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INSILICO STRUCTURE BASED DRUG DESIGNING OF A POTENT INHIBITOR FOR HUMAN NOTCH1 - A THERAPEUTIC TARGET FOR LYMPHOBLASTIC LEUKEMIA

[2]Navkiran Dosanjh, [1]Anju Sharma, [1]Bhardwaj, S.V., [2]Vinay Randhawa, [2]Harpreet Singh
[2]Department of Biotechnology, HMV College, Jalandhar (Punjab)
[1]Department of Biotechnology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan 173230, Himachal Pradesh. India

ABSTRACT
Proteins are functional molecules in cells and are major targets for drug action. Designing a single drug through laboratory methods may take minimum ten years. Therefore, the insilico approach for designing a drug can be more efficient as it minimizes the time period to provide the patients with an effective drug. To design a rational drug, we must firstly find out which proteins can be the drug targets in pathogenesis. Proteomics has great promise in identification of protein targets and biochemical pathways involved in disease processes. Proteomics as a whole increasingly plays an important role in the multi-step drug-development process. The process includes target identification and validation, lead selection, small-molecular screening and optimization, and toxicity testing. Furthermore, sub-disciplines such as computational proteomics, chemical proteomics, structural proteomics and topological proteomics offer significant contributions especially in computer-aided drug design. This paper makes an attempt to design a computer assisted, structure based drug of a potent inhibitor for Human Notch1. It also summarizes the recent progress in pharmaco-proteomics and the discipline's potential application in insilico drug design. Acute lymphoblastic leukemia (ALL) is a malignant proliferation of lymphoid cells. The target protein Notch1 plays important role in tumour formation. Protein sequence of Notch1 was retrieved from NCBI and its homology was found by Blastp. Model of the target protein sequence was then generated by homology modeling. Models were further analysed by using SAVS (PROCHECK) and validated by loop-building and energy-minimization. Best model likely to act as receptor was selected. LIGSITE was used to search the best pocket in the receptor, where the inhibitor could bind. For insilico 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 OSIRIS, MOLSOFT and MOLINSPIRATION server, an inhibitor with no toxicity risks was selected and was docked with the receptor by using AUTODOCK 4.1. Molecular property prediction was done by using ADME-boxes. Though this drug had a low drug score of 0.25 but had no toxicity risks and was good enough to inhibit the expression of the protein. This new inhibitor can be used for drug development for further use.

KEYWORDS: Proteomics, drug design, drug target, protein target, target identification, lead validation, and computer-aided drug design, protein profiling.

INTRODUCTION

The long path from genomic data to a new drug can conceptually be divided into two parts. The first task is to select a target protein whose molecular function is to be moderated, in many cases, blocked by a drug molecule binding to it. Given the target protein, the second task is to select a suitable drug, that binds to the protein tightly, is easy to synthesize, is bio-accessible and has no adverse effects such as toxicity. The knowledge of the three-dimensional structure of a protein can be of significant help in both phases. The steric and physicochemical complementarity of the binding site of the protein and the drug molecule is an important, if not a dominating feature of strong binding. If the structure of the relevant binding site of the protein is known in detail, we can even start to employ structure-based methods in order to develop a drug binding tightly to the protein. Increased accessibility of genomic data and especially, that of large-scale expression data has opened new possibilities for search for the target proteins. This development has prompted large-scale investments into the new technology by many pharmaceutical companies. The respective screening experiments rely critically on appropriate bioinformatics support for interpreting the generated data. Specifically, methods are required to identify interesting differentially expressed genes and to predict the function and structure of putative target proteins from differential expression data generated in an appropriate screening experiment. In this communication bioinformatics methods for prediction of the protein structure are described and their use towards achieving the goal of drug designing is discussed. The possibilities and limitations of using protein structure knowledge towards the goal of developing new drug therapies are also discussed.

Structure-based drug design (SBDD) is one of the several methods in the rational drug design toolbox. Drug targets are typically key molecules involved in a specific metabolic or cell signaling pathway that is known, or believed to be related to, a particular disease state. Drug targets are most often proteins and enzymes in these pathways. Drug compounds are designed to inhibit, restore or otherwise modify the structure and behaviour of disease-related proteins and enzymes. SBDD uses the known 3D geometrical shape or structure of proteins to

assist in the development of new drug compounds. The 3D structure of protein targets is most often derived from x-ray crystallography or nuclear magnetic resonance (NMR) techniques. X-ray and NMR methods can resolve the structure of proteins to a resolution of a few angstroms (about 500,000 times smaller than the diameter of a human hair). At this level of resolution, researchers can precisely examine the interactions between atoms in protein targets and in potential drug compounds that bind to the proteins. This ability to work at high resolution with both proteins and drug compounds makes SBDD one of the most powerful methods in drug designing. Although the time devoted to SBDD may represent only a fraction of the total time towards developing a marketable drug product (Hill *et al*, 2004), yet it an essential and most powerful part of entire drug lead discovery process. In case a three dimensional structure of the target protein is not available from experimental procedures, molecular modeling offers the best alternative. The use of insilico methods in drug design has grown significantly in popularity over the past couple of years. Indeed many pharma companies have already adopted some type of virtual screening capability to complement high throughput screening (HTS) methods (Heal J, 2003).

