

**COMPUTATIONAL STUDY OF MARINE NATURAL PRODUCTS(MNP'S)
MODULATORY EFFECT ON HIV RESISTANT MUTANTS**

Submitted in partial fulfilment of the requirements for the award of
Bachelor of Technology degree in Bioinformatics

By

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DEPARTMENT OF BIOINFORMATICS

SCHOOL OF BIO AND CHEMICAL ENGINEERING

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A" by NAAC

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **NITEESHA ADAPALA(Reg. No.37220012)** and **TREESA K ABRAHAM(Reg.No.37220025)** who carried out the project entitled “**COMPUTATIONAL STUDY OF MARINE NATURAL PRODUCTS(MNP’S) MODULATORY EFFECT ON HIV RESISTANT MUTANTS**” under my supervision from August, 2020 to March, 2021.

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DECLARATION

We, **NITEESHA ADAPALA (Reg.No.37220012)** and **TREESA K ABRAHAM(Reg.No.37220025)** hereby declare that the Project Report entitled **“COMPUTATIONAL STUDY OF MARINE NATURAL PRODUCTS(MNP’S) MODULATORY EFFECT ON HIV RESISTANT MUTANTS”** done by us under the guidance of **Dr. JEMMY CHRISTY, MSC., MTECH., PhD.**, is submitted in partial fulfilment of the requirements for the award of Bachelor of Technology degree in Bioinformatics.

DATE: 19th May 2021

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**COMPUTATIONAL STUDY OF MARINE NATURAL
PRODUCTS(MNP'S) MODULATORY EFFECT ON HIV RESISTANT
MUTANTS**

ABSTRACT:

In Spite of the significance of various antiretroviral drugs and therapies, proper cure is not yet obtained due to safety and efficacy limitations, as well as high resistance rate of conventional therapies, many natural molecules are preferred over the synthetic drugs. In this study we have some marine natural products that act as antiretroviral drugs. The reason to choose marine is that the environment has a recognized distinctive compound with pharmacological properties such as antitumor, anti-inflammatory, analgesic, immunomodulatory, anti-allergy, and antiviral activities. Marine Natural products(MNPs) yield more effective anti-HIV compounds due to their higher efficiency (lower effective dose) and a better selectivity also they do not induce the development of resistance. By fetching the data from various literature studies, a list of marine natural compounds with anti-HIV activity was obtained. These compounds were screened based on their ADMET properties using SwissADME, an online web tool. The resistant mutants of these screened compounds were further compared with FDA compounds through Docking. This study aimed at the prediction that marine natural compounds can also be considered as lead molecules for designing the future classes of lead molecules to overcome the drug resistance.

TABLE OF CONTENTS

CONTENTS

PAGE NO.

- LIST OF ABBREVIATIONS

- LIST OF FIGURES

- LIST OF TABLES

1. INTRODUCTION	1
1.1 HUMAN IMMUNODEFICIENCY VIRUS	
1.2 ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS)	
1.3 LIFE CYCLE OF HIV VIRUS	
1.4 HIV ASSOCIATED PROTEINS	
1.5 CHALLENGES IN THE CURRENT THERAPEUTIC REGIMEN FOR HIV	
1.6 MARINE NATURAL PRODUCTS AS ANTIVIRAL DRUGS	
1.7 INTEGRASE	
1.7.1 INTEGRASE INHIBITORS	
1.8 REVERSE TRANSCRIPTASE	
1.8.1 NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS(NNRTIs)	
2. LITERATURE REVIEW	
3. METHODOLOGY	
3.1 PUBCHEM	
3.2 PROTEIN DATA BANK	
3.3 SWISS ADME	
3.4 HIV DRUG RESISTANCE DATABASE	
3.5 MOLECULAR DOCKING	
4. RESULTS	
4.1 INTEGRASE	
4.2 REVERSE TRANSCRIPTASE	

5. CONCLUSION

LIST OF ABBREVIATIONS

ABBREVIATIONS	EXPANSIONS
HIV	Human Immunodeficiency Virus
AIDS	Acquired immune deficiency syndrome
FDA	Food and Drug Administration

HAART	Highly Active Antiretroviral Therapy
NNRTI	Non-nucleoside reverse transcriptase inhibitors
NNIBP	NNRTI binding pocket
NIH	National Institutes of Health
ART	Antiretroviral therapy

LIST OF FIGURES

NO.	FIGURES	PAGE NO.
1.7	Domains of integrase	5
1.8.1	NNRTI mutations associated with acquired drug resistance by country	8
3.1	Methodology	14
4.1	Mutation energy of Integrase	22

