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RESEARCH ARTICLE

IN SILICO APPROACH DESIGNING AND MODELING ANTI CD4 AND GP120 DRUG CANDIDATE TO BLOCK HIV INFECTION

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ABSTRACT

Most of drugs designed to act on the target gp-120 which is the surface part of the envelope glycoprotein (Env) of HIV_1. The HIV_1 inter to the cell by temporary interaction between the viral exterior glycoprotein GP120 and human CD4 receptor. Binding to CD4 stimulate the start of a cascade of conformational changes in gp120 and gp41 that lead to the fusion of the viral with the host cell membrane. Three gp120s and gp41s combine in a trimer of heterodimers to form the envelope spike, which mediates attachment to and entry into the host cell. The binding of ligand to many cell surface receptors leads to intracellular signaling molecules termed as second messengers. Stimulation of some GPCRs and other cell-surface receptors leads to activation of secondary messengers. Blocking the CD 4 receptor access to GP 120 of HIV_1 halts Antigen presentation process at crucial step in those cell lines (CD4+/T Helper) .Attempts are made to control this. Here in this project an SCFV fragment was studied for its ability to block CD4 receptor in such a way that gp120 (HIV_1) could not get access to CD 4 receptor.

INTRODUCTION

The acquired immune deficiency syndrome (AIDS) is a pandemic disease. This disease was recognized in 1981 in the United States. Two decades later, nearly 50 million individuals are living with HIV/AIDS worldwide, according to figures released by the Joint United Nations Program on HIV/AIDS (UNAIDS) and the World Health Organization (WHO) (www.unaids.org). Thirty three years of research has yielded a large amount of information regarding the pathogenesis, structure, and immunobiology of HIV. From this knowledge has come the development of effective drugs for managing the disease and to deactivate the HIV. The virus responsible for the comparatively new and fatal viral disease, acquired immunodeficiency syndrome (AIDS). It was not long before the infectious agent, a retrovirus (an RNA virus that transcribes its genes into DNA) called human immunodeficiency virus (HIV), was identified by laboratories in France. Study of HIV revealed it to be closely related to a chimpanzee virus, suggesting a recent host expansion to humans in central Africa from chimpanzees (Tatt *et al.,* 2001). Infected humans have little resistance to infection, and nearly all of them eventually die of diseases that non-infected individuals easily ward off. Few who contract AIDS survive more than a few years untreated. HIV viral entry into a host cell requires three main stages: binding of the gp120 protein on the viral envelope to CD4 cell receptor, a conformational change in gp120 that permits binding to other receptors on the cell, and a conformational change in gp 41 that leads to fusion of the envelope and host cell membrane (Kwong *et al.,* 1998; Weiss, 2003; Huang *et al.,* 2005). A broad diversity of neutralizing antibodies has been generated from memory B cells in response to HIV in HIV-infected patients. Many of these high affinity neutralizing antibodies are targeted to the gp120 variable loops, the CD4-binding site, and the co-receptor-binding site (Scheid *et al.,* 2009).

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GP120

GP120 is a glycoprotein which forms the spikes sticking out of a HIV virus particle. Its main function is to bind to CD4 in human cells. The 120 in its name comes from its molecular weight of 120 KD. It exists on the surface of HIV in trimeric state along with GP41.This core gp120 comprises 25 beta-strands, 5 a-helices and 10 defined loop segments. The structure confirms the chemically determined disulphide-bridge. The polypeptide chain of gp120 is folded into two major domains, plus certain excursions that emanate from this body. The inner domain features a two-helix, two strand bundle with a small five-stranded beta-sandwich at its termini proximal end and a projection at the distal end from which the V1/ V2 stem spreads out (Modi *et al.,* 2013). The outer domain

is a stacked double barrel that lies alongside the inner domain so that the outer barrel and inner bundle axes are approximately parallel. The proximal barrel of the outer-domain stack is composed from a six-stranded, mixed-directional b-sheet that is twisted to embrace helix a2 as a seventh barrel stave. The two barrels share one contiguous hydrophobic core, and the staves also continue from one barrel to the next except at the domain interface. One half of the molecular weight of gp120 is due to the carbohydrate side chains. This dome of carbohydrate prevents gp120 from being recognized by the human immune response. As the HIV virus and the human CD4 cell come together, the gp120 binding site snaps open. Because of the important role of the gp120 glycoprotein in receptor binding, information about the gp120 structure is important for understanding HIV infection and for the design of therapeutic strategies (Patel *et al.,* 2012).

