Int J Pharm Bio Sci 2013 Jan; 4(1): (B) 1050 - 1056

Research Article **Bioinformatics**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

SQUALENE AS ALEAD MOLECULE AGAINST HIV INFECTION

MUTHUSAMY CHINNASAMY AND THIRUNALASUNDARI THIYAGARAJAN*

Department of Industrial Biotechnology, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India – 620 024.

ABSTRACT

HIV is a life-threatening virus, causes immune suppressive disease called AIDS. Researchers put enormous effort to find a remedy for HIV infection. Though there is a considerable success in developing drugs to combat HIV, the effectiveness of existing antiretroviral therapies is limited. Hence, an attempt was made in this study to find a plant based lead molecule, using *in-silico* method. The structure of gp120 and 4 selected bioactive molecules were downloaded and docking was done using AutoDock. Results showed that Squalene have lowest Binding Energy-6.73 Kcal/mol., Ligand Efficiency-0.22 and Inter Molecular Energy-11.2. Other molecules are not up to the mark. Interestingly it is bound to the V3 loop of gp120, which is considered as an antibody binding site. Study of physicochemical properties like XLogP (3.30525), LogS (-2.07933) and environmental toxicity showed Squalene is better molecule than others. Overall results of this study revealed that, Squalene would be a lead molecule for developing a drug against HIV.

KEY WORDS: **gp120, Env protein, Squalene and CD_4.**

THIRUNALASUNDARI THIYAGARAJAN
Department of Industrial Biotechnology, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India – 620 024.

INTRODUCTION

Human Immunodeficiency Virus (HIV) causes Acquired Immuno Deficiency Syndrome (AIDS). It is a major life-threatening virus around the world. It infects human and invades CD_4positive cells. HIV infection begins when there is an interaction between gp120, the trimericenvelope glycoprotein of HIV and CD_4- the primary receptor of the host cell[1]. Ultimately this interaction resulted in the exposure of the co-receptor binding sites of gp120, which in turn facilitate binding of chemokine receptors like CCR5 and CXCR4 present on the CD_4 subset[2]. gp120 structure, revealed the presence of highly conserved residues located in V3 region **(**Fig 1- B) that plays major role in the activation of its counterpart CD_4[3]. Deletion of most of the V3 residues from gp120 had no effect on CD_4 receptor binding, at the same time stabilization of major variable region enables immunogenic response for that specific region[2]. Since the highly conserved V3 region is the key for HIV infection and it may represent unique targets for antiviral drugs based on structure based drug designing[1]**.** In spite of knowing all these structural and functional evidences, finding a sole drug for HIV infection remains unassured. Currently many drugs like Rilpivirine (2011), Atazanavir (2003) and Zidovudine (2000) etc., are available in market as anti HIV drugs, but they provoke many side effects. In contrast to synthetic chemical compounds, naturally occurring human monoclonal antibodies targeting gp120 achieved greater rate of viral neutralization[4, 5, 6].

HJ16, a recently isolated antibody neutralizes the virus intensity of about 40%[7] and its neutralization is more sensitive and effective, but only minimal fraction of them are able to neutralize viruses. Though there is a considerable success in developing drugs to combat the HIV, the effectiveness of existing antiretroviral (ART) therapies is limited by mutation and increasing drug resistance. It has been found that approximately 20% of all new HIV infections are resistant to the currently available drugs. Consequently, concerted efforts towards the discovery and development of novel inhibitors of HIV infection must persist. Interestingly products derived from natural resources have been shown to inhibit HIV replication during various stages of the virus life cycle and represent a potential source of novel therapeutic agents. Natural bioactive compounds always promise ability to fight against viruses. A number of compounds exhibiting anti-HIV activity were isolated from natural resources & it is steadily increasing[8]. Betulinic acid, a bio-origin chemical compound reported to have a variety of biological activities including anti-inflammatory, *in-vitro* antimalarial effects, anti-HIV-1 activity and specific cytotoxic effect against a variety of tumor cell lines. These naturally occurring drugs are in a weak position because of lack of specificity. Thus, they are subjected to modification. In addition, several small molecules, like Squalene[9]**.** Lewis X[10], 6'-Sialyllactose and Galactosylceramide[11] are some natural products reported as antiviral compounds, but they are to be proved for their specific action against viral receptor. Therefore, targeting Env protein (gp120) or blocking the function of immunogenic region by either antibody neutralization or natural product will be a good start to achieve the ultimate goal.