4.2 Mutation stability analysis of Reverse Transcriptase

4.3 3D-Interactions of Integrase and Reverse Transcriptase

LIST OF TABLES

NO.	TABLE	PAGE NO.
2.1	Marine natural compounds that exhibited anti-HIV activity	9
3.2	Swiss ADME Results for Integrase compounds	17
3.3	Swiss ADME Results for Reverse transcriptase compounds	17
3.4	Resistant mutants of Integrase	19
3.5	Resistant mutants of Reverse transcriptase	19
4.1	Mutation stability analysis of Integrase	22
4.2	Mutation stability analysis of Reverse Transcriptase	

1. INTRODUCTION

1.1 HUMAN IMMUNODEFICIENCY VIRUS

Human Immunodeficiency Virus, HIV is one of the infectious diseases which was Globally challenged in the 1920s. HIV targets the immune system functioning resulting in the progressive decline in CD4+ cells and impairs the human defense against pneumonia, tuberculosis, and shingles as well as certain types of cancer. The failure of a patient's homeostatic mechanism as well as the reason for viral replication is due to the development of immunodeficiency. Hiv has two viral forms,

- Hiv 1 is the most common one (M-P)
- Hiv 2 (A-H).The human immunodeficiency virus[1]

There is a continuous increase in the number of people getting affected with HIV-1.Based on the latest statistical reports nearly there are 40 million HIV positive individuals in the whole world among which the major part is contributed by the developing countries i.e nearly 95%.Globally,14 thousand people are infected by HIV per day.It has been reported that there is more than 30 million death of people infected with HIV from the very first HIV positive patient.[2]

1.2 ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS)

The advanced and wild stage of HIV infection is the Acquired Immunodeficiency Syndrome (AIDS). This AIDS takes from two to 15 years to develop, depending on the individual's immune power.[3]

The virus that causes AIDS has spikes in its envelope. The spikes help the AIDS virus attach to molecules on the surface of T-lymphocyte.Without the T helper cell,the immune system is unable to organise a defense plan to invaders.Thus even the harmless organisms in the environment can cause life threatening infections in people with AIDS.The AIDS virus is present in body fluids and can be only spread through exchange of certain of those fluids.

1.3 LIFECYCLE OF HIV VIRUS

HIV attachment to host via

- 1)negatively charged cell-surface which is a nonspecific interaction
- 2)specific interaction
- 3)interaction with pattern recognition receptors.

Steps involved in viral life cycle,

Step 1:The interaction between the virus and the host cell membrane or receptor(s) called fusion or entry. Fusion/entry inhibitors have been more provided for HIV treatment. Step 2: Uncoating inhibitors. Step 3:nucleic acid synthesis which is mediated by viral enzymes, including RNA polymerase, DNA polymerase, and reverse transcriptase. So, these enzymes were considered as alternative targets in viral infections. Step 4:The release of new viruses from the host cell[1].

Conformational changes of the gp120 glycoprotein due to the binding of the HIV glycoprotein gp120 to the host cell CD4 receptor will uncover additional binding sites which in turn interacts with distinct proteins present on the host cell membrane commonly known as the β -chemokine co-receptors that facilitates the virus entry.A progressive decline of the CD4+ cells will lead to the failure of immune system functioning which causes the development of other opportunistic infections leading to the death of the infected individual[1].

1.4 HIV ASSOCIATED PROTEINS

HIV-1 is a Lentivirus of family Retroviridae that infects CD4+ T lymphocytes, monocyte or macrophages and dendritic cells, using the CD4 molecule and the chemokine receptors CCR5 or CXCR4 to enter the target cells. HIV-2 is less transmittable and is largely confined to West Africa. [4]

The HIV-1 genome has reading frames that codes for structural as well as regulatory proteins.The *gag* gene encodes the Pr55Gag precursor of inner structural proteins i.e the capsid protein,matrix protein, ,nucleoprotein and p6 which is involved in the release of virus particles . The *pol* gene encodes the Pr160GagPol precursor of the viral enzymes protease,reverse transcriptase, RNase H, and integrase.The *env* gene encodes the PrGp160 which is the precursor of the gp120 (surface glycoprotein) and gp41 the

transmembrane protein. Other genes include *tat*, that encodes p14 the transactivator protein, *rev*, that encodes p19 the RNA splicing regulator, *nef*, that encodes p27 the negative regulating factor, *vif*, that encodes p23 the viral infectivity protein, *vpr*, that encodes p15 the virus protein, *vpu*, that encodes p16 the unique virus protein, *vpx* in HIV2 that encodes p15 the virus protein x, and *tev*, that encodes p26 the tat/rev protein[1]. The main three proteins of HIV are Reverse Transcriptase, Integrase and Protease. These three play a major role in therapeutic drug design. RT of HIV is a multifunctional enzyme that converts the single-stranded viral RNA genome into dsDNA that is integrated into the host genome by the viral enzyme integrase. Without reverse transcriptase, the viral genome would not be able to incorporate into the host cell, resulting in failure to replicate. HIV-1 integrase (IN) is a multidomain enzyme which is required for the integration of viral DNA into the host genome.

