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Molecular Docking Studies of anti-HIV drug BMS-488043 derivatives using HEX and GP120 Interaction Analysis using Pymol

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***Abstract*-** AIDS is one of the most devastating pandemic. All drugs designed have targeted the viral protein the GP120. The entry of Human Immuno Virus (HIV) into the human cells is initiated by a temporary interaction between the viral exterior glycoprotein GP120 and human CD4 receptor. The primary receptor CD4 glycoprotein present on the cell surface of T-helper cells interacts with GP120. The GP120-CD4 complex involves the D1-D2 domain of the human CD4 and conserved regions of the viral protein GP120. The interaction leads to a number of conformational changes in the inner domains and bridging sheets of glycoprotein GP120 due to translocations and deletions, which creates a binding site in the V3 loop of GP120 for chemokine receptors (CCR5/CXCR4) present on the cell surface. This interaction brings the outer membrane of HIV in contact with the cell membrane of the host cell and initiates the entry of the viral genome in the human T-cells. Many drugs are being developed to inhibit this complex formation and one such drug is BMS-488043, which binds near the CD4 binding region and prevents disulphide reduction in GP120 which triggers the conformational change. Several analogs were designed for BMS-488043 using ChemSketch and were docked with the GP120 protein (PDB ID: 3DNN) using HEX. The analogs generated showed improvement in binding affinity and other properties in comparison to drug BMS-488043. Analog 8 had better binding affinity but analog 7 showed improved physico-chemical properties. The drug-protein complex showed interacting amino acids recognized to be Glutamic acid, Aspartic acid, Methionine, Phenylalanine, Lysine and Threonine.

***Index Terms*-** GP120, BMS-488043, Hex Docking, Chemsketch.

I. INTRODUCTION

The Human Immunodeficiency Virus leads to the disease Acquired Immune Deficiency Syndrome. This disease was lately recognized in 1981, in the 30 years since HIV/AIDS was first discovered, the disease has become a devastating pandemic. As per National Institute of Health there are 2.5 million newly infected people with HIV in 2011 [1].Till date around 31 drugs have been licensed by the United States Food and Drug Administration saving antiretroviral treatment, still around 1.7 million AIDS related deaths have occurred in 2011.This global epidemic of infection by HIV has created an urgent need for vaccines or new classes of antiretroviral agents [2].

Numerous drugs have been designed to deactivate the HIV by administering the drugs which act as inhibitors at various levels as Mavaviroc and Enfuvirtide [16]. Mostly the drugs designed and licensed act as Nucleoside Reverse Transcriptase Inhibitors (NRTIs), Nonnucleoside Reverse Transcriptase Inhibitors (NNRTIs), Protease Inhibitors (PIs), Fusion Inhibitors, HIV integrase strand transfer inhibitors and Entry Inhibitors - CCR5 co-receptor antagonist. The drug targeting the CCR5 approved till date is only one and research is on, to target this area more as it is the prime receptor involved in binding to the HIV envelop protein (http://www.fda.gov/ForConsumers/byAudience/ForPatientAdvocates/HIVandAIDSActivities/ucm118915).

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.

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.[10]

Figure 1: 3 Dimensional structure of GP120

MODE OF ACTION: The entry of HIV into susceptible cells involves the interaction of CD4 molecules present on the host cell surface, with the HIV envelope the glycoproteins GP120 and GP41. Also it was found, that interaction of the virus with co-receptors on the cell surface is very important for the entry. The predominant are the chemokine receptors CCR5 and CXCR4 [4]. The HIV-1 envelope glycoprotein complex is a trimer consisting of three GP120 exterior envelope glycoprotein and three GP41 transmembrane glycoprotein's [9]. These are derived by cleavage of a GP160 precursor glycoprotein by host cell proteases. The mature envelope glycoprotein complex is expressed on the surface of infected cells and is incorporated into virion membranes [9].