HIV entry into its host cell

CD4 Binding

Entry of primate immunodeficiency viruses into the host cell involves the binding of the gp120 envelope glycoprotein to the CD4 glycoprotein, which serves as the primary receptor. The gp120 glycoprotein binds to the most amino-terminal of the four immunoglobulin- like domains of CD4. Structures of both the N-terminal two domains (Ryu *et al*., 1990; Wang *et al*., 1990) and the entire extracellular portion of CD4 (Wu *et al*., 1997) have been determined, and mutagenesis indicates that the CD4 structure analogous to the second complementarity-determining region (CDR2) of immunoglobulins is critical for gp120 binding (Moebius *et al*., 1992; Sweet *et al*., 1991).

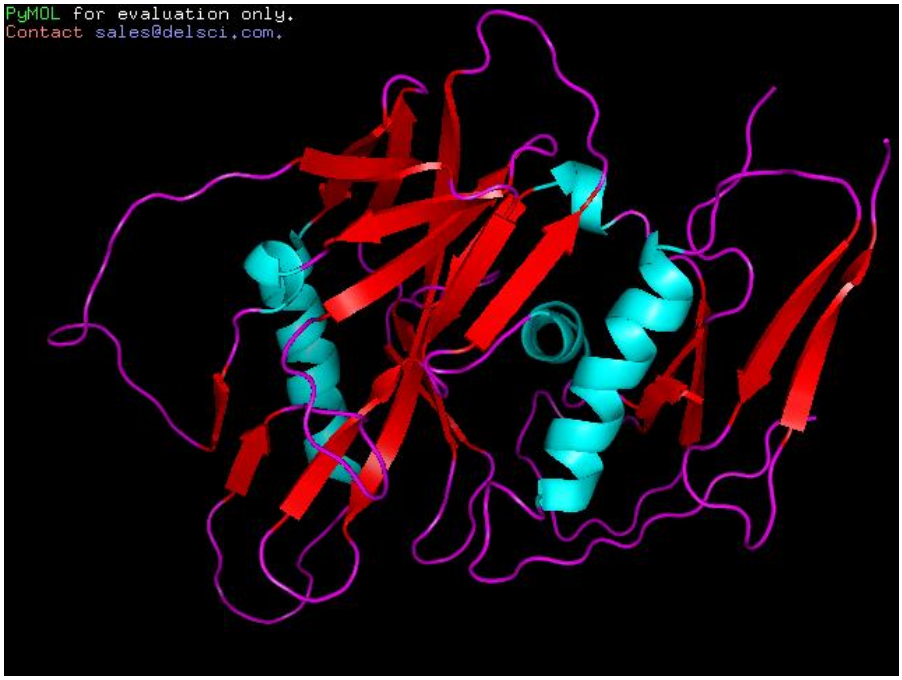


Figure 1. 3 Dimensional structure of GP120

Figure 2. GP120-CD4 complex (cd4 in green, Gp120 in red color)

Conserved gp120 residues important for CD4 binding have likewise been identified by mutagenesis3, (Olshevsky *et al*., 1990; Cordonnier *et al*., 1989). CD4 binding induces conformational changes in the gp120 glycoprotein, some of which involve the exposure and/or formation of a binding site for specific chemokine receptors. These chemokine receptors, mainly CCR5 and CXCR4 for HIV-1, serve as obligate second receptors for virus entry (Moore, 1997; Feng *et al*., 1996).

The gp120 third variable (V3) loop is the principal determinant of chemokine receptor specificity (Speck *et al*., 1997). However, other more conserved gp120 structures that are exposed upon engagement of CD4 also seem to be involved in chemokine-receptor binding. This CD4-induced exposure is indicated by the enhanced binding of several gp120 antibodies (Thali *et al*., 1993; Sattentau *et al*., 1993) which, like V3-loop antibodies, efficiently block the binding of gp120–CD4 complexes to the chemokine receptor (Wu *et al.,* 1996). These are called CD4-induced (CD4i) antibodies. CD4 binding may trigger additional conformational changes in the envelope glycoproteins. For example, binding of CD4 to the envelope glycoproteins of some HIV-1 isolates induces the release, or 'shedding', of gp120 from the complex (Moore *et al.,* 1990), although the relevance of this process to HIV entry is uncertain. The gp120 viral envelope protein has an affinity for the CD4 receptor which is expressed on the cell surface of the CD4+ cell. CD4 binding inhibitors such as the experimental drug TNX-355 have been shown to inhibit the gp120 and CD4 receptor interaction and block HIV infection.