1.5 CHALLENGES IN THE CURRENT THERAPEUTIC REGIMEN FOR HIV

Currently used synthetic drugs develop resistance as the virus strains continue to evolve along with the serious side effects, cell toxicity and the high cost for the life long treatment, the need of the hour is the development of more potential drugs which can overcome this drug resistance.

Antibiotics often build resistance in our body, which could cause antibiotics to become less effective. The longer the treatment for antibiotics, the more damage to the body's immune system. Some antibiotics may also have side effects

1.6 MARINE NATURAL PRODUCTS AS ANTIVIRAL DRUGS

60% of the available drugs in the market is the contribution of nature. Marine organisms act as a potential source for the development of novel drugs. The marine species offered by our marine resources accounts for a total of half of the global biodiversity. The reason to choose marine is because they are prone to live in a competitive environment where they are forced to develop specific and active molecules which is necessary for their survival compared to the terrestrial environment. These bioactive molecules can be exploited for the development of novel antiviral drugs. 70% of the marine metabolites is from marine corals, sponges and microorganisms.[3].

The major barrier for the development of the marine compounds are the complexity of its structure, optimization required for its formulation, ADMET properties, scale up issues and its feasible supply[1].

The two strategies adopted for the development of marine derived antiviral drugs are: 1) screening of the extracts from different strains, 2) direct extraction of bioactive molecules and their purification and validation following the drug development protocol[3].

1.7 INTEGRASE

Integrase which is a 32 kDa(288 aa) protein is encoded by the end of pol gene. It is released by the viral protease-mediated cleavage and is produced as part of the Gag-Pol polypeptide precursor.[5]

HIV-1 integrase being a multidomain enzyme act as the means for the integration of viral DNA into host genome. It is considered as one of the targets for therapeutic drug design.

Integrase is much needed for retroviral replication also it has no functional analogue in the host.[6]

The major step in viral replication is the integration of the reverse-transcribed HIV cDNA into the host DNA. The initial DNA breaking and joining reactions for cDNA integration is catalyzed by the virus-encoded integrase protein.

There are three domains in Integrase which are the N-terminal domain with a His2 Cys2 motif which chelates zinc, the core domain with the catalytic DDE motif that is necessary for its enzymatic activity, and the C-terminal domain with an SH3-like fold that binds DNA nonspecifically.

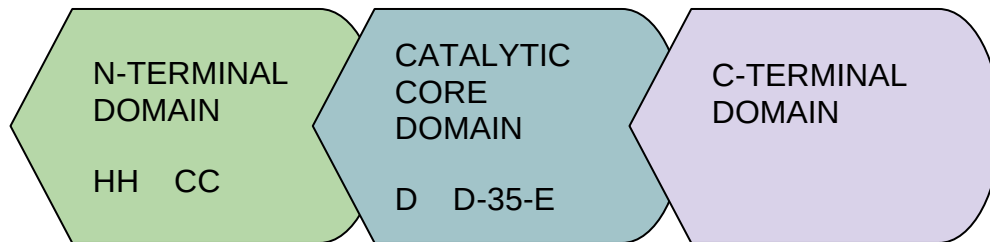


FIG 1.7 Domains of integrase

The three domains of integrase are:

The **N-terminal domain** of HIV-1 integrase contains a preserved combination of His and Cys residues, a motif almost like the metallic element zinc-coordinating residues of zinc fingers. Though this domain will bind with metallic element zinc, its structure is completely totally different from that of zinc fingers. It consists of 3 α -helices. It consists of SH3 fold, though there's no relationship in terms of its function with the SH3 domains of different proteins. The N-terminal domain favours protein multimerisation which is the key process in integration. The multimeric nature of integrase is indicated by the factors such as Zn^{2+} which also stimulate the specified Mg^{2+} dependent activity of the integrase.

The **catalytic core domain** contains the invariant triad of acidic residues, the D,D-35-E motif, comprising residues Asp64, Asp116, and Glu152 within the case of HIV-1 integrase. It has been proposed that coordination of divalent metal ions to these residues plays a key role in catalysis. Moreover, the residues comprising the active site region exhibit considerable flexibility, this suggests that the binding of DNA substrate is required to impose the precise configuration of residues that is required for catalysis. These structures of retroviral integrases belong to a superfamily of poly nucleotidyl transferases.