Acute lymphoblastic leukemia

Acute lymphoblastic leukemia is also known as acute lymphoid leukemia or acute lymphocytic leukemia (ALL). Malignancies in this disease can arise either in T-cell or B-cell lymphocytes. Although the cause of ALL is unknown in most patients, several factors are associated with its development. These factors trigger the malignant transformation of cells, perhaps due to action of one or more oncogenes, radiation exposure, exposure to toxins & drugs, genetic factors and syndromes. Defects in DNA repair mechanism also contribute to the development of acute lymphoblastic leukemia. The majority of childhood leukemia are of the ALL type. T-cell acute lymphoblastic leukemia/lymphoma (T-ALL) is an aggressive tumour that generally affects children but also arises in adults (Goldberg, *et al* 2003; Moorman *et al*, 2007; Pui *et al,* 2004). Activating mutations in NOTCH1 are present in about 50% of T-ALL cases and small molecule inhibitors of the γ-secretase complex (GSIs), which effectively abrogate NOTCH1 signaling, have been proposed for the treatment of T-ALL. 60% of childhood T-cell acute lymphoblastic leukemia (T-ALL) has mutations in the Notch1 gene (Weng *et al*, 2004).

Potential drug target

NOTCH1 was chosen as an inhibitor in the present insilico drug designing. Multiple genes involved in anabolic pathways are directly controlled by NOTCH1 and it further promotes cell growth via direct transcriptional upregulation of MYC (Palomero, 2006; Sharma *et al*, 2006; Weng *et al* 2006). Recent identification of activating mutations in NOTCH1 in the majority of T-cell acute lymphoblastic leukemias (T-ALLs) has brought major interest towards targeting the NOTCH signaling pathway in this disease (Ferrando, 2009). GSIs are the first family of drugs targeting NOTCH1 signaling in T-ALL (Tosello and Ferrando, 2009). The small molecules GSIs, which block a critical proteolytic step required for NOTCH1 activation, can effectively block the activity of NOTCH1 mutant alleles. However, clinical development of GSIs has been hampered by their low cytotoxicity against humans. Consistently, treatment of T-ALL cell lines harboring activating mutations in *NOTCH1* with CompE, a highly active GSI, induces cell cycle arrest in G1 and decrease rate of anabolic cell growth which results in a gradual reduction in cell size (Palomero, 2006; Weng *et al* 2004, 2006, Chaturvedi *et al*, 2010).

METHODOLOGY

Protein sequence of Notch1 was retrieved from NCBI and its homology was found by using Blastp. Model of target protein sequence was generated by homology modeling by using MODELLER. Models were then analysed by using SAVS (PROCHECK) and validated by loop-building and energy-minimization. Best model was selected, which acted as a receptor. LIGSITE was used to search the best pocket in the receptor, where the inhibitor could bind. For insilico drug designing, the ligand was generated from other existing drugs on that protein and HEX (docking software) was performed and was grown by LIGBUILDER. The molecular properties for absorption, distribution, metabolism, and excretion (ADME) are crucial for drug design (Butina *et al*, 2002). After checking their ADME properties on OSIRIS, MOLSOFT and MOLINSPIRATION server, an inhibitor with no toxicity risks was selected and was docked with the receptor by using AUTODOCK 4.1. Molecular property prediction was done by using ADME-boxes.

RESULTS AND DISCUSSION

The protein sequences of the NOTCH1 inhibitor were downloaded in FASTA format from NCBI and a check for structure of the query sequence in PDB was made. As the structure was not available, an attempt was made to build the same through homology modeling. The query sequence was compared with the library or database of sequences by using BLAST (Altschul *et al*, 1990). Protein sequences showing 40% or more similarity with the query sequences were taken as templates. By using this template sequences an alignment file and atom file were created and which acted as input for MODELLER (Sali *et al*, 1995; Sanchez *et al*, 1997; Eswar *et al*, 2006). It automatically calculates a model containing all non hydrogen atoms. MODELLER conducts comparative protein structure modeling by satisfaction of spatial restrains and can perform many additional tasks including de novo modeling of loops in protein structures, optimization of various models of protein structures with respect to a flexibly defined objective function, multiple alignment of protein sequences or structures, clustering searching of sequences etc. The large amount of available protein X-ray crystal structures, together with the development of more effective homology modeling techniques, has led recently to a steep increase in docking-based Virtual Screening studies (Tuccinardi, 2009).

