



## Insilico Drug Designing of Potent Inhibitors for PDE7B, A Therapeutic Target for Leukemia

Dwivedi Vivek Dhar<sup>1</sup>, Sharma Tanuj<sup>1</sup>, Shukla Swati<sup>2</sup>, Gaurav Naik<sup>2</sup> and Mishra Sarad Kumar<sup>2</sup>

<sup>1</sup>Department of Bioinformatics, U.C.S.T., Dehradun, UK, INDIA

<sup>2</sup>Department of Biotechnology, DDU Gorakhpur University, Gorakhpur, UP, INDIA

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### Abstract

Leukemia is a cancer of the blood or bone marrow and is characterized by an abnormal proliferation of leukocytes. However, it affects more adults than children. In leukemia, the bone marrow makes abnormal white blood cells known as leukemia cells. They crowd out normal blood cells and made it hard for them to function properly. Leukemia is of two types, acute and chronic. According to the recent research, the PDE7B (Phosphodiesterase 7B) cAMP-specific Phosphodiesterase controls the levels of cAMP, promote apoptosis, and a process that is defective in chronic lymphocytic leukemia (CLL). Recent studies and research have found that lower cAMP levels contribute to the decreased apoptosis that causes CLL. The catalytic region lies between 172-410 residues and the active site residue lies at position of 173 – Histidine which acts as a proton donor. The ligand was developed using ChemSketch, which was docked with the receptor (target protein) and then ligand was allowed to grow with the help of LIGBUILDER in to a potential drug candidate, the drug has mild toxicity risks and having drug score of 0.34. It also have  $IC_{50}$  9.88e-003 and delta G 6.59 which is good enough to inhibit the expression of the protein PDE7B and effective in curing Leukemia.

**Keywords:** Leukemia, cancer, cAMP, PDE7B, protein, ligand, docking.

### Introduction

Leukemia is a cancer of the blood or bone marrow and is characterized by an abnormal proliferation (production by multiplication) of blood cells, usually white blood cells (leukocytes). Leukemia is a cancer of the blood or bone marrow and is characterized by an abnormal proliferation (production by multiplication) of blood cells, usually white blood cells (leukocytes)<sup>1-3</sup>. Leukemia is a broad term covering a spectrum of diseases. In turn, it is part of the even broader group of diseases called hematological neoplasm. Most blood cells develop from cells in the bone marrow called stem cells<sup>4,5,6</sup>. Bone marrow is the soft material in the center of most bones. Leukemia is clinically and pathologically subdivided into several large groups. The first division is between its acute and chronic forms. Chronic leukemia is distinguished by the excessive build up of relatively mature, but still abnormal, White blood cells. Typically takes months or years to progress, the cells are produced at a much higher rate than normal cells, resulting in many abnormal white blood cells in the blood<sup>7</sup>. Chronic forms are monitored for some time before treatment to ensure maximum effectiveness of therapy. A human cAMP-specific Phosphodiesterase (PDE7B) cDNA was isolated from human caudate nucleus<sup>8</sup>. The human PDE7B composed of 450 amino acid residues with a molecular mass of 51,835 Da. The deduced amino acid sequence of human PDE7B was 64.1% identical to that of human PDE7A (67.1% identity in the catalytic region). The PDE7B exhibited unique tissue

distribution in humans and kinetic profiles. The specific type of enzyme, phosphodiesterase 7B (PDE7B) controls the levels of (cAMP), a molecule that can promote programmed cell death (apoptosis), and a process that is defective in CLL. It is also known as the 3', 5'-cyclic nucleotides cAMP and cGMP<sup>9</sup>. Cyclic nucleotide Phosphodiesterase (PDE) isoforms can influence disease pathogenesis because lower cAMP levels may contribute to the decreased apoptosis that occurs in chronic lymphocytic leukemia (CLL), on assessing the expression levels of PDE isoforms in peripheral blood mononuclear cells (PBMC) of healthy adults and patients with CLL<sup>10-11</sup>. A unique PDE mRNA signature was found in CLL: higher levels than in normal PBMC of PDE7B (increased approximately 23-fold). Increased PDE7B mRNA in CLL correlates with a 10-fold-higher expression of PDE7B protein and results in an increased contribution of PDE7 to total PDE activity. The increase in PDE7B expression and PDE7 inhibitor-promoted apoptosis implicates PDE7B as a drug target in CLL<sup>12</sup>. Considering the above facts, designing a drug for leukemia is quite challenging. In the present study, we performed the individual *in silico* structure based drug designing of potent inhibitors for PDE7B, which is a therapeutic target for leukemia.