MATERIALS AND METHODS

The present study carried out by using different bioinformatics' tools, biological databases like PubMed, pubchem, PDB (Protein Data Bank) and software's like gold, Pymol, Marvin Chemsketch 14.7.21.0.

Step of using gold: (Docking setup)

Molecular docking plays a crucial role in computational drug design. Docking predicts the preferred orientation of a lig and with the binding site on a receptor.

1- Target

From RCSB Protein Data Bank and the PDB we brought the data of GP120 which carry with ID: 2BF1 Length: 481 amino acids (residues 31 - 511) Molecular Weight: 53922 Da Theoretical pI: 9.05 was used as target complex structure in current study and CD4 carry ID: 1WIQ and save it within computer in pdb format.

2- Ligand selection

PubChem database is an online database contains many of chemical drugs (ligand) which provide the information and structure of chemicals. 12 antiviral molecules were taken from the National Centre for Biotechnology Information(NCBI) Pub-Chem compound database (URL:http://pubchem.nc-bi.nlm.nih.qov/) as ligand molecules. These molecules were downloaded in Structure Data File (SDF) format. Were selected for docking with GP120 and CD4 separately acting as inhibitors.

3- Setting up GOLD Parameter

The protein molecule of GP120, CD4 was uploaded into GOLD. The ligands were also uploaded. GOLD was run in a particular way such that a particular atom number was given from the identified active site. The GOLD was setup to run at an active site radius of 4.5 angstroms. The output folder was specified. All the other fitness function parameters and the genetic algorithm parameters were kept in default mode. We used Pymol to view the GOLD output.

4-Screening and optimization of inhibitors

The Chemicalize server was used for generating structure property prediction and calculations (ChemAxon product) to determine their ADME properties. To be evaluated at the primary stage at in vitro level. This reduces the chances of selecting the false positive results. The various basic physico chemical properties calculated in vitro to evaluate a molecule to act as drug involve logP, logD, H-bond donor, polar surface area, molecular, etc. The value of log P should be not more than 5,this is the distribution coefficient or partition coefficient important for finding the solubility of the drug that is lipophilicity. Molecular weight of the compound should not exceed 500 Da, as most of the drugs are small molecules.

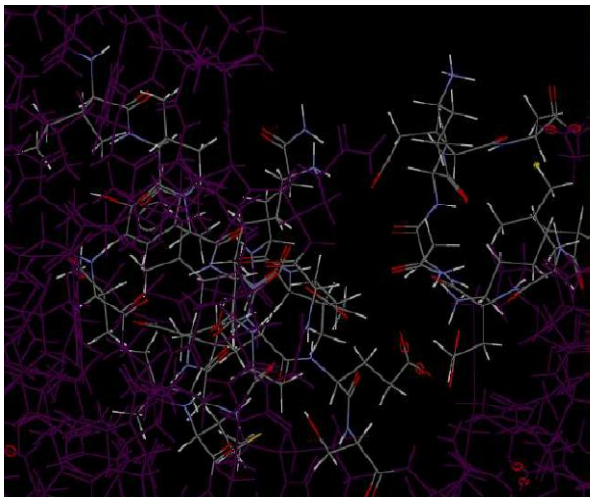


Fig. 3. Print View Result Of Interaction Between Tenofovir And Cd4 (GOLD)