The best model generated by MODELLER was analyzed by using Swiss PDB viewer (Guex *et al*, 1997). By using Ramachandran plot (Fig.1) in Swiss PDB viewer, the model was refined by building a loop and refine side chain packing for energy minimization (Fig.2). This refinement or energy minimization step was repeated until or almost all the residues which were in disallowed regions of the Ramachandran plot gone inside the allowed region. The

final model was evaluated using PROCHECK online software (Fig.3). It indicated the bad contacts in the model. It also showed the percentage of residues present in the Ramachandran plot i.e. core, allowed and disallowed regions. The model hence formed, acted as our receptor protein.

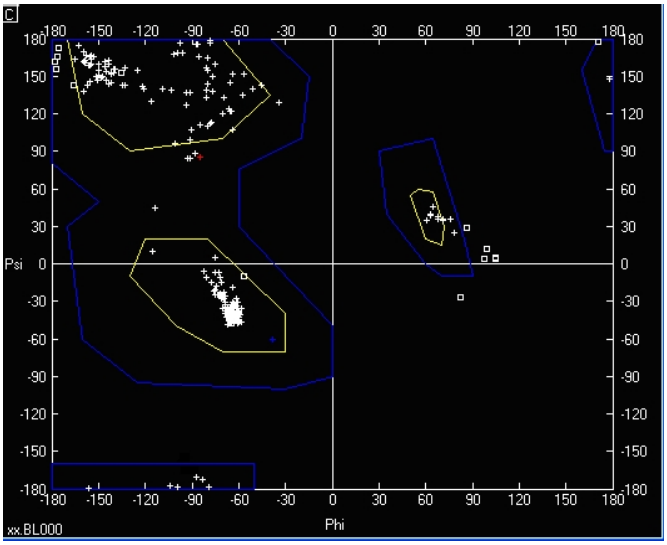

FIGURE1. Ramachandran Plot result from SPDBviewer

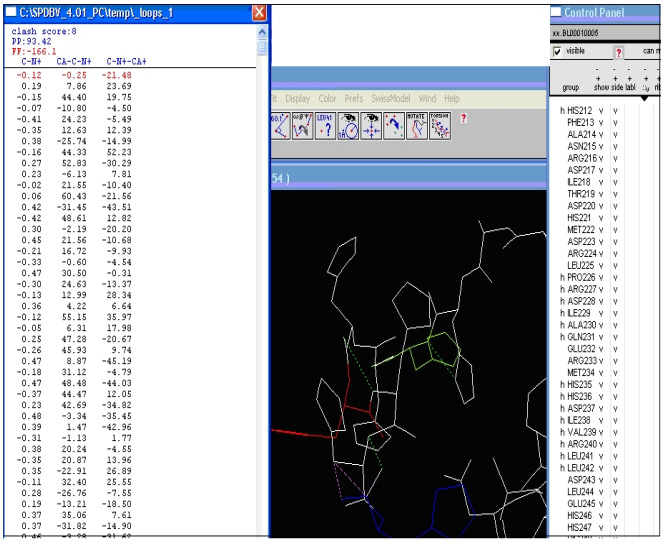

FIGURE 2. Loop Building using SPDBviewer

```
+----------<<<  P  R  O  C  H  E  C  K     S  U  M  M  A  R  Y  >>>----------+
|                                                                            |
| /var/www/html/Services/SAVES_3/jobs/4558636/a4.pdb   2.0      253 residues |
|                                                                            |
| Ramachandran plot:   91.3% core    8.3% allow    0.4% gener    0.0% disall |
|                                                                            |
| All Ramachandrans:    4 labelled residues (out of 251)                     |
| Chi1-chi2 plots:      6 labelled residues (out of 157)                     |
|                                                                            |
| Main-chain params:    6 better     0 inside      0 worse                   |
| Side-chain params:    5 better     0 inside      0 worse                   |
|                                                                            |
| Residue properties: Max.deviation:     5.6              Bad contacts:     1 |
|                     Bond len/angle:    4.7    Morris et al class:  1  1  2 |
|     1 cis-peptides                                                         |
| G-factors           Dihedrals:   0.94  Covalent:   0.22    Overall:   0.67 |
|                                                                            |
| M/c bond lengths:100.0% within limits   0.0% highlighted                   |
| M/c bond angles:  96.3% within limits   3.7% highlighted                   |
| Planar groups:    81.1% within limits  18.9% highlighted       2 off graph |
|                                                                            |
+----------------------------------------------------------------------------+
```