### Material and Methods

Target molecule PDE7B was selected as potential drug target for leukemia. Its protein sequence was retrieved from NCBI protein sequence database with accession number NP\_061818.

Homolog for this protein sequence was retrieved using BLAST program. Homology modeling was performed by the use of modeller tool. Validation of models was done by PROCHEK tool under the SAVS server. Pocket identification of model was done by ligsite to identify the best pocket. ChemSketch was used to draw ligand for inhibition of above model. Mol tracer was used to find the closest residues the pocket is determined. After drawing ligand, then rigid docking was performed by HEX. Ligbuilder was used to build ligand molecule inside the binding pocket by growing the small ligand into a large molecule. ADMET properties of derivative structure were validated with the help of online servers: OSIRIS and MOLINSPIRATION. The best structure was selected on the basis of its properties. Docking of the ligand to a set of grids describing the target protein was done by AutoDock. QUANTUM software was used for flexible docking.

## Results and Discussion

Protein sequence for leukemia was retrieved from the protein database available at the National Center for Biotechnology Information (NCBI) with accession number NP\_061818. Homology search for the query sequence was performed by using BLAST program against protein data bank. Five models were obtained for target protein using homology modeling. These models were analyzed through PROCHECK under the SAVS server and then each model was opened in SPDB viewer to check their Ramachandran plot for those amino acids which were in disallowed region (figure-1). Models were validated by loop building to remove those amino acids which were in disallowed region and by energy minimization to remove bad

contacts. After performing validations steps the model with zero bad contacts and highest core % was selected as the best model or the final receptor. Pockets were identified by using the online server LIGSITE. After running the ligsite 3 pockets were identified and selected the pocket 1 as active site residue his-173 lies in this pocket (figure-2). The structure of inhibitor was drawn on the basis of available drugs/inhibitors on the target protein. The functional group common to all inhibitors was taken to draw the ligand for inhibitor on ChemSketch (figure-3). After finding the best pocket for performing hex, the closest residue to the pocket was determined with the help of mol tracer program (figure-4), which is software used to find the closest residues and gives the first five closest residues. HIS-80, CE1-854 was preferred as first residue. After drawing ligand, then rigid docking was performed by HEX. Receptor for hex was the final model obtained after validation. Then hex docks the ligand in the free space near the closest residue - HIS-80, CE1 (figure-5). Ligbuilder was used to build ligand molecule inside the binding pocket by growing the small ligand into a large molecule (figure-6). After checking their ADMET properties on OSIRIS (figure-7) and MOLINSPIRATION server (figure-8), inhibitor10 was selected as for mild toxicity risks and then it was docked with the receptor by using AutoDock 4.1(figure-9). And their binding energies were calculated. The receptor was denoted by biomacromolecule and ligand was denoted by small molecule. Firstly in biomacromolecule, grid box was created and it was run till the active site analysis was 100%, then in small molecule the ligand was docked in the protein using Quantum 3.30 (figure-10).