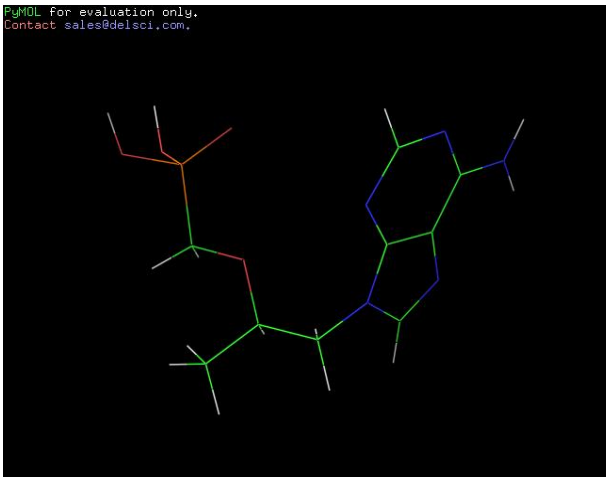


Fig. 4. 3D Structure Of Tenofovir As Seen In Pymol

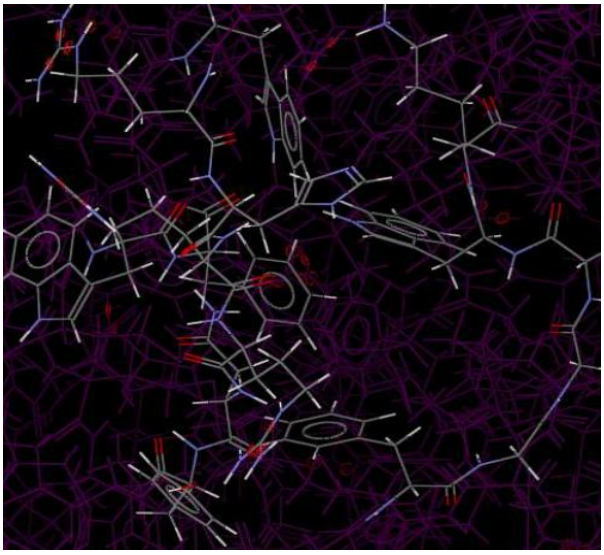


Fig. 4. Print View Result Of Interaction Between Lipinavir and HIV_1 (GOLD)

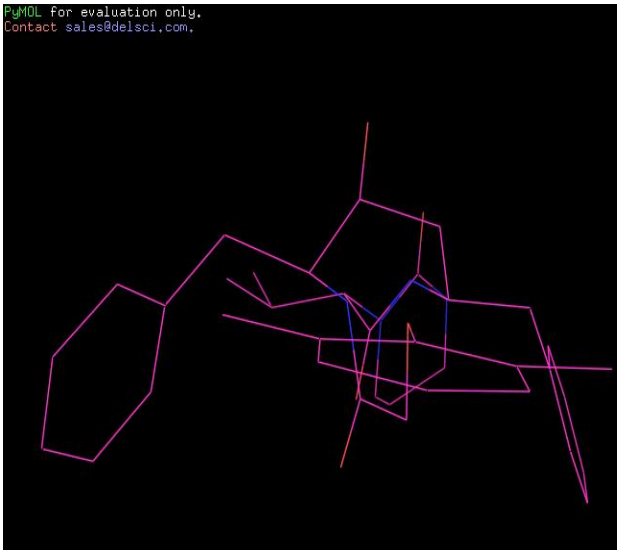
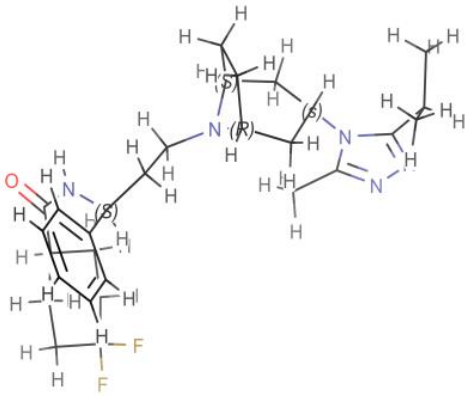
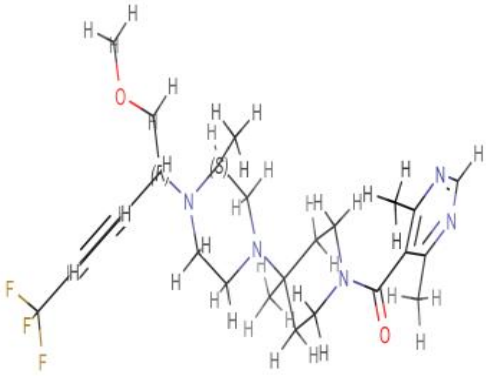