FIGURE 3. Ramachandran plot results from PROCHECK

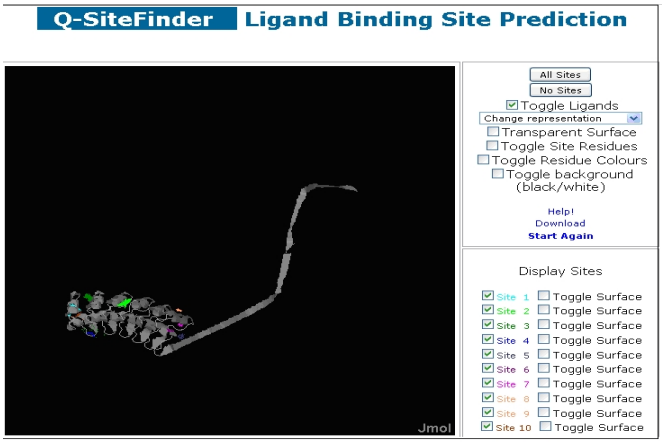

FIGURE 4. Determination of active site using Q-SiteFinder online software

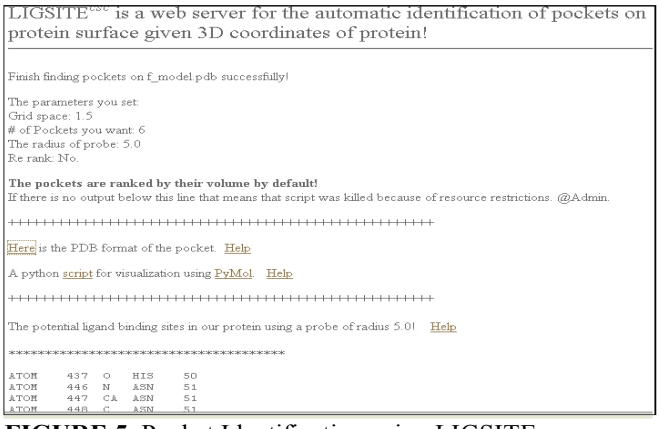

FIGURE 5. Pocket Identification using LIGSITE

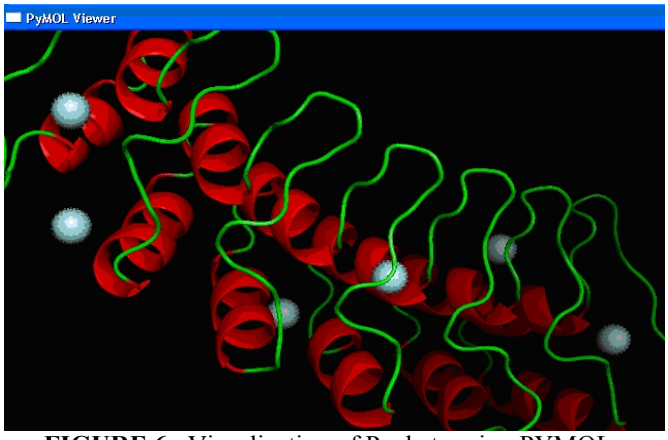

FIGURE 6. Visualization of Pockets using PYMOL

The potential active amino acid site was predicted by molecular cavities and Q-Site FINDER (Fig.4) based on energy and surface area. This site would act as best binding site where the ligand protein molecule would be targeted. LIGSITE (Hendlich et al, 1997) online software (Fig.5) was used to identify pockets (Fig.6) which were visualised by PYMOL.

The seed ligand structure which would bind with the active site of receptor protein was extracted from literature available on general information of inhibitors of NOTCH1. The ligand (inhibitor) structure was drawn using CHEMSKETCH software (Fig.7 a,b) which acted as final ligand.

Molecular docking is often employed to aid in determining

how a particular drug lead will interact with a binding pocket. The ligand and receptor PDB files were opened in **HEX** software (Fig. 8 a,b) and different parameters were adjusted before docking, such that, parameters did not bump or collapse with each other. The positions of ligand and receptor were also analysed using PYMOL (fig. 9) software.