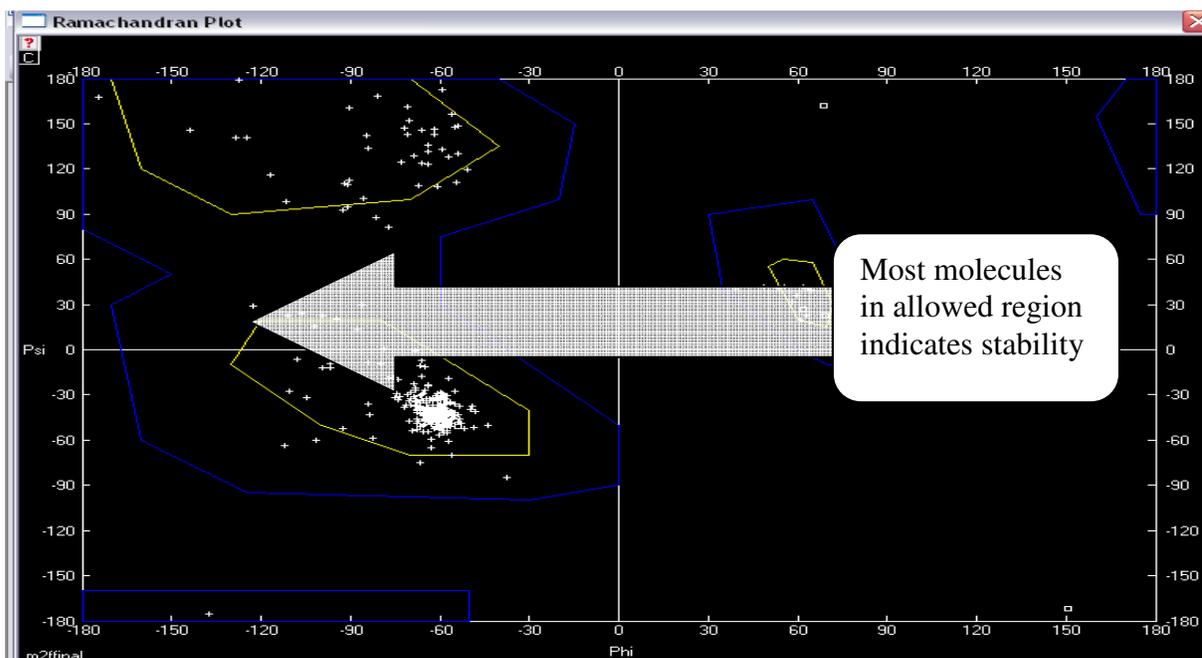
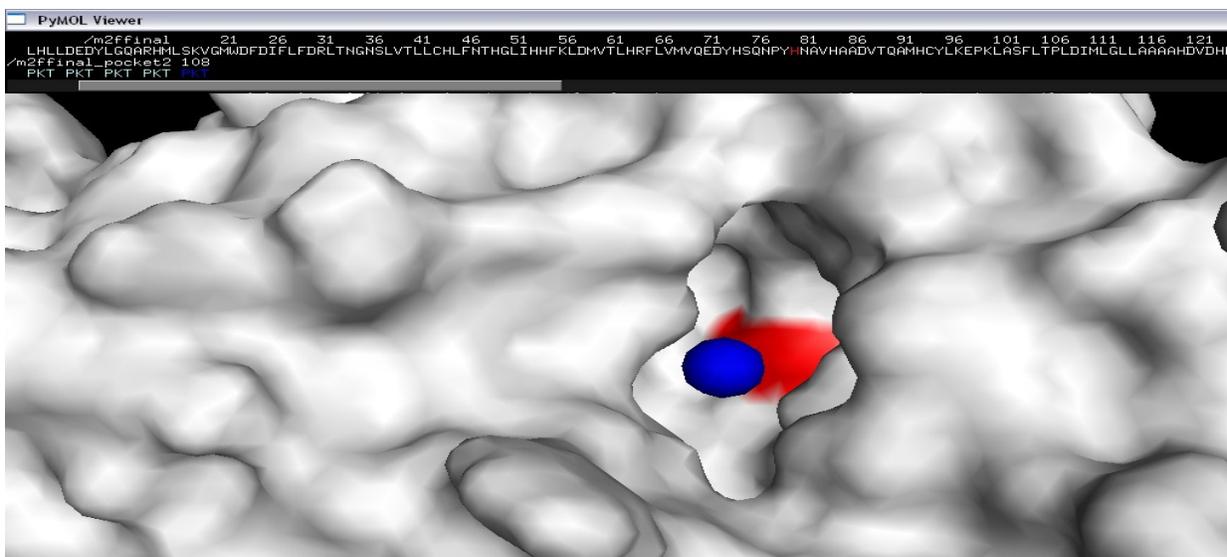
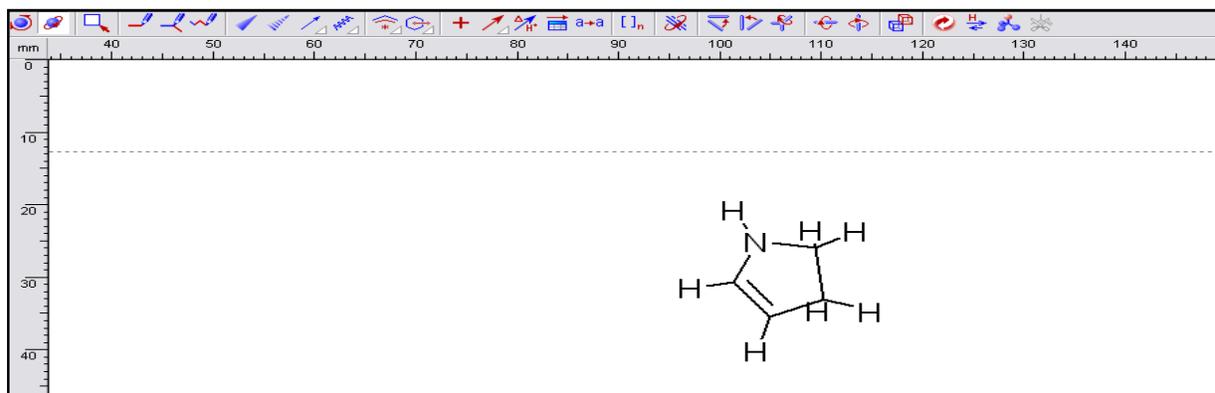