Science and Technology advancement helped to develop a handful of techniques to understand biological system. Among that *in-silico* screening like Computational searching and identification of bioactive molecules from a collection of uncharacterized compounds is most advanced. It also promises lead identification, drug designing, increased drug specificity and sensitivity. All the above it reduces the laborious time taken, manpower and money involved in the other methods of drug screening. The most promising compounds obtained from the screening are called "hits". Some of these hits are then promoted as lead compound that are further refined and modified in order to achieve more favorable interactions with fewer side effects. Lead identification is the first and foremost step in drug designing and it must have good ADMET (Absorption, Distribution, Metabolism,

Excretion and Toxicity) and physiochemical property[12]. Having known all these, an attempt was made in this study to identifying a lead molecule of natural origin against HIV by *in-silico* method. An attempt was also made to find out the molecular docking between gp120 (receptor) and small molecule (ligand). The problem can be looked as a "lock – and – key" concept. In this study, the orientation of the ligand was "fitted" to the receptor. All the selected small molecules were subjected for ADMET (Physiochemical properties) and GUSAR (General Unrestricted Structure-Activity Relationships) predictions.

MATERIALS AND METHODS

Receptor (gp120 core)

HIV uses its envelope protein gp120 (Fig 1-A) present on its surface as a ligand to infect host cell. Therefore it could be a target for drug or vaccine development (Ping Zhu, 2006. Girard 2006). But in this study it was taken as a receptor to dock against the ligands chosen. To accomplish the goal three-dimensional (3D) structure of gp120 was downloaded from structural database called Protein Data Bank[13] (PDB ID: 3DNN) and it was used as the receptor for docking study.

Core Structure of HIV gp120

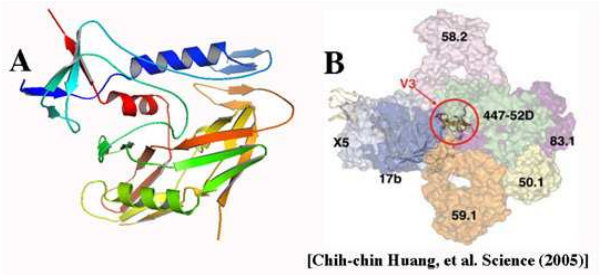

Figure 1
A: Three Dimensional Structure of gp120. B: Hyper Variable Region of gp120 marked as V3

Small Molecules (Ligands)

The structures of small molecule (Fig 2- A, B, C & D) chosen for this study were downloaded from online Databases. The structures *viz* i) Squalene (2,6,10,15,19,23 Hexamethyltetracosa-2,6,10,14,18,22-hexaene), precursor of steroid reported to have anti-HCV and anti-HIVproperty[9] and ii) Lewis X[10] a common trisaccharide were downloaded from NCBI Pubchem Database. The other two compounds are 6'-Sialyllactose and Galactosylceramide, an oligossacharide were downloaded from Human Metabolome Database version 2.5. Both the compounds are found in human breast milk and cow's milk, and have recently been shown to inhibit the infection of HIV-1 virus. Sialyllactose is also responsible for the inhibitory activity on cholera toxin. Finally, Galactosylceramide, a non-acidic monoglycosphingolipid, has high affinity towards gp120 and considered to be a potential alternative receptor for HIV[11].

Molecular Docking (AutoDock Tools)

All four ligands were subjected for Molecular docking, using AutoDock software, as it is one of the most cited docking software in the research community. The utility of automated AutoDock in virtual screening was successfully demonstrated using various target enzymes from chemical database with higher accuracy than any other docking tools[14].AutoDock consists of two main programs. They are AutoDock for docking of the ligand to a set of grids describing the target protein; and Auto Grid for pre-calculating these grids. The protein-ligand complexes were prepared with AutoDock Tools: Kollman charges were assigned for the proteins and Gasteiger charges for the ligand molecules. A grid box with a size of 90x90x90 points with a spacing of 0.375 A° was defined around the ligand.

