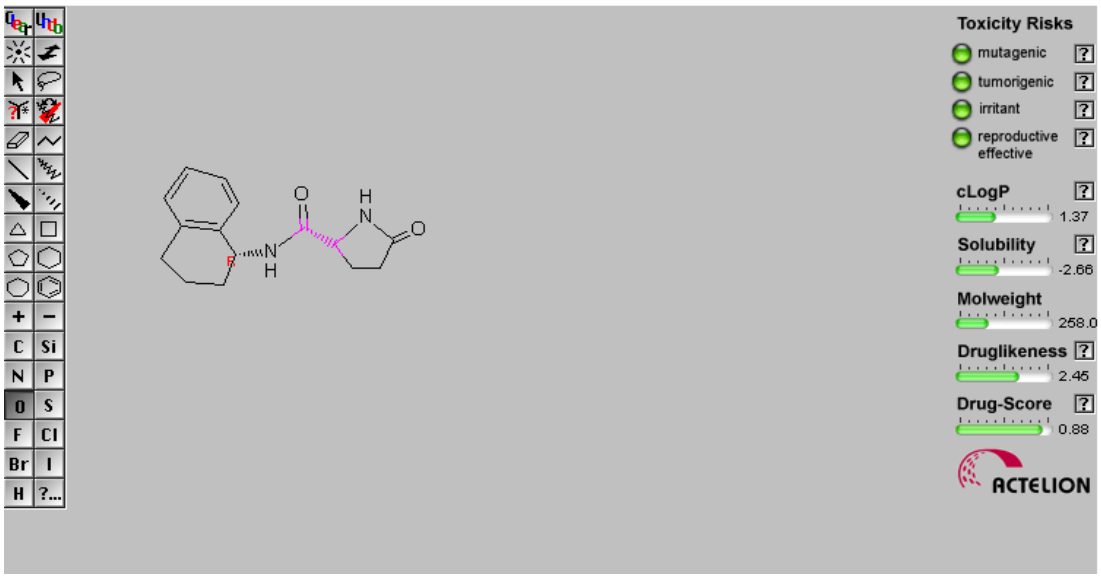
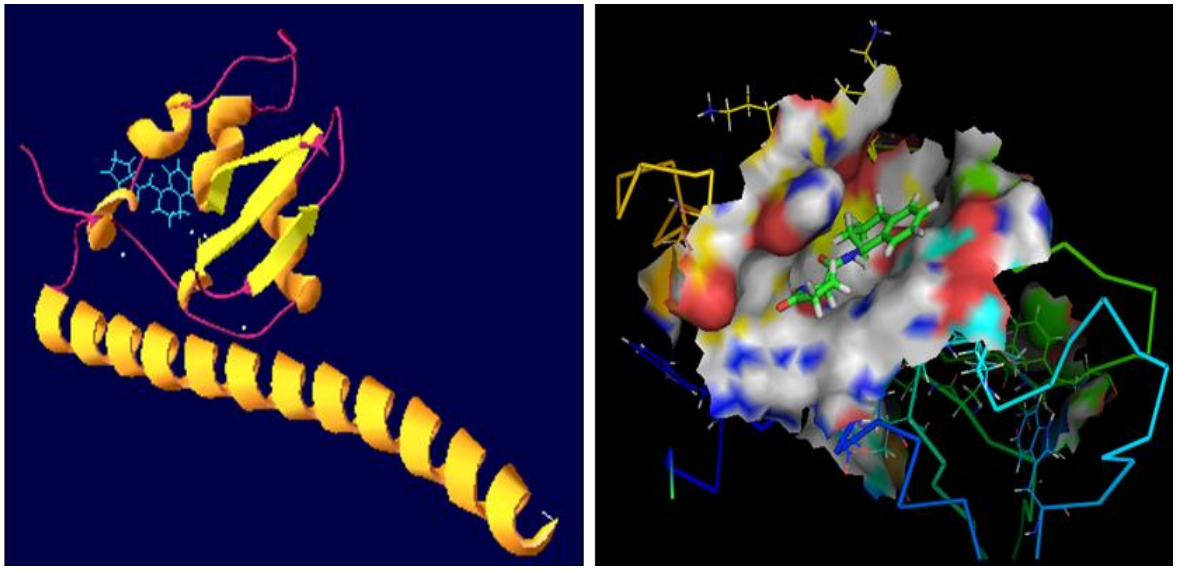


Fig 7: Showing toxicity, clogP, druglikeness, overall drug score of Ligand molecule .

The drug molecule was checked out with no toxic, non-irritant, and no bad effects on organism. The target molecule was than docked with ligand via HEX software (Fig 8). Final docking will be performed by Auto Dock vina (vina.scripps.edu/).



A. Swiss-PDB Viewer View **B.** PyMol View

Fig 8: ShowingDockingofSer-78with (2R)-5-oxo-N-[(1S)-1, 2, 3, 4-tetrahydronaphthalen-1-yl] pyrrolidine-2-carboxamide.

The putative drug molecule with molecular weight 258.31 and the smile notation O=C1CCC (N1) C (=O)N[C@@H]3CCCc2ccccc23.C have no toxicity, non irritant, non carcinogenic effect and have no adverse effect on organism.. Thus the overall drug score of ligand molecule is 0.88. The target molecule was improved and its desired properties were checked through various tools and servers. This putative lead molecule can be the good drug candidate against Cancer.

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3. http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_gor4.html, http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopm.html, http://www.sbg.bio.ic.ac.uk/phyre/, http://string-db.org/