The **C-terminal domain** binds dna nonspecifically. As a result the sites of integration into target dna are comparatively nonspecific. However, several cross linking- studies counsel that the c-terminal domain interacts with a subterminal just inside the viral DNA end. The

c-terminal of retroviral integrase could play an analogous role to that of the site specific DNA binding domain of transposases, this is also known as the subterminal region.[7]

1.7.1 INTEGRASE INHIBITORS

The motive of integrase inhibitors is to prevent the ability of HIV to make integrase that is required for replication. HIV inhibitors along with other medication is used to control the infection. These are usually used in combination with other drugs and is the initial medication used for HIV treatment. The usage of integrase inhibitors was approved in 2007 by the U.S. Food and Drug Administration (FDA). The integrase inhibitors that are currently used are raltegravir, dolutegravir, elvitegravir (available in combination with other drugs), bictegravir (available in combination with other drugs)

Integrase inhibitors is known to have less side effects. The reason behind this is that integrase inhibitors work on the virus itself rather than on the HIV infected cells. Few of its side effects are diarrhea, nausea, fatigue, headache, insomnia, dizziness. Severe skin reactions and widespread inflammation are among the serious side effects caused by integrase inhibitors. Often medications become less effective also the virus become resistant to the drugs altogether which demands for the need of new drugs to be discovered.[8]

1.8 REVERSE TRANSCRIPTASE

Reverse transcriptase (RT) is one of the three enzymes encoded by the HIV-1, the etiological agent of AIDS. The HIV-1 RT is a heterodimer composed of 66kDa subunit p66 and p51 kDa subunit. Drugs that are used for inhibiting the RNA- and DNA-dependant DNA polymerase activity of RT are the main components of highly active antiretroviral therapy (HAART), which has dramatically reduced mortality and morbidity of people living with HIV-1/AIDS in developed countries. RT inhibitors belong to two main classes acting by distinct mechanisms. The 3' hydroxyl group on ribose is absent in NRTIs (Nucleoside RT inhibitors) and hence they act as chain terminators. Non-NRTIs bind into a hydrophobic pocket close to the polymerase active site and inhibit the chemical step of the

polymerization reaction. Mutations in the RT-coding region selected during treatment with nucleoside analogues confer resistance through different mechanisms: (i) altering discrimination between nucleoside RT inhibitors and natural substrates (dNTPs) or (ii) increase in the phosphorolytic activity of RT's, which in the presence of a pyrophosphate donor (usually ATP) allow the removal of chain-terminating inhibitors from the 3' end of the primer. Both mechanisms are implicated in multi-drug resistance. The mutations that confer resistance to nucleoside or non nucleoside RT inhibitors or the one's that affect RNase H activity and also the amino acid substitutions that interfere with the binding of template primer can be modulated by the excision reaction.[9]

1.8.1 NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS(NNRTIs)

NNRTIs is a keystone for the treatment of HIV. These are essential because of their high specificity, uniqueness in their antiviral activity and low toxicity. The inhibition by NNRTI's occur by binding to the RT's allosteric site called as the NNRTI binding pocket(NNIBP), which is approximately 10 Å away from the active site of DNA polymerase. The various conformations by which the NNRTI bind to NNIBP are "butterfly", "horseshoe" or "U" with a scaffold at the centre and two wings.[10]

HIV DRUG RESISTANCE REPORT 2019

The cause for HIV drug resistance (HIVDR) is the mutation/s in the genetic structure of HIV that affects the ability of a specific drug or combination of drugs to block replication of the virus. The emergence of drug-resistant virus has led to the partial or full inactivity of the antiretroviral(ARV) drugs. WHO commonly classify HIVDR into three main categories.

1. Acquired HIV drug resistance (ADR), this is developed because of viral replication in the presence of ARV drugs.
2. Transmitted HIV drug resistance (TDR), this is detected among ARV naive drug people with no history of ARV drug exposure.
3. Pretreatment HIV drug resistance (PDR), refers to the resistance that is detected among ARV drug-naïve people initiating ART or people with

previous ARV drug exposure initiating or reinitiating first-line ART.

