Ligand Based Pharmacophore Modeling and Virtual Screening of PARP2 Inhibitors

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1.Abstract

In whole world malignancy in prostate is most wide reprted reason of male casualties and sixth most known reason of deaths in men. To cure PCa, ADT is the advanced cure leading to initial response and durable remission, incurable castration-resistant prostate cancer (CRPC) invariably develops.PARP-2 the drug target protein, who increases AR-mediated transcription by binding with talready present factor FOXA, that is also a drug target. Taking PARP-2 potentially give best replacement therapy for AR inhibition without the involvement of AR ligand binding. NAD+coenzyme contesting inhibitor PARPat enzyme active site are new most effecting therapeutic treatmentsfor cure of malignancy having ability to repair DNA.

1.2 Introduction:

Almost 8 years ago, huge number of cases round about in lacsand many casualties in world were reported from PCa.It is good prostate cancer not only grow in slow speed but also spread slowly and can be cured at very first stage. It shows very confined aggressiveness.

Most of the cases don't show any type of initial symptom and delayed symptoms in many cases make involvement of aches due to anemia, disturbance in structural organs, and many others.

For PCa diagnostics,PSA diagnosis, and TRUS is done.

The advanced diagnostic techniques make involvement PSA levels, PCA3 urine testing, PHI, "4K" test, genomic analysis, MRI , PIRADS, and MRI-TRUS.

In start PCa is bengn and restructed to only prostate and not dangerous and can be cured very easily as it is considered on pre first stage of the prostate cancer.

Prostate cancer become malignant in some cases and start to spread in other parts and organs of body even can spread in bones.For malignant tumor the temproray pain medications, hormonal treatment, chemotherapy, radiopharmaceuticals, immunotherapy, focused radiation, and other targeted therapies are done.

The results of treatments depend on age of patient,other health problems(HYpertension,Diabetes) tumor histology and rate of cancer at which it can spread.

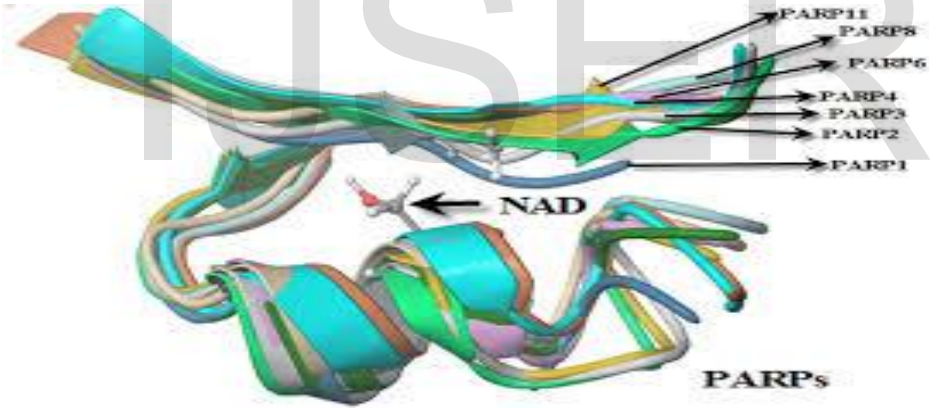
1.3 PARP2:

Polymerase (PARP) is main category of enzymes, this category of enzymes perform post-translational modification other huge enzymes and control many various cellular processes. PARP-1 and PARP-2, drug targets,the two main componentsof PARP family, who perform many important functions including catalytic activity created by DNA-strand breaks and are very important for multiple DNA damage repair pathways.

PARP inhibitors are promising therapeutic agents that show synthetic lethality against many types of cancer (including PCa) with homologous recombination (HR) DNA-repair deficiency.

Beyond DNA damage repair function, PARP-2, is main part in AR machinery by reacting with factor FOXA1.

PARP-1 and PARP-2 make utilization of β-NAD+ precursor, make and transfer ADP-ribose polymers onto glutamate substrate.



hPARP-2 a protein in nucleus having molecular weight of 62 kDa. Gene at 14q11.2 code it.

2.Methods:

2.1Selection and structural analysis of target protein:

The target protein PARP2 compsition was obtained and analysed from PDB and Drug bank. By using the PDB the protein was filtered again to Homo-sapiens only. The PARP2 PDB (ID: 4PJV).Catalytic family stick to inhibitor BMN 673 was then selected. We selected 15 known ligands from 4PYJ protein and downloaded in molfilefrom PDB.