Physicochemical Properties

To consider the action of a bioactive compound in the fields of high interest such as drug design, reaction prediction, and biodegradation demands, a complete understanding of fundamental physicochemical properties of a compound such as polarity or lipophilicity is essential. Its behavior in chemical &biochemical, or environmental processes is more important. In order to predict physicochemical property online tool ADRIANA.Code(MOSES.Descriptor) was used. It comprises a unique combination of methods for calculating molecular structure descriptors on a sound geometric and physicochemical basis. These descriptors can be used for a wide range of applications in all areas of chemistry in general, lead discovery & optimization, diversity assessment of compound libraries and drug designing in particular and prediction of ADME/Tox properties.

Environmental Toxicity prediction

Finally GUSAR software was used to measure the environmental toxicity. GUSAR creates QSAR/QSPR models on the basis of the appropriate training sets represented as SDfile containing data about chemical structures and endpoint in quantitative terms[15].

RESULTS

Molecular Docking Studies

To achieve suitable conformational flexibility all four small molecules chosen for this study *viz*. Squalene, Lewis X, 6'-Sialyllactose and Galactosylceramide were processed by Auto Dock tools and allowed to dock with gp120 receptor individually (Fig 2 – E, F, G & H). This AutoDock program “adjust” the conformation of the ligand and the side chains of the receptor binding site - the site of interaction that results in the increase of binding energy and produced a total of 10 different conformations for each compound and each of them ranked according to degree of binding energies (Table 1). To check the reproducibility of docked conformations, docking experiment was carried out in triplicate average value has been taken as final. Among the 4 selected ligands, Squalene was found to have lowest binding energy ie.-6.73 Kcal/mol.,Ligand Efficiency-0.22 and Inter Molecular Energy-11.2(Fig 2 – E).

Structure of ligands and Docking Conformations with gp120

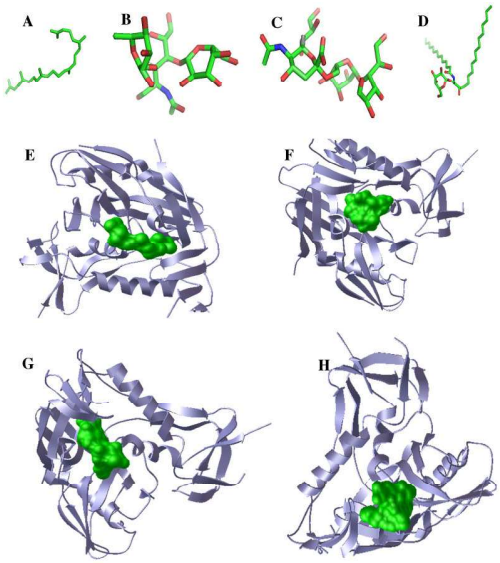

Figure 2

A: Squalene. B: Lewis X. C: Sialyllactose. D: Galactosylaceramids. E: gp120 and Squalene Complex. F: gp120 and Lewis X Complex. G: gp120 and Sialyllactose Complex. H: gp120 and Galactosylceramids Complex.

Earlier research also stated that Squalene has important biological activity like immunologic adjuvant in several vaccines[16]. Based on its binding energy towards gp120Squalenemay be considered as a potential lead for drug designing among the small molecules selected in this study, other molecules Lewis X, 6'-Sialyllactose and Galactosylceramide showed binding energy valuesie-3.15 Kcal/mol. -3.9 Kcal/mol. and 57.61 Kcal/mol. respectively (Table 1).