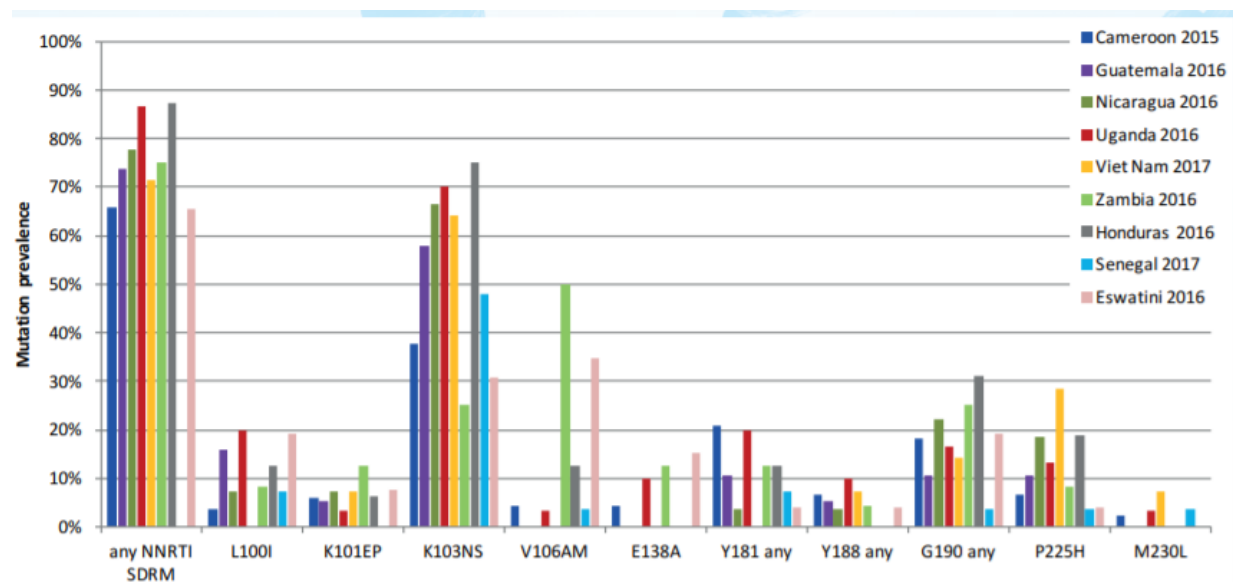


Fig. 1.8.1 NNRTI mutations associated with acquired drug resistance by country (early time point survey, 12 months)

2. LITERATURE REVIEW

1.Teng. et al, 2020 has reviewed that viruses belong to a class of pathogenic microorganisms that threaten the health and safety of human life worldwide. Since a long time, the treatment of viral infections has been slow to develop, and only a few antiviral drugs have been used clinically. Marine-derived microorganisms can produce active substances with more novel structures and unique functions compared with those of the terrestrial environments. [11]

2.VonRanke NL et.al ,2020 studied that the natural products can be used as an alternative to anti-HIV drug design.As the isolation of natural product is a major difficulty,the best way to study these compounds is by molecular modeling.The various aspects that helped them in this study is the structure activity relationship,molecular docking and teh molecular dynamic studies that directed the study of these marine natural products for their anti-HIV activity.These compounds were evaluated by molecular docking, and they showed interaction at the allosteric site when a conformational change was made in the enzyme.The *in silico* ADMET simulations predicted that these compounds have a high potential to be developed successfully as a drug. [12]

Few marine natural compounds that exhibited anti-HIV activity:

Compound	Extracted from	Functions
marine dolastanes (1–3) and secodolastane diterpenes (2)	brown alga namely <i>Canistrocarpus cervicornis</i>	This inhibits HIV-1 replication in a dose- dependent manner [1]
Equisetin	fungus <i>Fusarium heterosporum</i>	Equisetin inhibit 3' end processing and also strand transfer as well as disintegration[13]
Polyacetylenetriol	Mediterranean sponge <i>Petrosia sp.</i> ,	It inhibits RNA- and DNAdependent DNA polymerases. It showed selective inhibition of the DNA polymerase activities of HIV-1 RT, without affecting the RNase H activity of HIV-1 RT.[14]
Thalassiolins A–C	Caribbean seagrass <i>Thalassia testudinum</i>	It displays in vitro inhibition of the integrase catalyzed strand transfer reaction and an antiviral activity.It has a favorable binding mode at the catalytic core domain of HIV-1 integrase.[15]
AcDa-1 & Da-1:	the two diterpenes obtained from <i>Dictyota menstrualis</i>	The restriction of the RNA- dependent DNA- polymerase activity of HIV- 1 RT was observed during dose-dependent fashion. It was justified that diterpenes inhibit HIV-1 RT and consequently virus replication. [16]

Dolabelladienetriol	isolated from brown algae marine algae <i>Dictyota paffii</i>	This blocks the synthesis and integration of HIV-1 provirus and completely revokes the viral replication in primary cells. This Compound also blocks HIV-1 replication at the post transcriptional step.[17]
Fucans: sulfated fucan Galactofucan	<i>Dictyotaceae</i>	It showed a noticeable inhibitory effect on reverse transcriptase. [18]
Diphlorethohydroxycarmalol	marine brown alga, <i>Ishige okamura</i>	This compound shows the inhibitory effects on both the HIV-1 reverse transcriptase and integrase.[19]
N-carboxymethylchitosan N,O-sulfate:	polysaccharide derived by a random sulfation reaction of N-carboxymethyl chitosan	It has the ability to inhibit the propagation of HIV-1 in CD4+/immune cells, they prevent the invasion of HIV-1 by inhibiting the viral adsorption to the CD4 receptor.[19]
Lamellarin	Marine mollusk <i>Lamellaria</i>	It is also inhibited integration in vitro by authentic HIV-1 replication intermediates isolated from infected cells.[20]