Figure-1  
Indicating allowed region of target protein



**Figure-2**  
 Indicating best pocket identified



**Figure-3**  
 Indicating structure of ligand in ChemSketch

The image shows the MolTracer software interface. At the top, the MolTracer logo is displayed next to a 3D model of a protein structure. Below the logo, the following information is shown:

**Protein Information** C:\Documents and Settings\All Users\Documents\batch2\joita\m2ffinalpdb

**Pocket Co-ordinates** [ X : 93.200 ] [ Y : -10.795 ] [ Z : 8.470 ]

No.	Residue	Atom	Distance (Angstrom)
1	HIS - 80	CE1 - 854	4.98472720685472
2	HIS - 80	NE2 - 855	5.01737699704626
3	HIS - 124	H - 1255	5.99834114558361
4	HIS - 80	CD2 - 853	6.33006924940056
5	HIS - 80	H - 856	6.79008424229569

**Figure-4**  
 Indicating closest residues found by moltracer

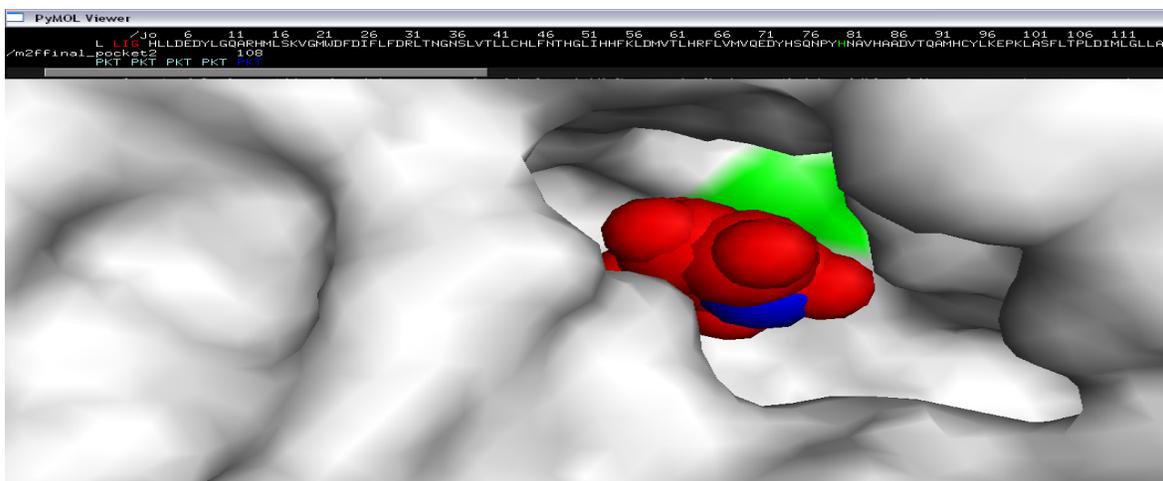


Figure -5  
Indicating docked residue of target protein

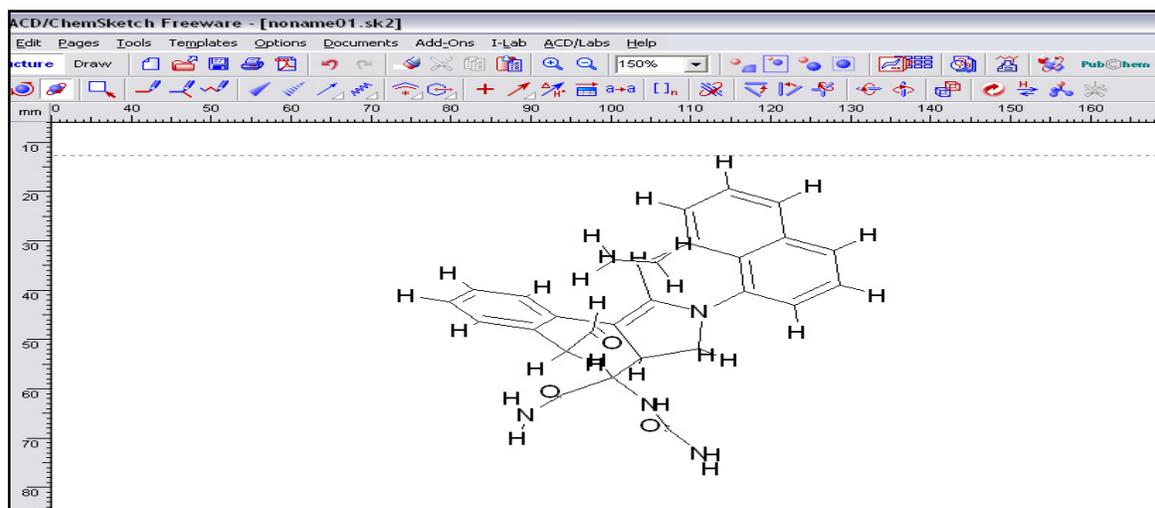


Figure-6  
Structure of inhibitor in ChemSketch obtained by ligbuilder

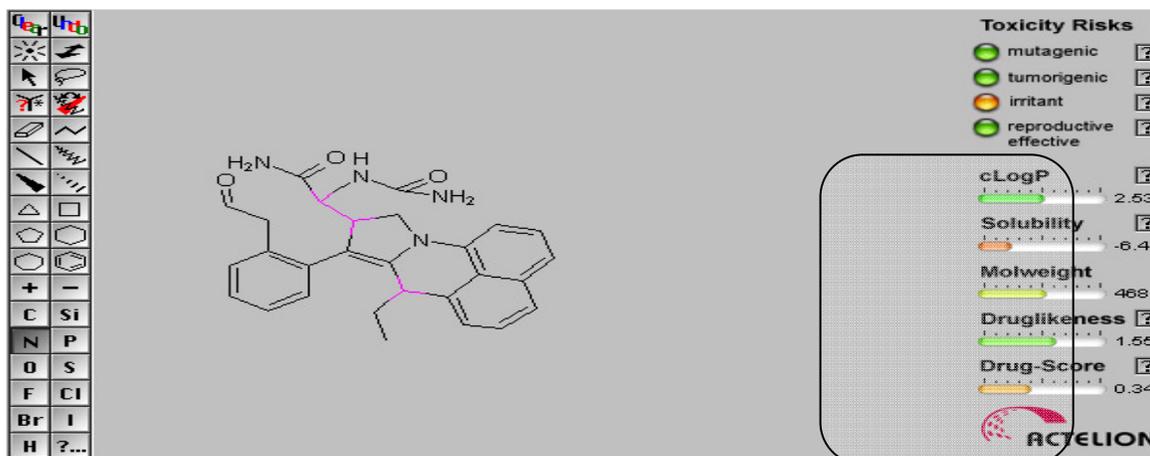
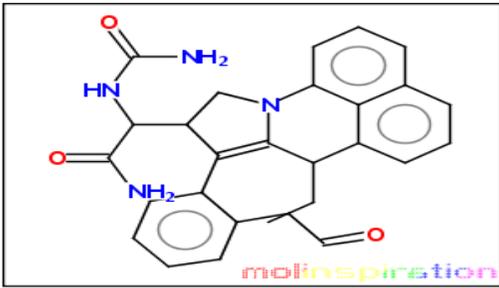