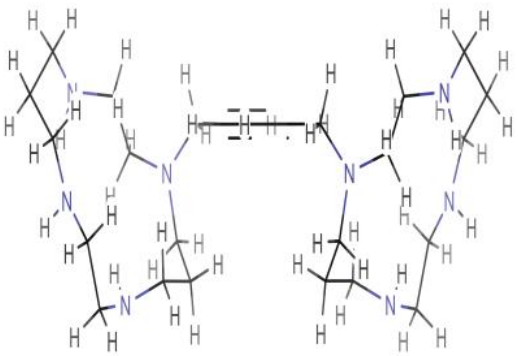
Fig. 5. 3d Structure of Lopinavir as seen in Pymol



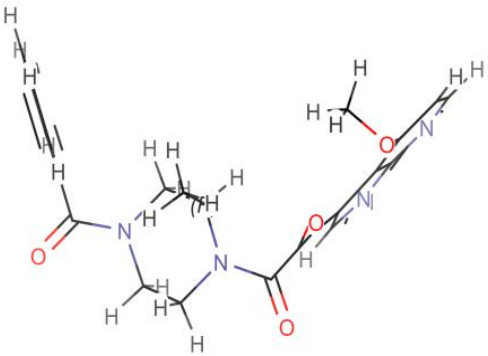
2D Structure of Maraviroc



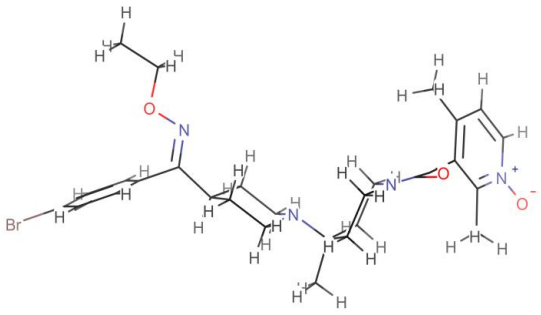
2D Structure of Vicriviroc



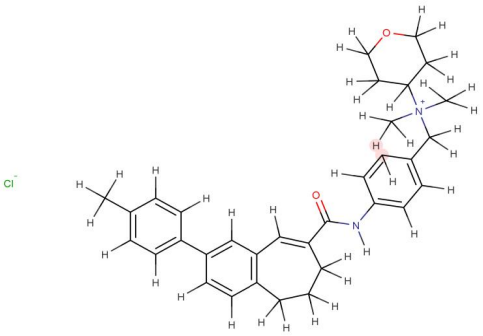
2D Structure of Plerixafor



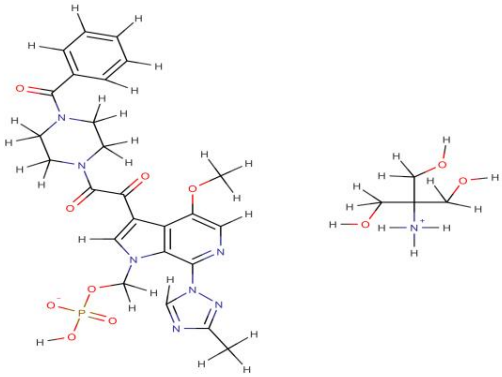
2D Structure of BMS-806



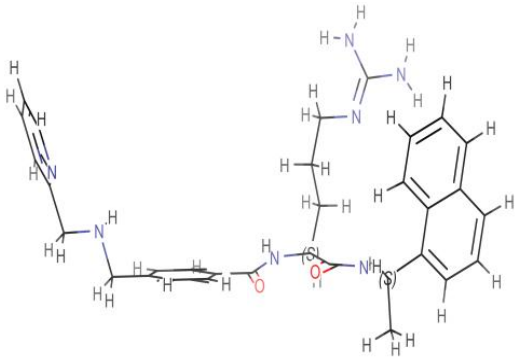
2D Structure of SCH-C



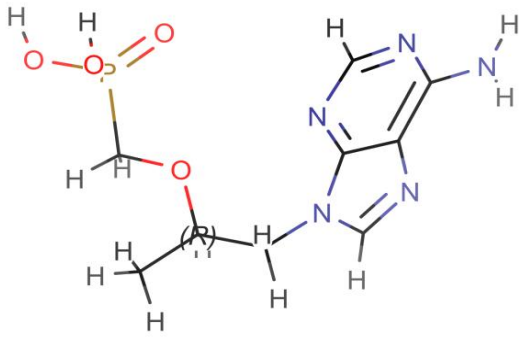
2D Structure of TAK-779



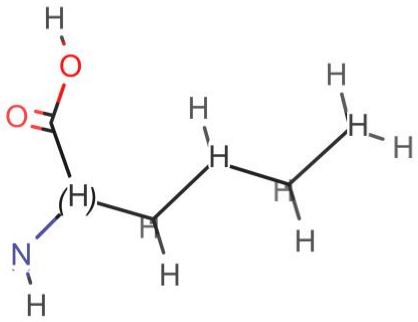
2D Structure of BMS-663068



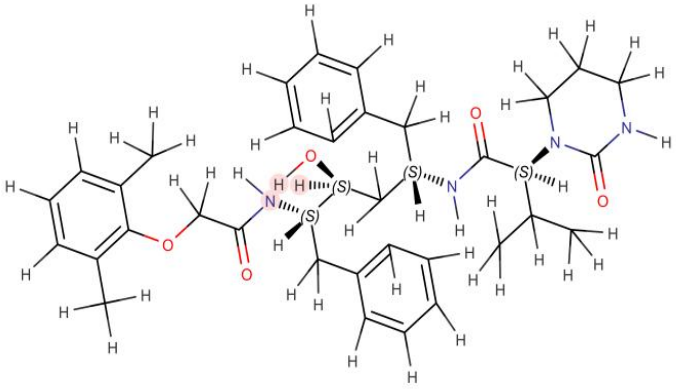
2D Structure of KRH- 1636



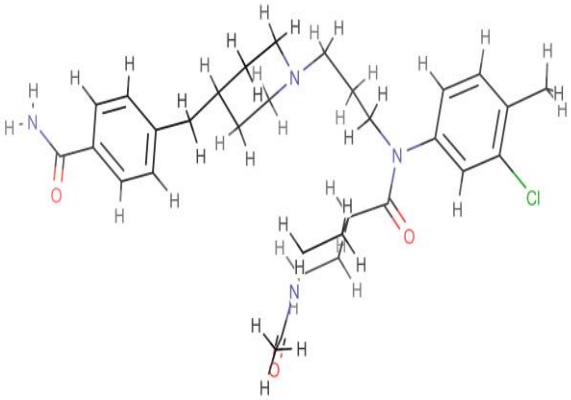
2D Structure of TENOFOVI R



2D Structure of CAPRINE



2D Structure of Lopinavir



2D Structure of TAK-220

RESULTS

The protein- Ligand complex with the most favorable score among the top-scoring complexes was used for further studies. The main active site residues of CD4 were Phe43, Arg59, Asp368, and Glu370. Its volume was 56.83.The main interacting residues of gp120 Ile3, Val8, Arg16, Glu4, Pro10 and Glu21. We can observe this amino acid with in 4.5 angstroms residues by using Accelry discovery studio visualization software, after virtual screening of 12 compounds against CD4 and gp120 separately, we got 6 best compounds to CD4 and 6 best to gp120. There are 3 compounds common to both. Among 4 common compounds, namely Plerixafor, Maraviroc, Lopinavir, Tenofovir are the promising ligands. We also got best compounds which are target specific (CD4 and gp120). Tenofovir and Lopinavir are the CD4 specific best compounds. Lopinavir and TAK-220 are the gp120 specific best compounds.