2.2Pharmacophore modelling and virtual screening:

A pharmacophore model was generated using ligand scout. This was done by uploading the 3D structures of 15 known ligands obtained from PDB database into the ligand scout. Training set of these 15 combination of atoms were selected. The library for unknown group of atoms was obtained from Aurora. IDB databases were then inserted into the ligand scout. By performing screening, the potential unknown ligands similar to known ligands in terms of properties, toxicity and structure index were obtained. The top 5 candidate ligands with the greatest pharmacophore fit score were then selected for inhibiting PARP2 in prostate and to further validate the generated pharmacophore.

2.3Determination of properties:

The Lipinski rule ADMET qualitieswere determined using mcule as well as molinspiration software. The toxicity of top 5 candidate ligands was determined using Mcule. Mcule is an online drug discovery platform to determine substructures commonly found in toxic and poisonous ligands. Molinspiration is used to calculate the bioactivity of a ligand. Both these softwares also provide property calculator to determine molecular properties of a ligand along with numerous other features necessary for drug designing.

2.4Molecular Docking:

For further analysis, the structures of top 5 candidate ligands were drawn and downloaded in molfile form via Pubchemsketcher. The structures were later uploaded in UCSF Chimera for further processing. UCSF chimera, a molecular modelling system.Chimera was used for the removal of natural residues from 4PJV dimer. 4PJV protein structurewas fetched into chimera from PDB and searched for possible natural ligands. In order to dock our candidate ligands to our target protein we usedPyRx. Pyrx is a virtual screening software.The process of dockingwas done by uploading the PDB file of our target protein 4PJV. After which imported the five structures of ligands made in PubChemsketcher. This was followed by Auto docking the ligands. Running Vina wizard, the results obtained should be negative.

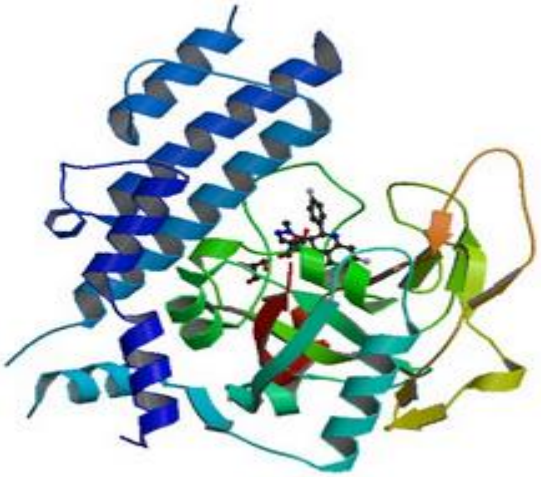
2.5Visualization:

Then we use PyMOL software for visualization of our results. PyMOL provides animated 3D molecular visualization of our target protein and candidate ligands docked to it.

Results and Discussion:

PARP1 and PARP performing activity in DNA damage response.A new PARP1/2 inhibitor is BMN 673in next levels of medical growth for BRCA-deficient breast malignancy. New two structured scaffold of BMN 673 enhance binding reactions. Crystallographic structural resultsanalysed here give molecular basis for high dose of medically growing candidate BMN 673, and also novel availabilities for increasing inhibitor selectivity.

ThePARP2 structure retrieved from PDB (ID: 4PJV).



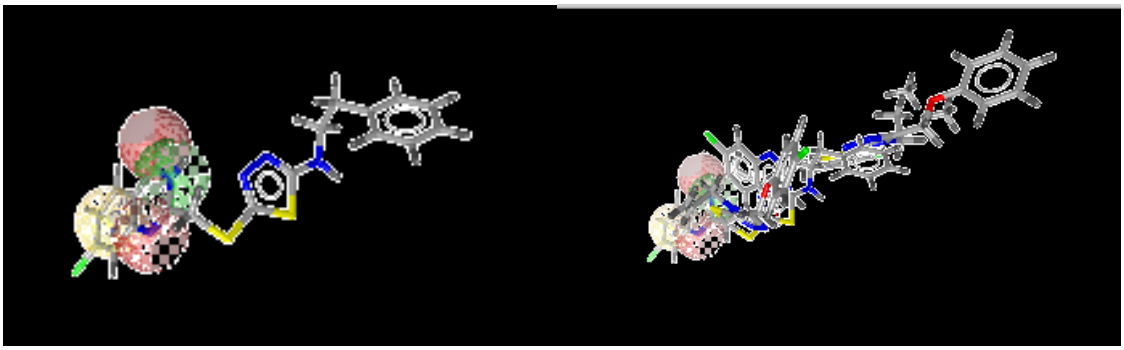
3.1 Lead identification and validation

- ***Dataset collection***

Compounds which have strong racting capability with molecular proteinare reactive than others. Active most compounds should involved in the training set and all biologically relevant data should be obtained by homogenous procedures.
Lying on structural variation and activity range, 15 compounds were used for training set compounds and others were utilized as test set for validating model.