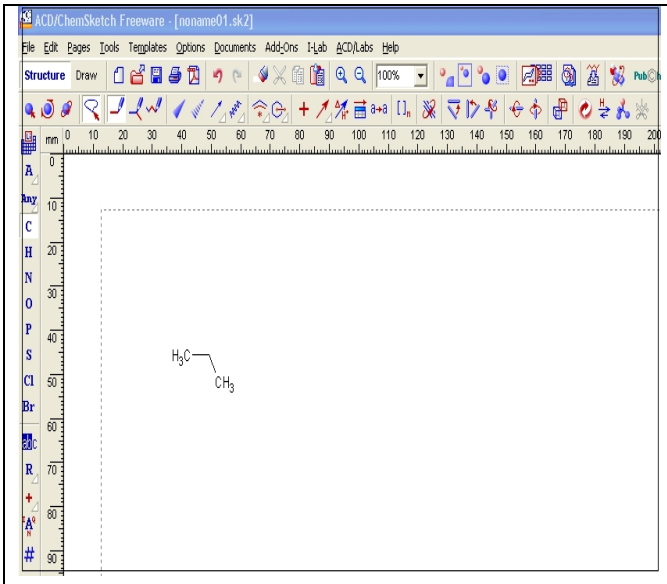

FIGURE7(a) Usage of CHEMSKETCH

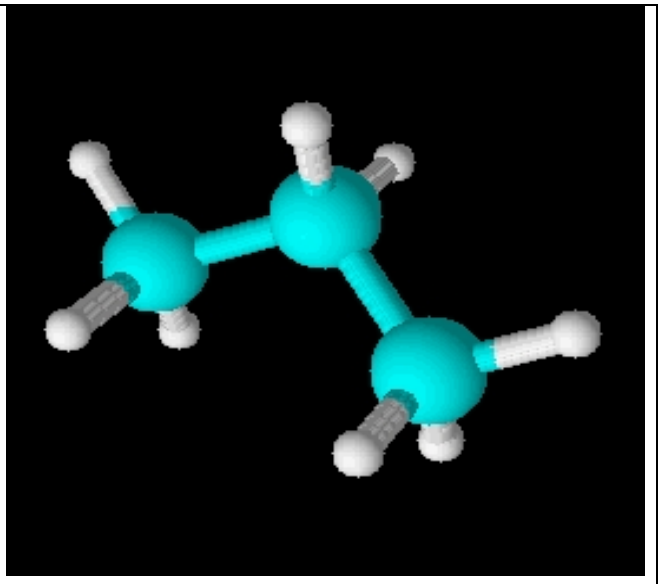

FIGURE 7(b) 3-D view of Ligand (seed)

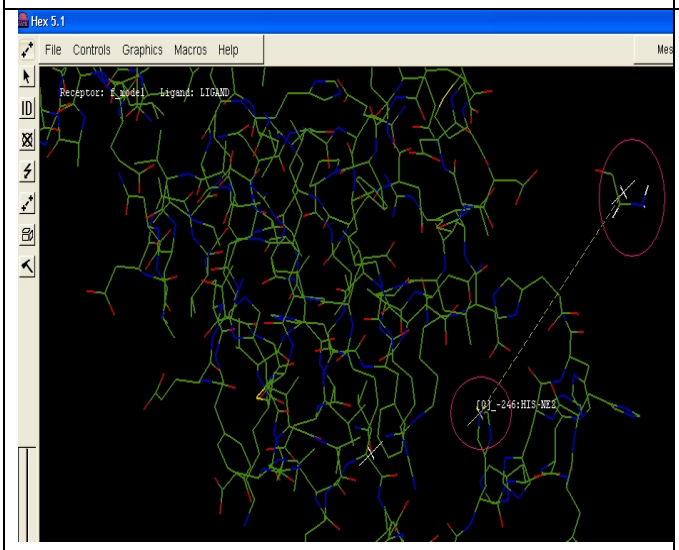

FIGURE 8(a). HEX Docking Software

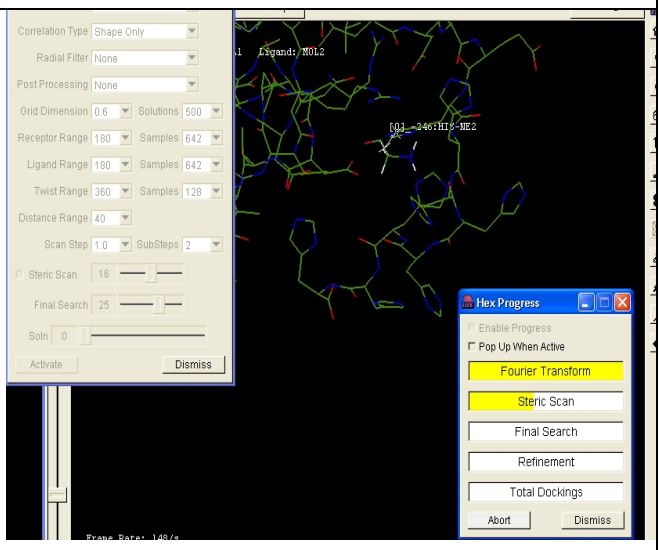

FIGURE8(b). Running Hex

LIGBUILDER is a general purpose program package based on the 3-D structure of the target protein and can automatically build ligand molecules within the binding pocket. The HEX result (saved in PDB format) was used as input for LIGBUILDER. The predicted ligand molecule was drawn in CHEMSKETCH (fig.10) and viewed its 3-D structure (fig.11).