HIV binding receptor for human T-cells. Crystal structures of HIV gp120-CD4 complexes reveal a close interaction of the virus receptor with CD4 Phe43, which is embedded in a pocket of the virus protein. Blocking the cellular entry of the virus is more practical and exhibit less side effects compared to a drug operating at intracellular level, e.g. a protease inhibitor. Without inhibitory activity against HIV-1 reverse transcriptase, protease and integrase and it has been proposed as a good candidate against HIV-1 infection. A clinical study of gp120 inhibition has not been conducted so far; however an oligopeptide CD4 mimic has been used to study the inhibition of the HIV-1 entry using a cell-based fusion assay. Several computational studies have been performed on docking and molecular dynamics of small ligands with either gp120 or CD4 to identify suitable inhibitor candidates. Another investigation applied molecular dynamics on the gp120-CD4 complex targeting to predict a mimic for the natural Phe43 conformation in the complex. This is the first study that conducts

Table 1. Properties of ligand

S.No	Compounds	CID no.	Molecular Weight (g/mol)	Molecular formula	H-bond donor	H-bond Acceptor	Polar surface area A^2	Log P	Log D
1	MARAVIROC	3002977	513.665546	$C_{29}H_{41}F_2N_5O$	1	4	64.25	3.63	1.47
2	Vicriviroc	3009355	533.628830	$C_{28}H_{38}F_3N_5O_2$	0	6	63.00	2.80	0.77
3	Plerixafor	65015	502.781960	C28H54N8	6	8	93.60	0.43	-10.09
4	TAK-779	183789	531.127960	C33H39CIN2O2	1	2	38.33	0.83	1.87
5	SCH-C	9574343	557.52238	$C_{28}H_{37}BrN_4O_3$	0	5	73.28	2.73	1.83
6	BMS-806	5495818	406.43448	$C_{22}H_{22}N_4O_4$	1	5	95.60	1.57	1.12
7	BMS-663068	56843675	704.624842	$C_{29}H_{37}N_8O_{11}P$	1	10	187.87	-1.65	-2.65
8	TENOFOVIR	464205	287.212322	$C_9H_{14}N_5O_4P$	3	8	139.21	-1.2	-3.68
9	KRH- 1636	465968	551.68188	$C_{32}H_{37}N_7O_2$	5	7	153.71	2.82	0.18
10	CAPRINE	21236	131.17292	$C_6H_{13}NO_2$	2	3	67.77	0.83	-2.10
11	TAK-220	5275766	553.13524	$C_{31}H_{41}CIN_4O_3$	1	4	88.15	3.55	1.47
12	Lopinavir	92727	628.80082	$C_{37}H_{48}N_4O_5$	4	5	120	4.69	4.69

Table 2. Gold docking results of GP120 and cd4 with ligand

S.No	Compounds	CID no.	GOLD SCORE CD4	GOLD SCORE GP120
1	Maraviroc	3002977	13.28	18.36
2	Vicriviroc	3009355	-2715.80	-2706.51
3	Plerixafor	65015	-2715.80	-705.86
4	TAK-779	183789	-2114.92	-2118.44
5	SCH-C	9574343	-2110.10	-2087.82
6	BMS-806	5495818	-552.88	-548.20
7	BMS-663068	56843675	-1870.63	-1856.57
8	Tenofovir	464205	54.55	43.74
9	KRH- 1636	465968	-1482.45	-1471.93
10	Caprine	21236	-815.23	-818.26
11	TAK-220	5275766	-1722.57	-1724.98
12	Lopinavir	92727	27.86	44.68

DISCUSSION

To analyze the promising ligands, we used Similarity ensemble approach that relates proteins based on the set-wise chemical similarity among their ligands. This will forecast the role of our proposed ligands in any vital pathways as due to chemical similarity it may affect any useful pathway. We have set our threshold > 0.5 to human proteins. The protein - Ligand complex with the most favorable score among the top-scoring complexes was used for further studies. The main active site residues of CD4 were Phe43, Arg59, Asp368, and Glu370. Its volume was 56.83. The interactions between HIV-1 gp120 and mutated CD4 proteins were investigated in order to identify a lead structure for therapy based on competitive blocking of the computational protein-protein interactions to propose the design of a therapeutic strateg

Conclusion

The gp120-CD4 complex targeting to predict a mimic for the natural Phe43 conformation in the complex. This is the first study that conducts computational protein- Ligand interactions to propose the design of a therapeutic strategy. Crystal structures of HIV gp120-CD4 complexes reveal a close interaction of the virus receptor with CD4 Phe43, which is embedded in a pocket of the virus protein. Blocking the cellular entry of the virus is more practical and exhibit less side effects compared to a drug operating at intracellular level.

Finally we conclude that there is scope to inhibit the viral entry by developing new drugs using this interaction information.

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