4-hydroxyquinoline-3-carbohydrazide	<i>pipestelide C</i>	4-hydroxyquinoline-3-carbohydrazide that may serve as the metal binding pharmacophore. [21]
Integric acid	acylated eremophilane sesquiterpenoid	They belong to a unique class of integrase inhibitors. These combinations of reactions are catalyzed by pre-integration compounds derived from HIV-1-infected cells.[22]
Avarol	marine sponge <i>Dysidea cinerea</i>	This repairs damage after exposure to DNA-damaging agents and inhibitors of this enzyme can potentiate cytotoxic activity[23]
Robustaflavone	<i>Rhus succedanea</i>	In in vitro assay, they exhibit strong inhibition of the polymerase of HIV-1 reverse transcriptase[24]
Polycitone A	isolated from an ascidian, <i>Polycitor sp.</i>	It is a powerful inhibitor of reverse transcriptase and also retroviruses as an inhibitor of DNA polymerases.[24]
Phomasetin	marine fungi <i>Fusarium heterosporum</i> and <i>Phoma sp</i>	It exhibits <i>in vitro</i> inhibitory activity against the

		integrase enzyme of HIV-1.[25]
Depsidone	Lichens	Its core structure is necessary for the inhibition of aromatase [26]
Alginic Acid	alginate	“911” drug inhibits the viral replication of HIV by reducing the activity of reverse transcriptase, decreasing the virus adsorption and improving the defense functioning of the host cells[27]
Eckol 6,6'-bieckol: 8,8'-bieckol & 8,4'''-dieckol	<i>Ecklonia cava</i>	They exhibit HIV-1 reverse transcriptase and protease inhibiting activity.[28]
Rezishanone	Caribbean sponge <i>Agelas dispar</i>	act as HIV entry inhibitors [29]

Table 2.1 Marine natural compounds that exhibited anti-HIV activity

3. METHODOLOGY

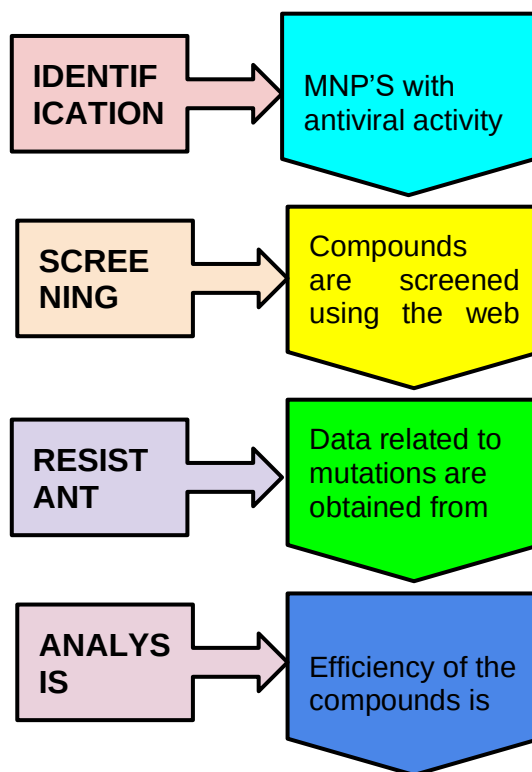


FIG 3.1 Methodology

3.1 PUBCHEM

PubChem is a public repository containing information about various chemical substances and their biological activities. It was launched in 2004 as one of the components of the Molecular Libraries Roadmap Initiatives of the US National Institutes of Health (NIH). This database has now rapidly grown to a key chemical information resource that serves scientific communities in many areas such as cheminformatics, chemical biology, medicinal chemistry and drug discovery.

PubChem is one of the largest repositories of chemical information that is available publically. It covers ten thousand unique protein target sequences and has more than 157 million chemical substance descriptions provided by various depositors also a count of 60 million chemical structures which are unique and nearly descriptions of one million for biological assays. The entire data of PubChem is inter-linked to three databases i.e Substance, Compound and BioAssay. The depositor-contributed information are stored in

Substance from where the unique chemical structures are obtained and stored in Compound database. The descriptions of biological assays on chemical substances are stored in BioAssay database. SID, CID, AID i.e SubstanceID, CompoundID and AssayID are the respective primary identifiers. [30]

