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"Analysis of binding properties of integrase protein to find out potential drug candidate for HIV-2 through insilico molecular docking "

Vijay Laxmi Saxena[1,*,] Shrasti Gupta[1] , Shweta Maurya[2,*]
Bioinformatics Infrastructure Facility Centre of D.B.T, Dept of Zoology, D.G (P.G.) ,College, Kanpur
Bioinformatics Infrastructure Facility Centre of D.B.T, D.G (P.G.), College, Kanpur
CSJM, Kanpur university,Kalyanpur, Kanpur

Abstract: *Human immuno deficiency virus is a lentivirus (slowly replicating virus) that cause acquired immuno deficiency syndrome "(**AIDS**). **HIV** attached to the human CD4 receptor and leads to the infection. The most notable, the T-helper cells which plays a central role in immune response are disabled and killed during the typical course of infection.**HIV-2** infection is characterised by higher CD4 cell count & lower viral RNA level. The main objective of this project work is to find out potential compound that can help to interrupt the function of **HIV-2**integrase (IN) protein. In this study, we employed the integrase protein sequence to build suitable drug by homology modeling , which has been checked for high reliability by verify score ,energy minimization for stability and active side prediction. Its accuracy has been verified by the Saves server and Ramachandran plot. The model structure was employed for docking. Known inhibitor taken from the literature docked, and docked at the binding site. Obtain molecule **2'-Deoxyuridylic acid,2-(Ethoxymethyl)-4-(4-Fluorophenyl)-3-[2-(2-Hydroxyphenoxy)Pyrimidin-4-yl]isoxazol-5(2H)-one,{4-[2,2-BiS(5 methyl-1,2,4-oxadiazole-3-YL)-3-phenyl pyro]phenyl}sulfamic acid,2-(2-{2-[(Biphenyl-4-ylmethyl)-amino]-3-Meracapto-pentanoylamino}-acetylamino)-3-methyl-butaric acid methyl ester, N-[2-(2,4-diaminopyrido[2,3-d]pyrimidin-7-yl)-2-methylpropyl]-4-phenoxybenzamide** *have been given score of -136.401 kcal/mol , -143.584 kcal/mol , -136.863 kcal/mol , -135.008 kcal/mol , -156.243 kcal/mol.*

Keywords: *HIV- 2,CD4 receptor,Integraseprotein,Computer aided drug designing, Docking.*

I. Introduction:-

The human immunodeficiency virus is the causal agent of AIDS. AIDS morbidity and mortality have led to efforts to identify effective inhibitors of the replication of this virus.Integration of retroviruses like the Human Immunodeficiency Virus-1 (HIV-1) & Human Immunodeficency virus-II (HIV-II) establishes a provirus in the host genome, embodying the point-of-no-return in the viral replication cycle.**[13]**. Viral replication is driven by a molecular motor consisting of the three viral enzymes: the reverse transcriptase, protease and integrase (IN).(**2**).Our study is mainly deals with Integrase enzyme.Integrase possesses two major catalytic activities: an endonucleolytic cleavage at each 3'-OH extremities of the viral genome, named 3'-processing, and a strand transfer reaction leading to the insertion of the processed viral DNA into the target DNA by a trans-esterification mechanism. These catalytic functions of the integrase are essential for the overall integration process and have thus been the object of intensive pharmacological research.**[13]**

Drugdesigning is a time consuming and expensive process. The first stages of this process are lead discovery and lead optimisation.Traditionally lead compound have been discovered serendipitously, by chemically modifying andimproving existing drugs orby isolating the active ingredients in herbal remedies. More recently, pharmaceutical companies have focussed on high throughput screening (HTS). This involvesscreening a large chemical libraryagainst a protein target.**[1]**The choice of a drug target is primarily made on a biological and biochemical basis. The ideal target macromolecule for structure-based drug design is one that is closely linked to human disease and binds a small molecule in order to carry out a function. The target molecule usually has a well defined binding pocket. In our study the drug target is integarse enzyme.