Figure-7  
Indicating Osiris result of inhibitor

**molinspiration**

SMILES CCC3C2=C(c1ccccc1CC=O)C(C(NC(N)=O)C(N)=O)CN2c4cccc5cccc3c45



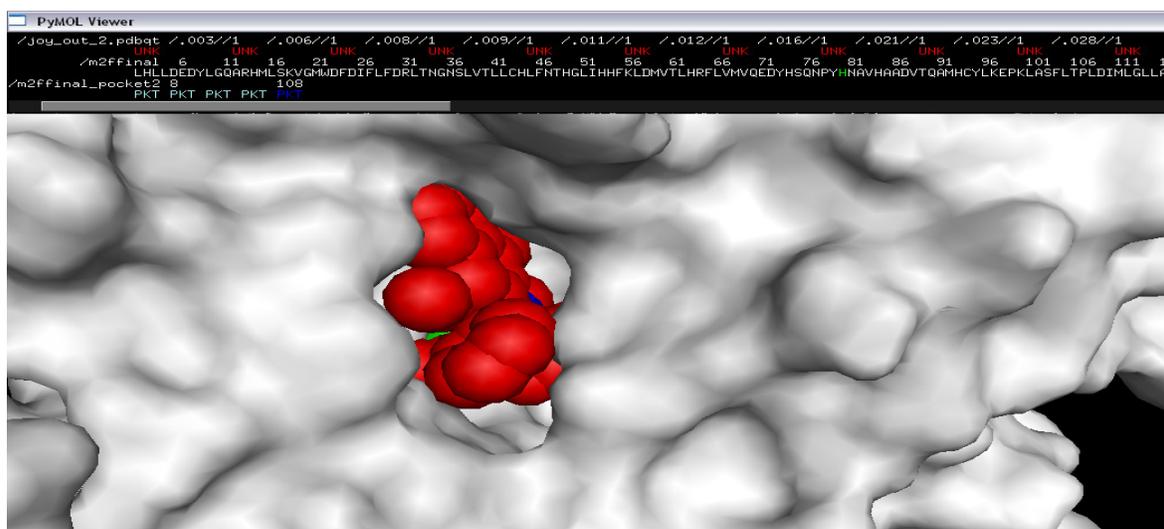
**Molinspiration property engine** v2009.01

<a href="#">miLoqP</a>	4.349
<a href="#">TPSA</a>	118.524
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MW	468.557
nON	7
nOHNH	5
nviolations	0
nrotb	7
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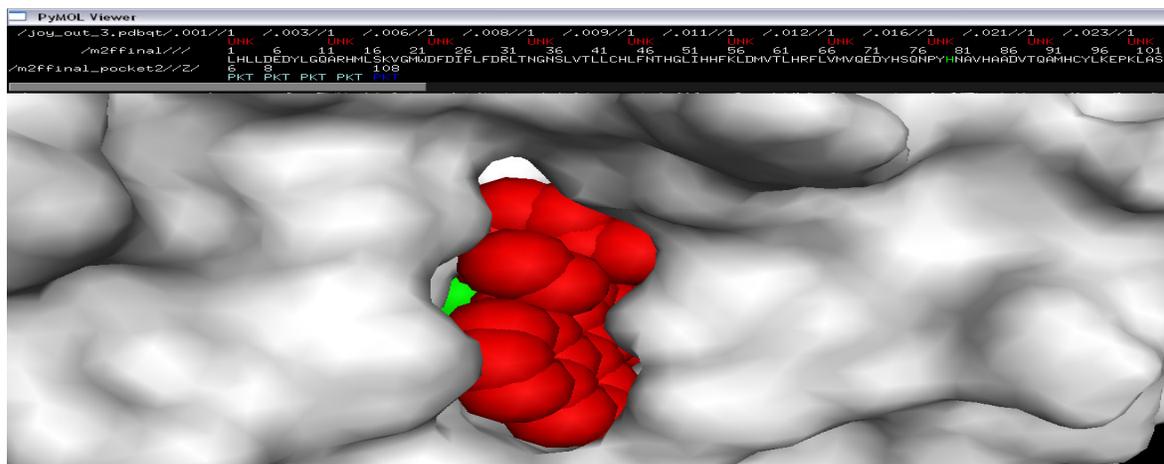
[Get data as text](#) (for copy / paste).

[Get 3D geometry](#) BETA

**Figure -8**  
 Indicating MOLINSPIRATION result of inhibitor



**Figure-9**  
 Result of AutoDock



**Figure-10**  
 Indicating result of Quantum

## Conclusion

Leukemia is a cancer which begins in blood forming tissues of the body and produces large number of abnormal blood cells. The target Protein PDE7B plays important role in the tumor formation. Protein sequence of PDE7B has been retrieved from NCBI protein sequence database and its homology was found by BLASTp. Then Model of target protein sequence was generated by homology modeling. Models were then analyzed by using SAVS (PROCHECK) and validated by Loop building and energy minimization, then best model was selected which act as receptor. Ligsite was used to search the best pocket in the receptor where the inhibitor could bind. For *In silico* drug designing, the ligand was generated from other existing drugs on that protein and hex was performed and was grown by ligbuilder. After checking their ADMET properties on MOLINSPIRATION, and OSIRIS server, inhibitor 10 was selected as there were mild toxicity risks and then it was docked with the receptor by using AutoDock 4.1 vina and Quantum 3.30 and their binding energies were calculated. Though, this drug has mild toxicity risks and having drug score of 0.34 which is low but good enough to inhibit the expression of the protein. This new inhibitor can be used for drug development for future use.

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