Table 1
Binding Energy of Receptor and Ligand

S. No	Receptor	Ligand	Docking Values			
			Binding Energy	Ligand Efficiency	Inter mol. Energy	Inhib. Constant Units
1.	GP120	Squalin	-6.73	-0.22	-11.2	uM
2.	GP120	Lewis X	-2.72	-0.08	-6.0	mM
3.	GP120	Sialyllactose	-3.9	-0.09	-8.38	mM
4.	GP120	Galactosylceramids	57.61	1.23	48.4	-

(uM: Micro Molar, mM: Milli Molar)

The chosen molecules were bound to within and near the V3 loop of gp120, which is considered as antibody binding site having the amino acid sequence as follows THR 283, MET 426, GLY 472, ASN 474 and ASP 477[1].Therefore, Squalene has lowest binding energy, optimal physicochemical property and inhibitory concentration at micro molar level and hence it could be considered as a lead molecule.

Prediction of Physicochemical Property

Having known the binding capacity of these ligands against gp120 receptor, it is important to measure physicochemical properties in order to make sure whether it can be a lead molecule.The MOSES Descriptors Community Edition web service calculates a set of molecular descriptors by processing the input file of chemical structures, Particularly for Squalene and the results showed suitable physicochemical property like XLogP(lipophilic efficiency)value 3.30525 and LogS(compound´s solubility)value -2.07933(Table 2).

Table 2
Physiochemical properties of the Chosen Ligand

Ligand Name	Molar Refractivity (cm^3)	Index of Refraction	Surface Tension (dyne/cm)	Dielectric Constant	XlogP	LogS	Complexity
Squalin	140.43 ± 0.3	1.491 ± 0.02	29.2 ± 3.0	0438051 ± 0.1	3.30525	-2.07933	528.405
Lewis_X	117.73 ± 0.4	1.629 ± 0.03	96.6 ± 5.0	3.52863± 0.1	-5.89693	2.17127	648.235
Sialyllactose	137.76 ± 0.4	1.669 ± 0.03	119.6 ± 5.0	8.74683± 0.1	-9.03611	3.84704	814.577
Galactosylceramids	192.13 ± 0.4	1.515 ± 0.03	48.2 ± 5.0	2.58928± 0.1	8.29635	-7.42719	673.594

XlogP:Octanol/Water Partition Coefficient.LogS: Aquous Solubility

Compared to Squalene, Lewis X and 6'-Sialyllactose does not show suitable Log P and Log S values, these are the criterion used in medicinal chemistry to assess the drug likeness of a given molecule, and used to evaluate the quality of research compounds.

Environmental toxicity prediction

Further, to verify environmental toxicity of the compounds chosen for this study, all four compounds were processed with GUSAR software. The results revealed that Squalene showed applicability domain of models for environmental Toxicity testing whereas others showed out of range (Table 3).

Table 3
Environmental toxicity Prediction

Activity	Squalene		Lewis X		6'-Sialyllactose		Galactosylceramide	
	Prediction	Applicability	Prediction	Applicability	Prediction	Applicability	Prediction	Applicability
Bioaccumulation factor Log10 (BCF)	1.508	In AD	-0.529	In AD	-1.297	In AD	-1.312	Out of AD
Daphnia magna LC50 -Log10 (mol/L)	6.140	In AD	4.379	In AD	4.686	In AD	5.849	In AD
Fathead Minnow LC50 Log10 (mmol/L)	-7.557	Out of AD	-1.459	In AD	-2.624	Out of AD	-7.310	Out of AD
Tetrahymenapyriformis IGC50 -Log10 (mol/L)	3.518	In AD	-1.430	In AD	-0.985	In AD	1.931	In AD

In AD - compound fall in applicability domain of models Out of AD - compound out of applicability domain of models

DISCUSSION

Developing a vaccine is an extremely important task to protect human kind against viral and bacterial pathogens. Currently drug design follows several paths to the development of new leads. Computational models derived from ligand and structure-based drug designs were screened with five million compounds. For instance known non-nucleoside reverse transcriptase, protease inhibitors and new putative hits were selected by making use of docking method. This study also successfully attempted for optimum conformational space search for each ligand using AutoDock tools and obtained decent conformations with maximum binding energy for Squalene, it is naturally occurring substance found in plants, animals, and humans. It has been widely reported as anti HCV and anti HIV compound[9]. This study also confirmed its competence to be a future leadmolecule for HIV, only when it is being taken to the next level (Lead optimization).