- ***Pharmacophore Generation and Validation***

From 15 active training set pharmacocore models designed by using ligand scout.The compounds with pharmcophore fit score upto 50% were chosen.



Pharmacophore Fit Score Table

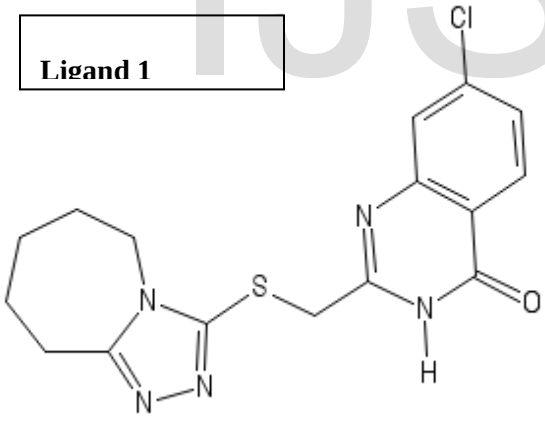
Name	#	Matching Features	Pharmaco-phore Fit-score	Mol. Index	Active/ Decoy	Best Match	Source Database	ID Number	# confs.	Score

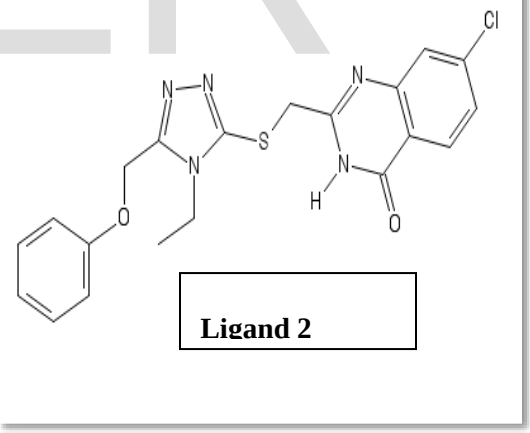
Ligand 1	**807**		**46.40**	**841**	**Active**	**Model-1**	**Aurora.idb**	**Z21577886**	**33**	**46.40**
Ligand 2	**2384**		**46.39**	**2572**	**Active**	**Model-1**	**Aurora.idb**	**Z217788864**	**50**	**46.39**
Ligand 3	**2216**		**46.39**	**2395**	**Active**	**Model-1**	**Aurora.idb**	**Z151842528**	**50**	**46.39**
Ligand 4	**2099**		**46.39**	**2264**	**Active**	**Model-1**	**Aurora.idb**	**Z194332162**	**50**	**46.39**
Ligand 5	**1305**		**46.39**	**1367**	**Active**	**Model-1**	**Aurora.idb**	**Z54952232**	**50**	**46.39**

Pubchem Sketcher:

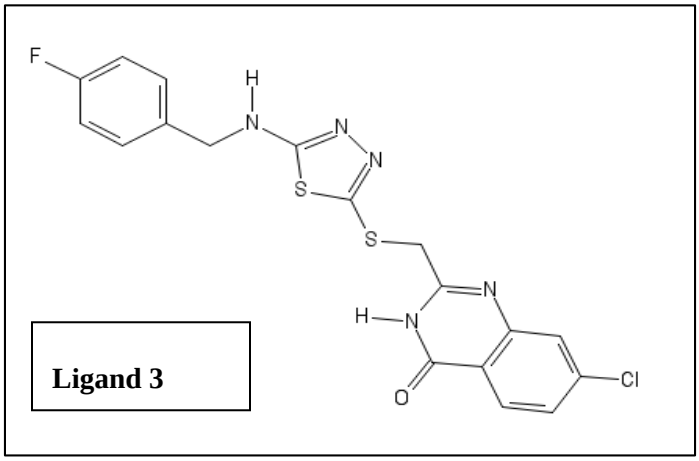
After pharmacophore generation on ligandscout, we obtain many structures, from which we selected top 5 and draw them with help of pubchem sketcher as shown below:

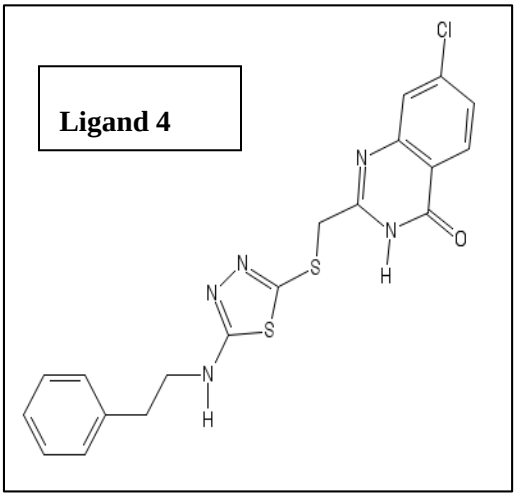
Ligand 1

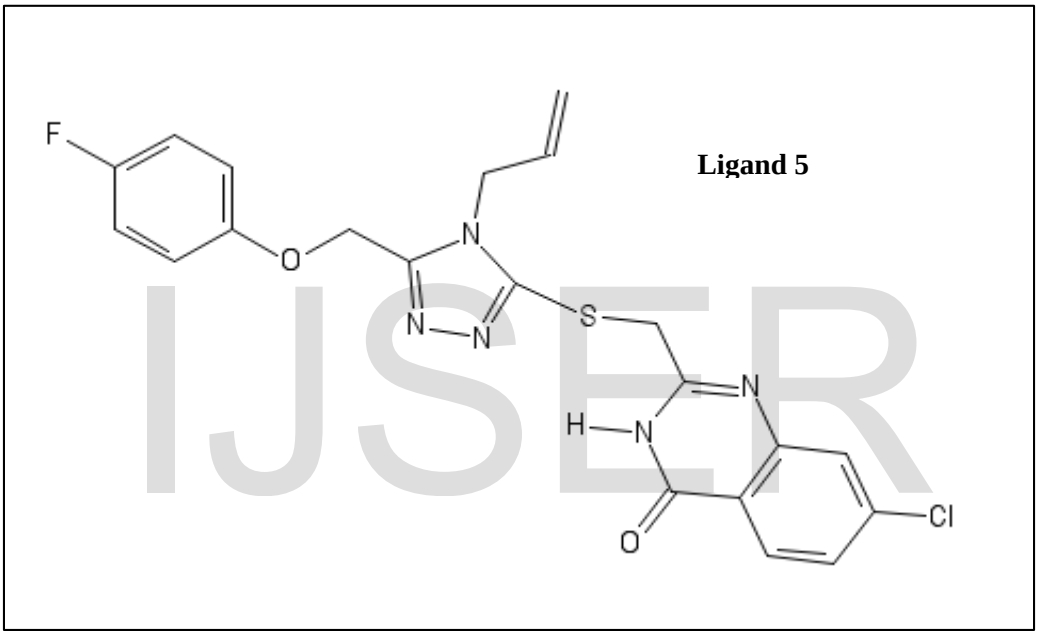




Ligand 2







3.2 Determination of ligand properties:

The ligand properties were determined using molinspiration and mcule software. The ligands ADMET qualities confirmed with the help ofmcule software and Lipinski's rule of five. These are used to verify the properties of drug-likeness or for the purpose to find out that if there is any chemical complexhavingspecific pharmacological or biological activity that has physical and chemical properties that would bring it to a possible orally active drug in humans.

1. MOLECULAR PROPERTIES:

Lipinski's rule declares that, commonly, an orally active drug has certainly no additional than one contravention of the subsequentconditions:

- More than 5 hydrogen bond donors (the entire number of oxygen-hydrogen and nitrogen-hydrogen bonds) should not be present.

LIGAND	GPCR Ligand	Ion channel modulator	Kinase inhibitor	Nuclear Receptor ligand	Protease inhibitor	Enzyme Inhibitor	Bioactivity Status

- More than 10 hydrogen-bond acceptors (eachoxygen or nitrogen atoms) should not be present.
- Molecular mass has to be less than 500 Daltons.
- Water-partition co-efficient (log *P*) that is octanol must not exceed than 5.