TYPES OF HIV AND DIFFERENCES BETWEEN THEM:-

Two types of HIV have been characterized: HIV-1 and HIV-2. HIV-1 is the virus that was initially discovered and termed both LAV and HTLV-III. It is more virulent, more infectiveand is the cause of the majority of HIV infections globally. The lower infectivity of HIV-2 compared to HIV-1 implies that fewer of those exposed to HIV-2 will be infected per exposure. Because of its relatively poor capacity for transmission, HIV-2 is largely confined to West AfricaSimilar in many ways, there are important differences between HIV-1 and HIV-2 that provide insights into virus evolution, tropism and pathogenesis. Major differences include reduced pathogenicity ofHIV-2 relative to HIV-1, enhanced immune control of HIV-2 infection and often some

degree of CD4-independence.Despite considerable sequence and phenotypic differences between HIV-1 and 2 envelopes, structurally they are quite similar. Both membrane-anchored proteins eventually form the 6-helix bundles from the N-terminal and C-terminal regions of the ectodomain, which is common to many viral and cellular fusion proteins and which seems to drive fusion . HIV-1IIIB gp41 helical regions can form more stable 6-helix bundles than HIV-2SBL gp41 helical regions ; however HIV-2 fusion occurs at a lower threshold temperature (25°C). **[11]**HIV type I (HIV-1) and type 2 (HIV-2) are very closely related but differ in pathogenicity, natural history and therapy. HIV-1 is more easily transmitted and consequently accounts for the vast majority of global HIV infections. The less transmissible HIV-2 was thought to be largely confined to West Africa (where it is thought to have originated)but has spread to parts of Europe and India.Whencompared to HIV-1, HIV-2 infected individuals have a much longer asymptomatic stage, slower progression to AIDS, slower decline in CD4 countlower mortality, lower rate of vertical transmissionand smaller gains in CD4 count in response to antiretroviral treatment (ART).Serologic reactivity to HIV-1 and HIV-2 (HIV-1/2) has also increased in HIV-2 endemic areas over the past decade. In terms of antiretroviral drug regimens, HIV-2 is intrinsically resistant to non nuclesoside reverse transcriptase inhibitors (NNRTI) such as nevirapine and efavirenz and not all the protease inhibitors (PIs) provide good viral suppression15.The genome of HIV-2 is 9,671 nucleotides is long,whereas HIV-1 is isolated about 9200 nucleotides long.The genetic organization of HIV-2 is analogous to HIV-1**.[12]**

5'Ltr-gag-pol-central region-env-orf-F-3'Ltr

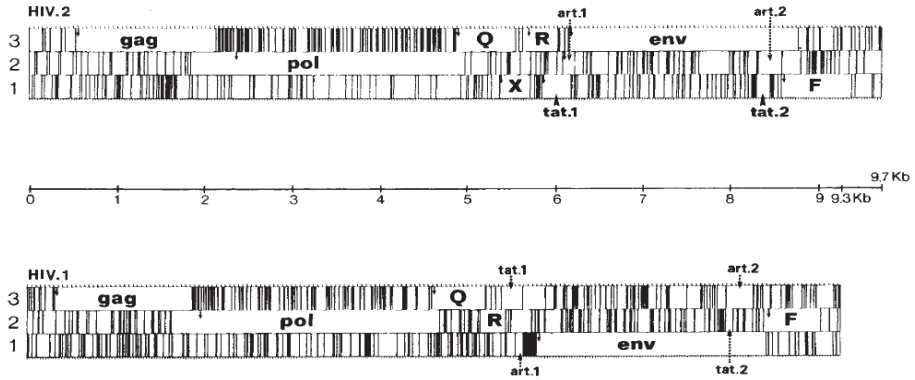


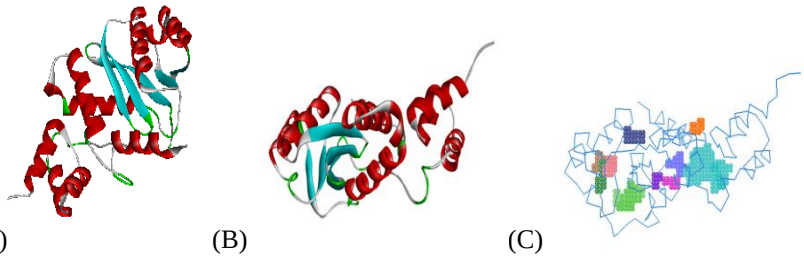
FIG :1HIV-2 has been found to be more infectious in later stages,causing a number of alignments in a very short spans.**[11]**