HIV-1 infection is initiated by GP120 binding to CD4 on the target cell surface. CD4 also has a binding site for Protein Disulfide Isomerase (PDI) and forms a PDI–CD4–GP120 complex. In that complex, PDI can reach and reduce GP120 disulfide bonds (s-s bonds), causing major conformational changes in GP120, which allows it to interact efficiently with one of the chemokine receptors, CCR5 or CXCR4. The interaction of GP120 with its receptors is thought to promote conformational rearrangements in GP41 that drive the fusion of the viral and target cell membranes [6].

The binding of HIV to CD4 is an attractive drug target, both because the CD4 binding site is highly conserved and because it is known that neutralizing antibodies can effectively block this step. Preventing the virus from binding to its primary receptor is the most obvious and direct way to prevent infection. BMS-488043 is low-molecular-weight inhibitors of HIV-1 entry that were recently identified by using a viral infection-based screen.

Figure 2: GP120-CD4 complex

BMS-488043 works by binding to the HIV-1 envelope glycoprotein GP120, thereby interfering with its attachment to the CD4 receptor (http://www.aidsmap.com/Attachment-inhibitor-BMS-663068-potent-and-well-tolerated-in-early-study/page/1681506/) [12]. GP120-CD4 interaction is blocked but GP120 binds to different residues in the same binding pocket. BMS-488043 prevents the reduction of disulfide bonds of the protein GP120 which inhibits the conformational changes occurring in GP120 and prevents it from binding to chemokine co-receptors [6]. This further inhibiting the GP41 interaction with the target cell membrane and prevents the HIV infection mechanism. BMS-488043 binds to residues 112,113,121,375,422and 426 which are adjacent to the disulfide bonds in the GP120 protein [7].

BMS-488043 has recently been replaced with BMS-663068, a compound derived on the basis of structure-activity relationship studies. It is currently being studied in Phase II clinical trials [25].

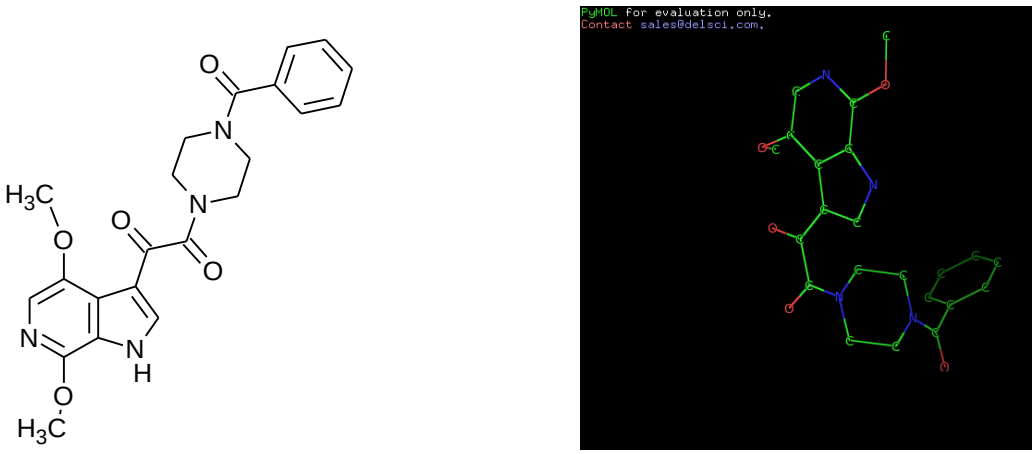

Figure 3: 2-Dimensional and 3-Dimensional structure of BMS-488043

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II. MATERIALS AND METHOD

The present study was performed using bioinformatics tools, biological databases like PubMed, ChemSpider, PDB (Protein Data Bank) and software's like Hex, ACD ChemSketch. From the literature review using PubMed it was recognized that GP120 interaction with BMS, and numerous of its analogues have been recognized of which BMS-488043 was selected to work upon.

ChemSpider, the chemical database that contains more than 26 million unique molecules from over 400 data sources resource that includes FDA, ZINC, Drug Bank etc, gave the chemical formula and structure of the BMS drug along with the used analogue [14]. ACD/ChemSketch which is a powerful all-purpose chemical drawing and graphics package from ACD/Labs developed to help chemists quickly and easily draw molecules, reactions, and schematic diagrams, calculate chemical properties, and design professional reports and presentations, was used to develop all the possible analogues of BMS-488043(Reference).Numerous analogues were created using the ACD/ChemSketch. When the properties were analyzed only 9 analogues were selected for docking with the protein acting as inhibitors [13].