3.2 PROTEIN DATA BANK

PDB was established in 1971, accounts as the global archive of experimentally determined 3D structure data of biological macromolecules and their complexes. It is one of the first open source database in biological science. The PDB has been managed by the Worldwide Protein Data Bank organization which includes its four PDB partners they are, the RCSB PDB, PDBj, PDBe and BMRB. is an international consortium that collaboratively manages the deposition, validation, biocuration, of ~11,000 new PDB entries annually and open access dissemination of 3D macromolecular structure data. Data from 2017, it has around 130,000 entries. The 3D atomic coordinates of more than 144 000 structural models of proteins, DNA/RNA as well as their complexes with metals and small molecules and also the related experimental data are present in the PDB Core Archive ensuring disaster recovery of PDB data and coordinating weekly updates. The PDB Core Archive has the structure and experimental data/metadata, which evolve as data/metadata incorporated by the wwPDB from new experimental techniques and structure determination methods. The PDB act as the repository for experimental data, associated metadata, and 3D-atomic level structural models that are derived from three different methods: crystallography, electron microscopy (3DEM) and nuclear magnetic resonance spectroscopy (NMR). [31,32]

3.3 SWISS ADME

A drug is said to be effective only at a particular concentration that should be in the bioactive form and must reach the drug target for the biological events that have been expected to occur. Drug development involves properties of absorption, distribution,

metabolism and excretion (ADME). SwissADME is a web tool that gives free access for physicochemical properties, pharmacokinetics, drug-likeness and medicinal chemistry friendliness and also methods such as the BOILED-Egg, iLOGP and Bioavailability Radar. Easy input as well as user understandable output is generated which can be predicted not only by specialists but also the non experts from computational chemistry or chemoinformatics. [33]

The compounds were screened by considering various properties like:

- Physicochemical properties -Molecular weight between 150 and 500 g/mol. , TPSA : (Topological polar surface area) between 20 and 130 Å².
- Lipophilicity - here the log value between “– 0.7 and + 5.0”
- Water solubility - here the class compound should be moderately soluble
- Drug Likelihood - here the bioavailability score should be a minimum of 0.55
- Pharmacokinetics - here the GI absorption should be “High”.
- Medicinal Chemistry - here the value of synthetic accessibility should be in between 1 to 10

Table 3.2 SWISS ADME RESULTS FOR INTEGRASE COMPOUNDS

COMPOUND	Equisetin	4-hydroxyquinoline-3-carbohydrazide	Integric acid	Depsidone	Phomasetin
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MOLECULAR WEIGHT (g/mol)	373.49	203.20	430.53	212.20	413.55
TPSA(Å²)	77.84	87.98	97.74	35.53	77.84
LIPOPHILICITY	2.93	0.65	3.94	2.78	3.74
WATER SOLUBILITY (class)	Moderately soluble	Moderately soluble	Moderately soluble	Moderately soluble	Moderately soluble
DRUG LIKELINESS (bioavailability)	0.56	0.55	0.56		0.56
PHARMACOKINETICS (GI absorption)	High	High	High	High	High
MEDICINAL CHEMISTRY(synthetic accessibility)	5.16	1.74	5.51	0.55	5.56

3.3 SWISS ADME RESULTS FOR REVERSE TRANSCRIPTASE COMPOUNDS

COMPOUND	Dolabelladienetro I	rezishanone	AcDa-1	Da-1
MOLECULAR WEIGHT (g/mol)	322.48	348.43	360.49	318.45

TPSA (Å ²)	60.69	83.83	60.44	54.37
LIPOPHILICITY	3.05	2.85	3.98	3.70
WATER SOLUBILITY (class)	Moderately soluble	Moderately soluble	Moderately soluble	Moderately soluble
DRUG LIKELINESS (bioavailability)	0.55	0.56	0.55	0.55
PHARMACOKINETIC S (GI absorption)	high	high	high	high
MEDICINAL CHEMISTRY (synthetic accessibility)	5.67	5.99	5.87	5.74

3.4 HIV DRUG RESISTANCE DATABASE

The HIV Drug Resistance Database was formed in 1998 with HIV reverse transcriptase (RT) and protease sequences by genotype-treatment correlations. In 2001, the genotype-phenotype correlations i.e the correlations between genotype and in vitro susceptibility test results were added. In 2004, the genotype-outcome correlations i.e correlations between genotype and the virological response to a new treatment regimen were added. The correlations that are present in the database have been obtained from nearly 600 published studies. [34]

The clinical usage of INSTI started in the year 2007. The latest class of antiretroviral drugs used for the treatment of HIV employs the usage of INSTIs. The preferred first-line

antiretroviral therapy (ART) regimens are INSTIs because of their higher efficiency and tolerability. Raltegravir (RAL) is a first generation INSTI that has shown long term efficiency and safety in the treatment. [35]

Table 3.4 Resistant mutants of INTEGRASE

Sites	Amino acids	Resistant Mutants
66	T	K
92	E	Q
118	G	R
138	E	K,A,T
140	G	S,A,C
143	Y	R,C,H
155	N	H