CONCLUSION

Thus the biologically active compound Squalene showed lowest binding energy and consists of proper physiochemical property it could be a one of the lead molecule for future drug discovery. In addition prediction of environmental toxicity test for Squalene by GUSAR&MOSES Descriptorare shows it is highly fit for bioaccumulation factor etc., where as other compounds showed out of range. With these findings, it is proposed that a naturally available small molecule, Squalene would be a future antiviral drug if worked further to improves its bioactivity.

REFERENCE

1. Huang C, Tang M, Zhang MY, Majeed S, Montabana E, Stanfield RL, *et al*. Structure of a V3-containing HIV-1 gp120 core. Science, 310 (5750): 1025-8, (2005).
2. Dey B, Svehla K, Xu L, Wycuff D, Zhou T, Voss G, *et al*. Structure-based stabilization of HIV-1 gp120 enhances humoral immune responses to the induced co-receptor binding site. PLoS pathogens, 5(5):e1000445, (2009).
3. Rizzuto CD, Wyatt R, Hernández-Ramos N, Sun Y, Kwong PD, Hendrickson WA, *et al*. A conserved HIV gp120 glycoprotein structure involved in chemokine receptor binding. Science, 280(5371):1949, (1998).
4. Zhou, T., Georgiev, I., Wu, X., Yang, Z. Y., Dai, K., Finzi, A., Do Kwon, Y., Scheid, J. F., Shi, W. and Xu, L. Structural basis for broad and potent neutralization of HIV-1 by antibody VRC01. *Science*, 329, 5993, 811-817, (2010).
5. Wu X, Yang ZY, Li Y, Hogerkorp CM, Schief WR, Seaman MS, *et al*. Rational design of envelope identifies broadly

neutralizing human monoclonal antibodies to HIV-1. Science, 329(5993): 856-61, (2010).
6. Audigé A, Schlaepfer E, Joller H, Speck RF. Uncoupled anti-HIV and immune-enhancing effects when combining IFN-α and IL-7. The Journal of Immunology,175(6):3724, (2005).
7. Pietzsch J, Scheid JF, Mouquet H, Klein F, Seaman MS, Jankovic M, *et al*. Human anti–HIV-neutralizing antibodies frequently target a conserved epitope essential for viral fitness. The Journal of experimental medicine, 207(9):1995-2002, (2010).
8. Sabde S, Bodiwala HS, Karmase A, Deshpande PJ, Kaur A, Ahmed N, *et al*. Anti-HIV activity of Indian medicinal plants. Journal of natural medicines, 65(3):662-9, (2011).
9. Elsherbini SH. Squalene is an antiviral compound for treating hepatitis C virus carriers. Google Patents; 1999.
10. Naarding MA, Ludwig IS, Groot F, Berkhout B, Geijtenbeek TBH, Pollakis G, *et al*. Lewis X component in human milk binds DC-SIGN and inhibits HIV-1 transfer to CD4^+ T lymphocytes. Journal of Clinical Investigation, 115(11):3256, (2005).
11. Colson P, Ravaux I, Yahi N, Tourrès C, Gallais H, Tamalet C. Transmission of HIV-1 variants resistant to the three classes of antiretroviral agents: implications for HIV therapy in primary infection. AIDS, 16(3):507, (2002).
12. Paul Gleeson M, Hersey A, Hannongbua S. In-silico ADME models: a general assessment of their utility in drug discovery applications. Current topics in medicinal chemistry, 11(4):358-81, (2011).
13. http://www.rcsb.org/pdb/explore/explore.do?structureId=3DNN (Structure downloaded on 18th May2011).
14. Cosconati S, Forli S, Perryman AL, Harris R, Goodsell DS, Olson AJ. Virtual screening with AutoDock: theory and practice. Expert opinion on drug discovery, 5(6):597-607, (2010).
15. Rao CV, Newmark HL, Reddy BS. Chemopreventive effect of farnesol and lanosterolon colon carcinogenesis. Cancer detection and prevention, 26(6):419-25, (2002).
16. http://www.pharmaexpert.ru/GUSAR/environmental.html (Online tool accessed on 30th July 2011).