The PDB (Protein Data Bank) is used to download the target protein the 3DNN is the PDB ID of the GP120 protein. The structure is visualized using the molecular graphics program PyMol intended for the structural visualization of proteins, nucleic acids and small biomolecules [25].

The docking analysis of BMS-488043 and its analog with glycoprotein GP120 was carried out using HEX 6.3 docking software, which is an Interactive Molecular Graphics Program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules Docking allows predicting the ligand with best scores and identifying the drug-receptor complex with lowest free energy [26].

The parameters used for the docking process were:

1. Correlation type – Shape + Electrostatics
2. FFT Mode - 3D
3. Post Processing- MM Energies
4. Grid Dimension - 0.6
5. Receptor range – 180
6. Ligand range – 180
7. Twist range – 360
8. Distance Range – 40

The drug and analogs generated were docked with the receptor using the above parameters.

After docking the analogues of BMS-488043, the various analogue's which gave the best affinity results were evaluated using chemical properties viewer of ChemAxon available at www.chemicalize.org.[13]

- Generate the canonical SMILES notation of the analogs.
- Submit the SMILES as query in the query box of the Chemicalize (ChemAxon) server.
- Click on the required property to observe the value.

III. RESULTS AND DISCUSSION

Docking results of the HIV membrane glycoprotein GP120 and the drug BMS-488043 (1-(4-Benzoyl-1-piperazinyl)-2-(4, 7-dimethoxy-1H-pyrrolo [2, 3-c] pyridin-3-yl)-1, 2-ethanedione) as well as the analogs generated are shown in the table.

Table 1: Hex docking results of GP120 with BMS-488043 and generated analogs.

COMPOUND	E-VALUE
BMS-488043	-256.27
Analog 1	-221.01
Analog 2	-241.67
Analog 3	-247.09
Analog 4	-222.80
Analog 5	-219.01
Analog 6	-233.59
Analog 7	-252.27
Analog 8	-263.29

The HEX docking results reveal that the e-value of Analog 8 (-263.29) is better as compared to that of original drug BMS-488043(-256.27). The analog showed an increase in the free energy of the complex with the receptor but bonded the receptor in the same binding pocket. This indicated that the functional group involved in the complex formation were same as that of the BMS drug and the difference was due to the increase in the steric compatibility and pharmacological properties of the analog. The analogue was created adding azole ring and a phosphate group.

The structure of the analogue of BMS-488043 is:

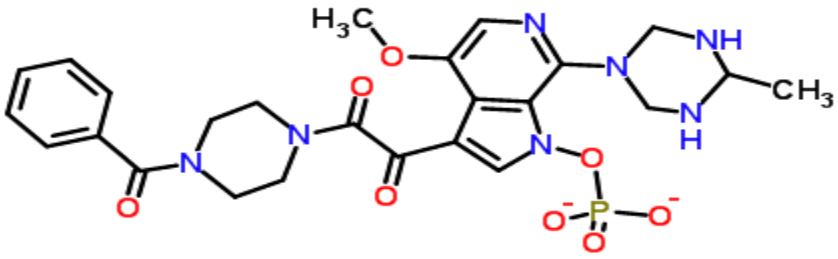

Figure 4: 2-methyl-1,3,5-triazinane and a phosphate group has been added to the basic BMS structure (1-(4-Benzoyl-1-piperazinyl)-2-(4, 7-dimethoxy-1H-pyrrolo [2, 3-c] pyridin-3-yl)-1, 2-ethanedione), to develop the analogue which gave the best result as inhibitor (Analog-7).