Every one of the ligands satisfied the conditions of being an orally active drug clearly shown in the table given below:

Ligands	H-bond Donors	H-bond Acceptors	Molecular Mass(Daltons)	Logp	Toxicity
Ligand 1	2	6	429.9492	4.4479	NO
Ligand 2	1	6	361.8499	3.1867	NO
Ligand 3	1	7	427.9076	4.0592	NO
Ligand 4	2	6	433.9131	4.5445	NO
Ligand 5	1	7	457.9087	4.3644	Yes

2. TOXICITY:

Mculesoftware that we used just for the purpose to measure toxicity according to which only ligand 5 was reported to be potentially toxic among other ligands.

3. BIOACTIVITY:

Drug bioactivity could be tested by analyzing the activity count of GPCR of ligand,nuclear receptor ligand, ion channel modulator,protease inhibitor,kinase inhibitor, enzyme inhibitor. To check the bioactivity, structures taken from Pubchem sketcher were uploaded on mcule and it provide the restrictions shown in the table given below. To know the bioactivity, subsequent criteria is being followed:

a) Bioactivity score if is>0 then it is active.
b) Bioactivity score if ranges from -5.0 to 0.0, then it is moderately active.
c) Bioactivity score if it is < -5.0, then it is inactive.

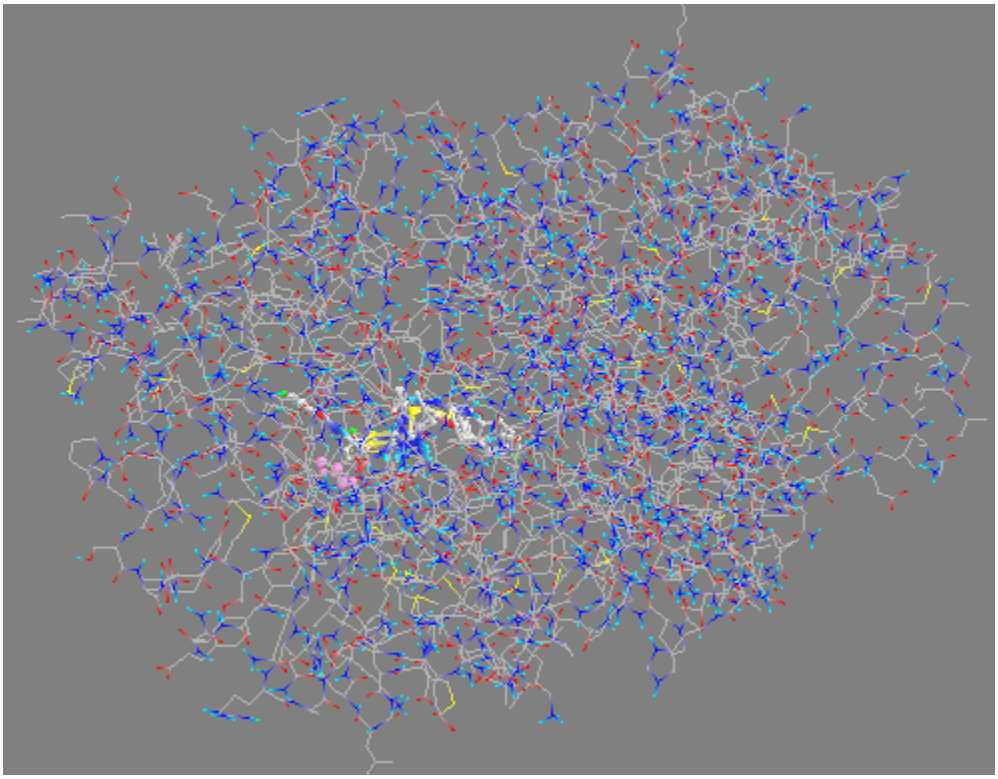
Ligand 1	-0.41	-1.01	-0.63	-0.99	-0.91	-0.28	Moderately active
Ligand 2	-0.58	-0.93	-0.53	-0.90	-0.86	-0.43	Moderately active
Ligand 3	-0.58	-0.69	-0.18	-0.71	-0.68	-0.21	Moderately active
Ligand 4	-0.51	-0.71	-0.20	-0.76	-0.59	-0.20	Moderately active
Ligand 5	-0.59	-0.84	-0.62	-0.87	-0.86	-0.48	Moderately active

3.3 Molecular Docking:

Utmost active compounds given in the training set and those which have been screened best hit compounds were identified. Finally, five compounds were selected as shown in the table given below. These particular ligands were uploaded on PyRX along with edited form of macromolecule of 4PJV. After running the vina wizard on PyRx, 5 hits were obtained. According to convention, more negative value of binding affinity, more strongly that ligand compound will attach to the target protein. As the _uff_E=505.42 show the most negative value, so this ligand will bind efficiently with the protein molecule.