The ligand hence designed was drawn in OSIRIS software and drug properties were analysed. Properties with high risks of undesired effects like mutagenicity or a poor intestinal absorption are shown in red, whereas a green colour indicates drug-conform behaviour (fig. 12). MOLSOFT (Fig. 14) offers complete solutions customized for a biotechnology or pharmaceutical company in the areas of computational biology and chemistry. Activity score and drug likeness for GPCR ligands, ion channel modulators and kinase inhibitors (interactive virtual screening), were calculated using MOLINSPIRATION by choosing the "Predict Bioactivity" option (Fig. 13). ADME Boxes software from pharmaAlgorithms was used to predict Physchem properties and absorption from the chemical structures (Fig. 15).

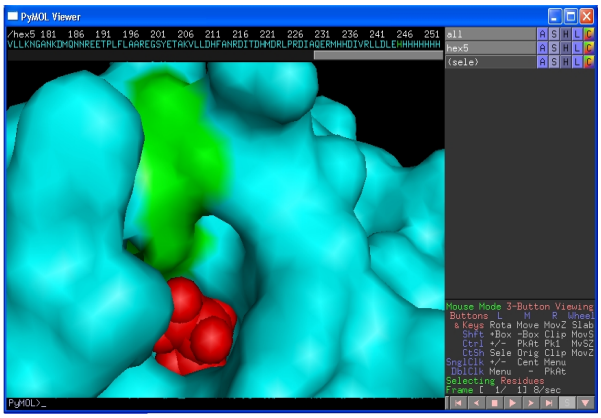

FIGURE 9. Visualization of Pockets using PYMOL

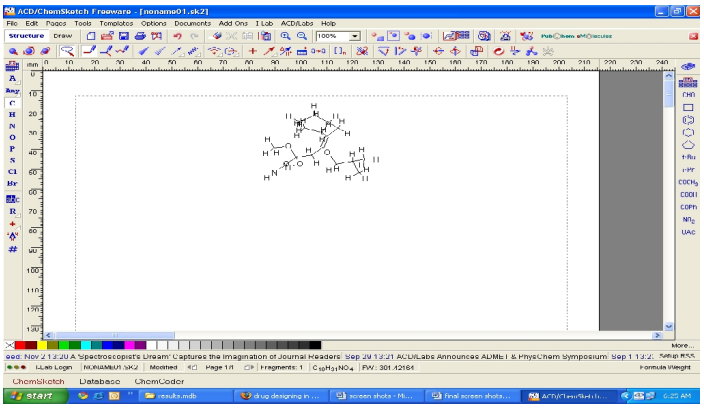

FIGURE10. Drawing Ligand Molecule using CHEMSKETCH

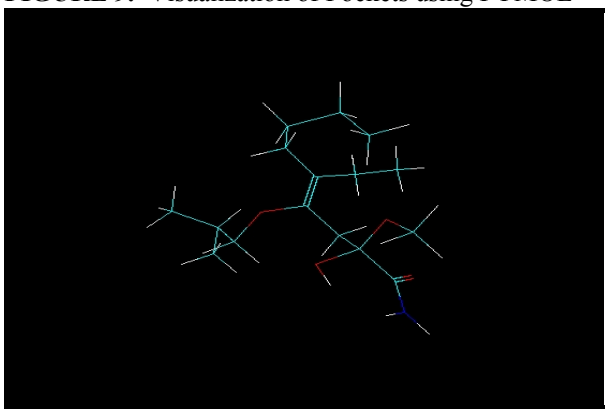

FIGURE 11. 3-D view of Ligand.