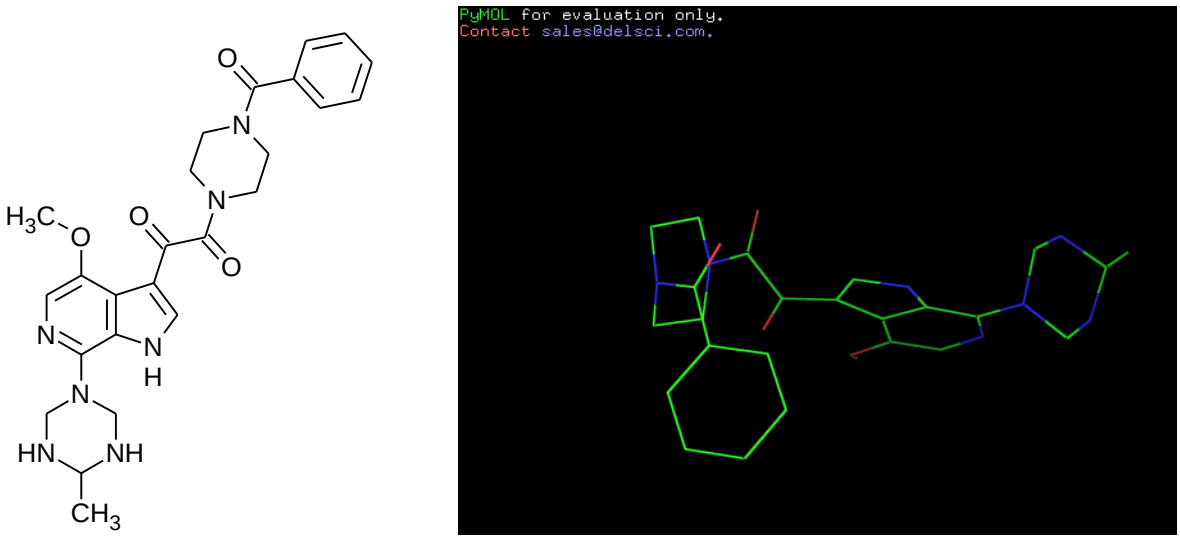

Figure 5: 2-Dimensional and 3-Dimensional structure of Analog-7.

The chemical structure of BMS-488043 was obtained from the Chemspider database [14] and can also be extracted from the Bristol Myers Squibb server available at www.bms.com .

The drug-likeness is necessary 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 refractivity, no. of atoms, rotatable bonds etc. The value of logP should be ≤ 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.

The Chemicalize server was used for generating structure property prediction and calculations (ChemAxon product) to determine their ADME properties [13].

Table 2: Properties of BMS-488043, Analog-7 and Analog-8

Property	BMS-488043	Analog-7	Analog-8
Molecular weight	421.4259	490.5343	585.5067792
Log P	2.561	1.82	2.8
Log D	1.05	1.58	1.59
H-bond acceptor	9	9	10
H-bond donor	1	0	0
Polar surface area	101.93 A^2	120.00 A^2	184.46 A^2
Molecular refractivity	111.524 cm^3	132.24 cm^3	145.553 cm^3

No. of atoms	52	64	69
Rotatable Bonds	5	5	7

The various properties when analyzed and it were found that the Analog-8 though has very good binding affinity and has good complex formation ability but it has some properties which violate the drug likeliness rules. The Analog-8 has higher molecular weight of 585.506779 Da as acceptable to be a promising drug. Analog-7 follows the Lipinski's rule of five and also has oral-bioavailability, but has low complex formation possibility compared to analog-8.

When the docking results were analyzed in PyMol, the GP120 complex with the analogue 8, the interacting amino acids were recognized.