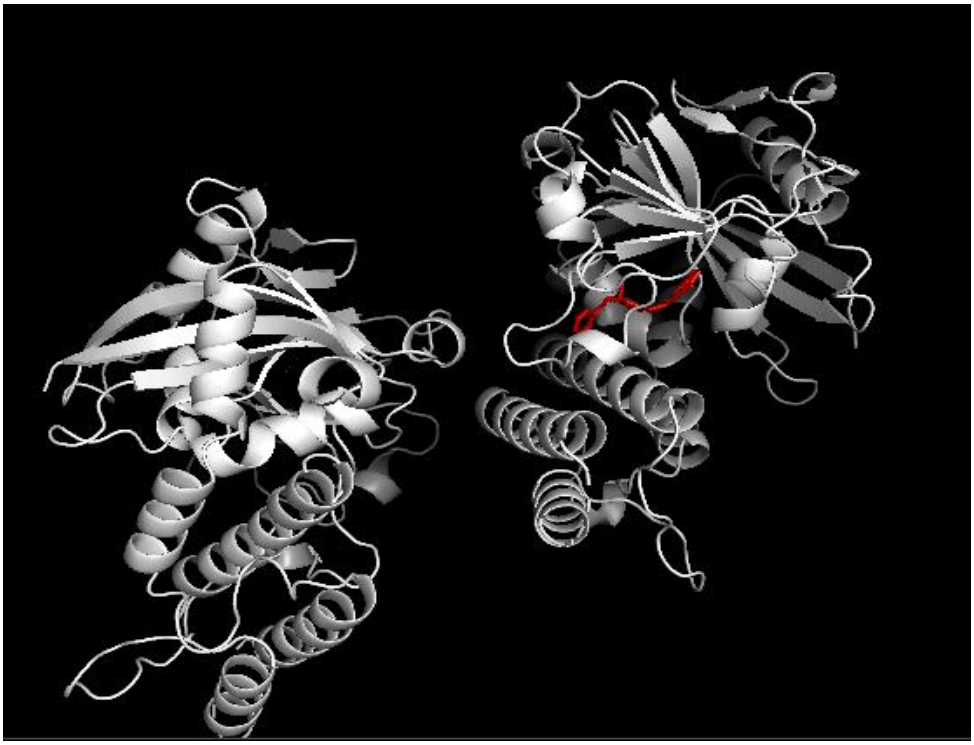
Ligands	**Binding Affinity Kcal/mol**
4PJV_uff_E=501.88	-10.0
4PJV_uff_E=533.33	-10.1
4PJV_uff_E=505.42	-10.3
4PJV_uff_E=499.67	-10.2
4PJV_uff_E=508.11	-10.2

It apperas on pyRxas shown below.