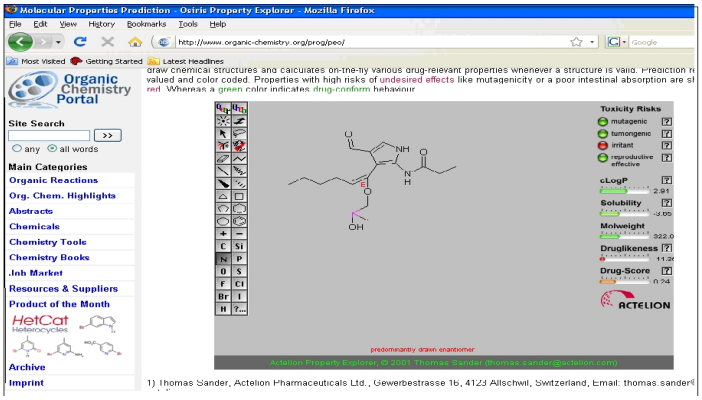

FIGURE12. Analysis of Drug Properties using OSIRIS

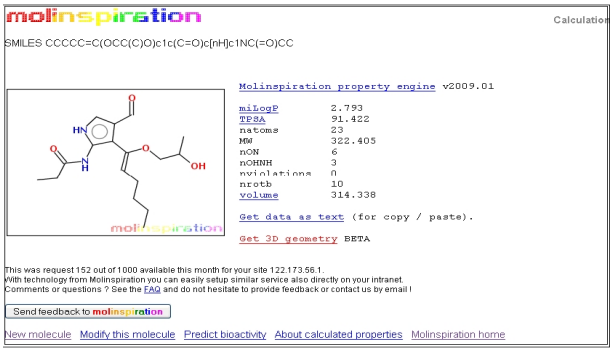

FIGURE13. Analysis of Physicochemical Properties using MOLINSPIRATION

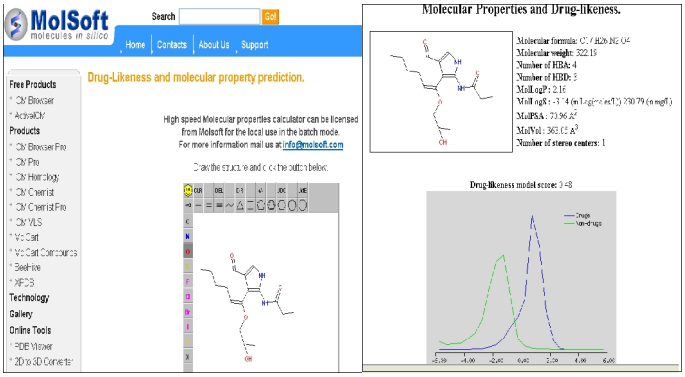

FIGURE14. Analysis of Drug-Likeness and Molecular Property Prediction using MOLSOFT

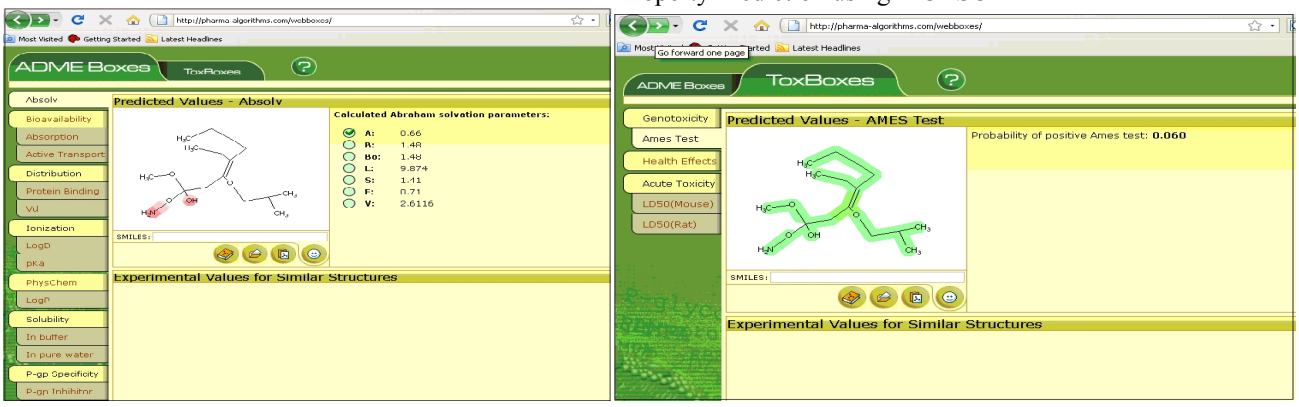

FIGURE15. Prediction for Physchem properties and absorption from the chemical structure using ADME boxes.