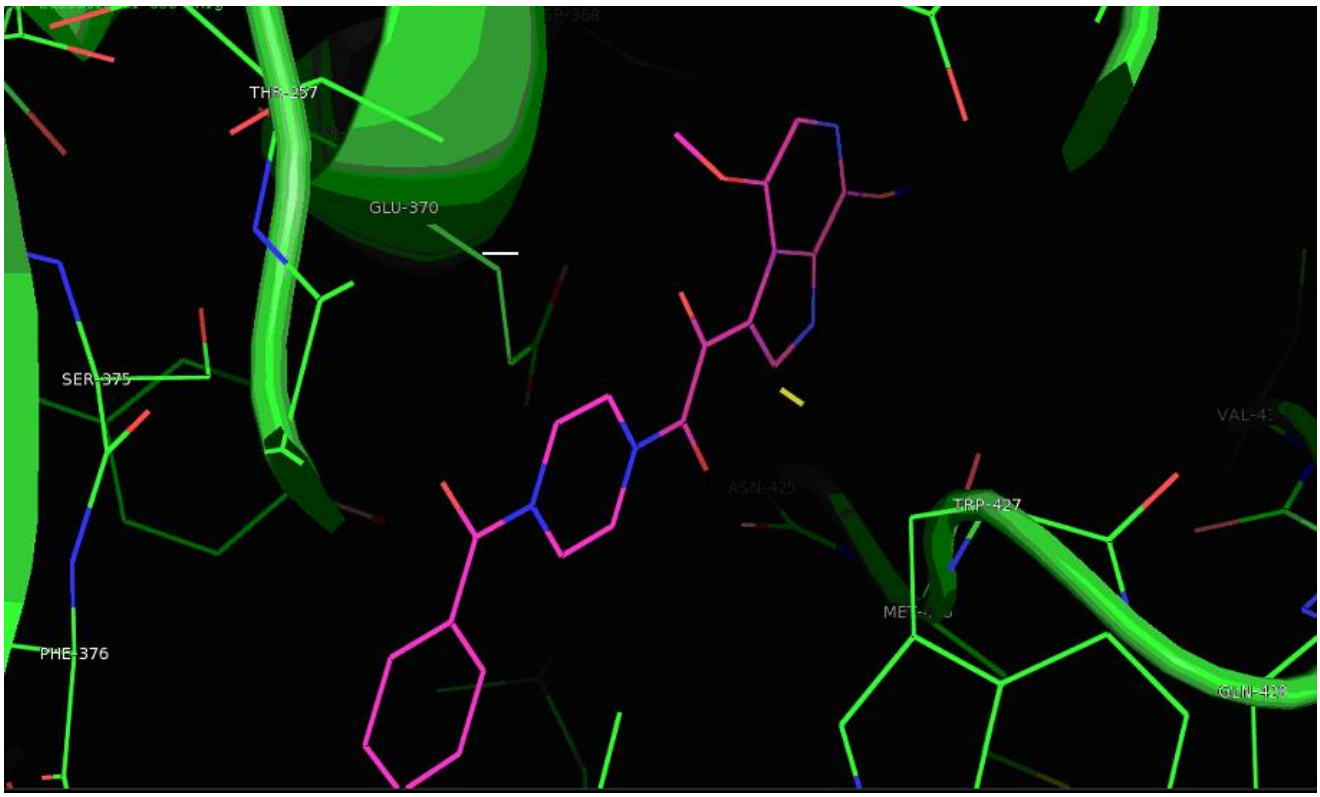

Figure 6: Snapshot of interacting amino acids in GP120 with the analog-8 of BMS-488043 as seen in PyMol.

As the GP120 is a homotrimer, with all the subunits of the complex it was found that the same amino acids are seen to interact with the drug like molecule. The interacting amino acids include the Glutamic acid, Aspartic acid, Methionine, Phenylalanine, Lysine and Threonine. All these amino acids are seen to interact with the GP120 disabling it to attach to the chemokine receptor.

IV. CONCLUSION

Since BMS-488043 is one of the most promising drug showing antiviral activities against HIV, it can be studied with further modifications to produce some useful analogue which can act as promising drug against the HIV. GP120 being the main interacting component for the virus to interact, the drug targeted for it are the most promising to look for. The binding of the drug inhibits the gp120 to bind to the chemokine receptor. Clinical Trials are proving to be good with few drawbacks which could be reduced once the effect of drug is studied under trial. In silico drug designing and interaction of the drug and the target protein provides a good understanding of the mechanism in which they bind and which residues are involved in the interaction. The drug like properties also give us a clue that the analogues designed with the BMS-488043 as the seed molecule are promising drug compounds. This provides us the understanding what and where to target the drug with enhanced activity of inhibiting the GP120-chemokine receptor complex formation.

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