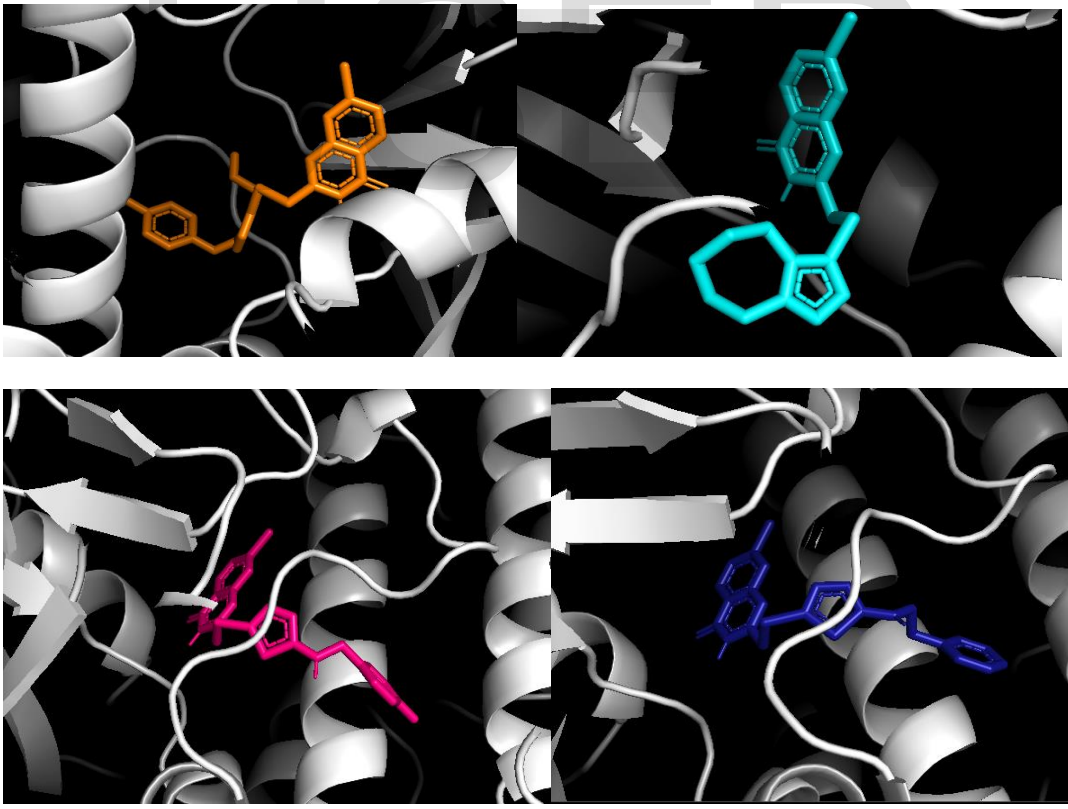


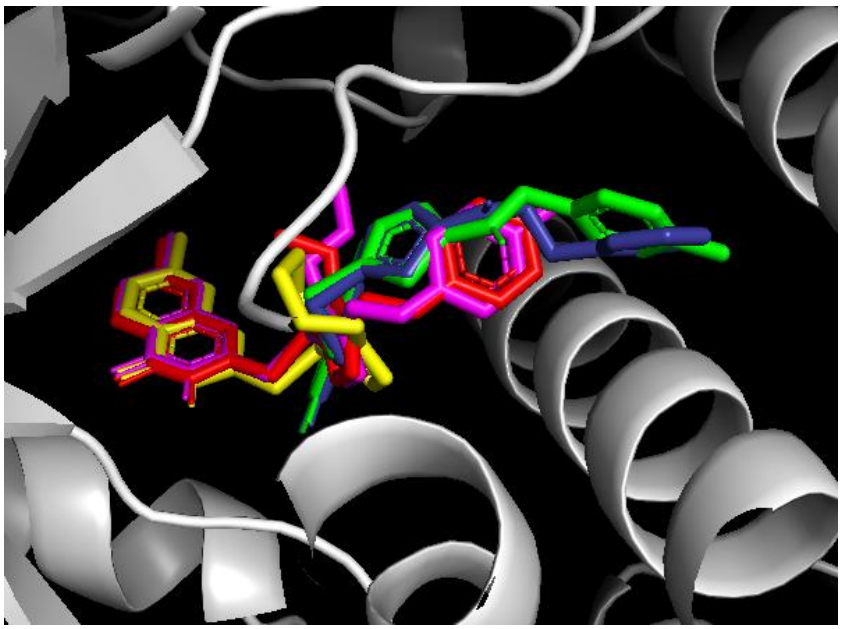
3.4Visualization on PyMOL:

PyMOL is a molecular graphics system and used for the visualization of the interaction between the compounds. In the image given below, it shows the interaction between the ligand(shown in RED) having best binding affinity with the 4PJV component of the APP. The other ligands are also shown below separately having binding affinity with 4PJV. In the last picture below all ligands are shown together bonded with the 4PJV.



Ligand: 505





Conclusion

The data from the study indicates that the potential drug to treat the leading Prostate cancer can be produced through pharmacophore modelling. The pharmacophore model illustrates the selective binding of 5 candidate ligands on target 4PJV homodimer fragment of PARP2 protein. Thus pharmacophore model is useful for insilico screening of potential drugs. The library of potential ligands were obtained from PDB and aurora.idb databases. The result of the study indicates,ligand uff_E=505.42 with moderate activity best docks with the target protein 4PJVfragment and act as an effective inhibitor of BMN673 accumulation in prostate with preferably less toxicity among all ligands taken.This drug can be taken orally.

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