Thus for insilico drug designing, the ligand which could act as a drug against the Receptor (Notch1), and that binds with active binding sites of the Receptor was generated from other existing drugs on that protein and HEX was performed and was grown by LIGBUILDER. The effectiveness of drugs was analysed by online softwares viz., OSIRIS, MOLSOFT, MOLINSPIRATION**.** The observations on Mutagenicity, Tumourogenicity,

Irritanting effects, Reproductive effects, cLogP, Solubility (logS), Molecular Weight, Drug Likeliness and Drug score using Osiris are presented in table 1. All the four models showed irritating effects but no mutagenicity or tumourogenicity was observed in any of the models. The cLogP (measure of compound's hydrophilicity) value was less than 5 in all models, which indicated reasonable probability of being well absorbed. The absorption is significantly affected by the aqueous solubility of a compound. Lower the solubility, lesser the absorption. The second compound depicted maximum solubility (-2.79) while the fourth compound was least soluble. Molecular weight was noted to be lowest in case of second model (approx. 301 as calculated by all the mentioned softwares). Compounds with less molecular weight are more likely to be absorbed faster, therefore the low molecular weight is the desired criteria of every drug. Though the drug likeliness score was found to be negative for all compounds but the second compound recorded the highest value (-2.48). Similarly the second model obtained the highest drug score (0.25) value. MOLSOFT online tool calculated the chemical properties like Molecular Formula, Molecular Weight, Number of Hydrogen Bond Acceptors (HBA), Number of Hydrogen Bond Donators (HBD), molLogP (octanol/water partition coefficient), molLogS (water solubility), Polar Surface Area (molPSA), Volume, Number of Stereo Centers, Drug Likeness Model Score of four models as shown in table 2. MOLINSPIRATION calculated the molecular physicochemical properties relevant to drug design and QSAR, including logP, molecular polar surface area (PSA), and the rule of five descriptors as depicted in table 3. LogP was calculated by the methodology developed by Molinspiration as a sum of fragment-based contributions and correction factors. This method is very robust and is able to process practically all organic, and most organometallic molecules. The second model observed the maximum miLogP value (3.395). TPSA is calculated based on the methodology published by Ertl *et al*, 2000 as a sum of fragment contributions. The second model had minimum volume (313.258) and TPSA (81.79). "Rule of 5" properties are a set of simple molecular descriptors used by Lipinski in formulating his "Rule of 5". The rule states, that most "drug-like" molecules have logP <= 5, molecular weight <= 500, number of hydrogen bond acceptors <= 10, and number of hydrogen bond donors <= 5. Molecules violating more than one of these rules may have problems with bioavailability. The rule is called "Rule of 5", because the border values are 5, 500, 2*5, and 5. All the four models were observed to follow this rule. Number of rotatable bonds (nrotb) were found to be 11(1^{st} and 2^{nd} model) or 10 (3^{rd} and 4^{th} model).

Finally the second model was docked with the receptor by using AUTODOCK 4.1 vina and its molecular property prediction was done by using ADME-boxes. It can therefore be concluded that second model can act as a better drug as this drug had no toxicity risks like mutagenecity, tumourogenecity and had highest drug score of 0.25 which is, however, otherwise low, but good enough to inhibit the expression of protein. This new inhibitor can be used for drug development for further use.

TABLE 1: Various properties of different drug models developed as depicted by Osiris software

Ligand Model	Mutagenicity	Tumourogenicity	Irritating Effects	Reproductive Effects	cLogP	Solubility logS	Molecular Weight	Drug Likeliness	Drug Score
1.	No	No	Yes	Mild	4.12	-2.87	312.0	-7.39	0.18
2.	No	No	Yes	No	4.28	-2.79	301.0	-2.48	0.25
3.	No	No	Yes	No	2.91	-3.65	322.0	-11.35	0.24
4.	No	No	Yes	No	2.58	-3.82	306.0	-9.54	0.24

TABLE 2: Chemical properties of different drug models developed as depicted by Molsoft software

Molecular Formula	Molecular Weight	No. of Hydrogen Bond Acceptors (HBA)	No. of Hydrogen Bond Donators (HBD)	molLogP	molLogS	Polar Surface Area (molPSA)	Volume	Number of Stereo Centers	Drug Likeness Model Score
$C_{17}H_{28}O_5$	312.19	5	2	2.22	-1.76	65.90	356.02	2	-0.88
$C_{16}H_{31}NO_4$	301.23	4	3	3.15	-2.31	66.73	352.06	1	0.13
$C_{17}H_{26}N2O_4$	322.19	4	3	2.16	-3.14	70.96	363.05	1	0.48
$C_{16}H_{22}N_2O_4$	306.16	4	2	1.55	-4.18	69.75	337.75	1	-0.30

TABLE 3: Physico-chemical properties of different drug models developed as depicted by Molinspiration software

Molinspiration Property Engine	miLogP	TPSA	natoms	Molecular Weight	nON	nOHNH	nviolations	nrotb	Volume
v2009.01	2.819	83.832	22	312.406	5	2	0	11	314.364
v2009.01	3.395	81.79	21	301.427	5	3	0	11	313.258
v2009.01	2.793	91.422	23	322.405	6	3	0	10	314.338
v2009.01	2.813	88.265	22	306.362	6	2	0	10	291.94

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