Comparision of HIV-1 and HIV-2 characteristics

Characteristics	HIV-1	HIV-2
Infectivity	High	Low
Virulence	High	Low
Heterosexual spread	Higher	Lower
Vertical Transmission	20-50%	≤5%
Genetic Diversity	–	Lower
Prevalence	Global	West Africa
Time to AIDS	≤10 Years	≥20 Years

II. Materials And Methodology :-

PROTEIN MODEL:-

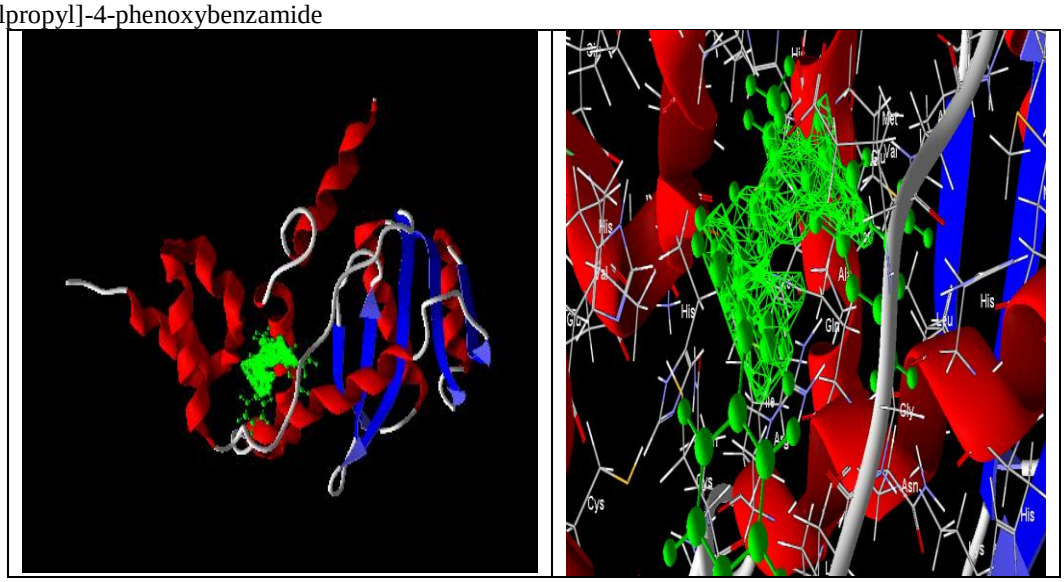


(A) (B) (C)

FIG1:(A)3D structure of integrase protein sequence,Red colours shows helical ,blue colurs shows sheet and green colours shows coiling in the structure of protein. **FIG:(B)**The model of integrase protein have been shown below which have minimum energy and is most stable. **FIG:(C)** 9active sites for the ligand binding sites. Among of them two active sites have larger pocket size **.**His, Tyr, Ser, Asn, Val, Gyl, Lys, Cys, Glu, Ala, Metamino acid residues are present at largest pocket site

Sites	Min cords	Max coords	Site volume	Protein volume
Site 1(Blue)	(19,-2,-6)	(1,-6,-17)	3017[Å³]	19894[Å³]
Site 2(Green)	(1,-6,-17)	(15,7,-4)	118[Å³]	19894[Å³]

BEST INTRACTION SHOWS WITH LIGAND 30:- N-[2-(2,4-diaminopyrido[2,3-d]pyrimidin-7-yl)-2-methylpropyl]-4-phenoxybenzamide



(a) **(b)**

FiG2:-(a) Ligand**N-[2-(2,4-diaminopyrido[2,3-d]pyrimidin-7-yl)-2-methylpropyl]-4-phenoxybenzamide**binding with integrase protein**. (b)** Structure containing **His, Met, Glu, Arg, Val** amino acid residues at the binding site of ligand.

Cavity of active site	Surface of protein	Moldock Score	H-Bond
41.472(Vol)	160	-156.243	-7.07561

III. Result And Discussion:-

Human Immuno deficiency virus cause AIDS which leads to life threatening opportunistic infections..Integrase protein sequence has been retrived from NCBI and the structure is modeled by Raptor X server. Raptor X server generated models of Integrase protein, model has been considered best because it has maximum core region, and minimum disallowed region and minimum energy. Then we using Saves server to verify the structure by verify score plot, procheck and Ramachandran plot. After this active site have been predicted using the P – site finder which resulted in to 9 active site each having specific volume, area and amino acid residues. the site 1 which having maximum volume has following amino acid His(158),tyr(16), ser(18),asn (161), val (38) ,cys (44) , lys (47) ,gly (48) , glu (49) ,ala (50) , gln (162) , arg (188), cys (183), met (184) ,lys (187). Active site is the site were the ligand binds disturbed the activity of receptor. To analyse the active site of protein .It is selected their largest pocket size withe higher number of site volume and higher number of residues present at the active site. To the prediction of protein ligand interaction, first chemical compounds were selected from drug bank and sdf (structure data file) format from Zinc database and validated to the Lipinski rule of five. It's a rule for analysing the pysio-chemical property of drug and analysed that a drug is more likely to membrane permeable and easily absorbed by the body. All selected compound follow Lipinski rules of five.Towards finding suitable inhibitors for Integrase protein the binding energy of prescribe drug for HIV 2 has been done by Molegro Virtual Docker is an integrated platform for predicting protein – ligand interaction. It is analysed docking from MVD the Mol dock score value and hydrogen bonds value shows higher binding affinity with the lower binding energy. **N-[2-(2,4-diaminopyrido[2,3-d]pyrimidin-7-yl)-2-methylpropyl]-4-phenoxybenzamide** have energy binding value **(-156.2343 kcal/mol)** and hydrogen bindingvalue **(-7.07561).**The bioactivity of **N-[2-(2,4-diaminopyrido[2,3-d]pyrimidin7-yl)-2-methylpropyl]-4-phenoxybenzamide** shows maximum positive value of dug likeness property it shows positive number of kinase inhibitor , Enzyme inhibitor and higer score of GPCR ligand score.

IV. Conclusion:-

In study of HIV-2 integrase protein with various ligand determine and the interaction between Inegrase protein and selected ligand that bind on active site of the Integrase enzyme ,although docking process is very complicated because its depends on various parameters the main resultant obtained by Molegro virtual docker for identify the suitable HIV-2 integrase inhibitor in all the 30 ligands which are docked with the integrase only

5 numbers of ligand given the minimum energy and these are2'-Deoxyuridylic acid,2-(Ethoxymethyl)-4-(4 Fluorophenyl)-3-[2-(2-Hydroxyphenoxy)Pyrimidin-4-yl]isoxazol-5(2H)-one,{4-[2,2-BiS(5 methyl-1,2,4-oxadiazole-3-YL)-3-phenyl pyro]phenyl}sulfamic acid,2-(2-{2-[(Biphenyl-4-ylmethyl)-amino]-3-Meracapto-pentanoylamino}-acetylamino)-3-methyl-butaric acid methyl ester,N-[2-(2,4-diaminopyrido[2,3-d]pyrimidin-7-yl)-2-methylpropyl] phenoxybenzamide have been given score of -136.401 kcal/mol , -143.584 kcal/mol , -136.863 kcal/mol , -135.008 kcal/mol , -156.243 kcal/mol. Out of these5 ligands, it is analyased that **N-[2-(2,4-diaminopyrido[2,3- d]pyrimidin-7-yl)-2- methylpropyl]-4- phenoxybenzamide**is potential Integrase inhibitor. Perhaps the ultimate solution is to develop a potential drug candidate against this devastating epidemic.

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