Table 3.5 Resistant mutants of REVERSE TRANSCRIPTASE

Sites	Amino acid	Resistant mutants
100	L	I
101	K	E, P
106	V	A, M
181	Y	C, I, V
188	Y	L, C, H

3.5 MOLECULAR DOCKING

MTiAutoDock permits to dock compounds into a binding site that is defined by the user or blind docking using AutoDock 4.2. MTiAutoDock employ the Lamarckian genetic

algorithm to generate orientations/conformations of the compound. MTiAutoDock is a user friendly and it is most suitable for non-advanced users. It performs docking up to 10 ligands uploaded by the user. The putative druggable pockets can be identified at the protein surface by blind docking. The two formats in which the protein structure can be uploaded are PDB and MOL2. If the protein input is of PDB format then the structure cleaning and preprocessing i.e the removal of heteroatoms and the addition of hydrogens to the structure using MGLTools is performed automatically. Also there is an option in which the user can prepare the protein structure manually and upload in MOL2 format. If the protein input is of MOL2 format then the removal of the solvent molecules as well as the addition of the hydrogen have to be done by user. The binding site are provided by the user as the grid center and dimensions or a list of residues except for blind docking. The ligand is given in SDF or MOL2 file format. MTiAutoDock provides an interactive page that gives the 3D protein structure of the best scored poses for the docked ligands. [36]

3.6 PYMOL

PyMOL is one of the open-source molecular visualization systems which is a molecular graphics tool that is widely accepted for the 3D visualization of electron densities, macromolecules as well as the small molecules which was created and commercialized by Warren Lyford DeLano in DeLano Scientific LLC. PyMOL has the capability to edit molecules, ray tracing, and making movies. This is a Python-based software with various Python plugin tools that has been developed to facilitate the drug design in PyMOL. [37]

4. RESULTS:

4.1 MUTATION STABILITY ANALYSIS

It calculates the effect of mutations on the protein stability and performs combinatorial scanning mutagenesis of protein molecules. Depending on the selected Mutation Sites mode this protocol generates all single, double or triple mutation sites from the set of selected residues or use all of them to create mutants for all combinations of specified

amino-acid substitutions. For each mutant the protocol calculates the difference between the folding free energy of the wild type and mutated structure.

Depending on the electrostatic model, specified by the *pH-dependent Electrostatics* parameter, the calculations can be carried out either in a pH-independent or pH-dependent mode:

- pH-independent mode: All electrostatic energy terms are calculated based on the protonation state of input structure. This method uses GBIM (Generalized Born with Implicit Membrane) approximation to account for the effect of the solvent. The electrostatic terms are approximated as a sum of Coulombic interactions and polar contributions to solvation energy. The energy functional also contains van der Waals interaction energy, a side-chain entropy term and a non-polar surface dependent term.
- pH-dependent mode: The mutation energy is calculated as a function of pH for the defined pH interval. The pH-dependent electrostatic terms are calculated by integration over the proton binding isotherms, derived from fractional protonations of acidic and basic residues that are calculated based on the Residue pK protocol and Calculate Protein Ionization. The remaining terms are the same as in pH-independent method.

The following residue properties are reported:

- Electrostatic Term: The electrostatic contribution to the folding energy difference between the wild type and mutated molecules.
- VDW Term: This contributes to the binding energy differences.
- Entropy Term: This side-chain contribution to the binding energy differences.
- Non-polar Term: This contributes to the binding energy differences.
- Mutation Energy The total free energy difference between the wild type and mutated structures. It is calculated as the weighted sum of the VDW, Electrostatic, Entropy and Non-polar terms.

Notes:

- Negative values of mutation energy correspond to a stabilizing effect of the mutation and vice versa.
- Only standard amino acid residues can be subject to mutation.
- The molecule must be typed with the CHARMM or CHARMM Polar H forcefield. If the molecule is typed with a forcefield different from the one specified in Forcefield parameter, the molecule will be re-typed internally.

Table 4.1 Mutation stability analysis of Integrase

MUTATED AA	SITE	MUTATION ENERGY NEUTRAL	DESTABILIZING	STABILIZING	MUTATION STABILITY ANALYSIS GRAPH
Thr	66	-0.25 (Ala) 0.37 (Lys)	-	-	
Glu	92	-	0.84 (Gln)	-	
Glu	138		0.95(Ala) 1.55(Thr)	-0.50(Lys)	
Gly	140	-0.30 (Cys) 0.03 (Ser) 0.18 (Ala)	-	-	
Tyr	143	0.16 (Cys)	0.59 (Arg) 0.62 (His)	-	
Asn	155	0.08 (His)	-	-	

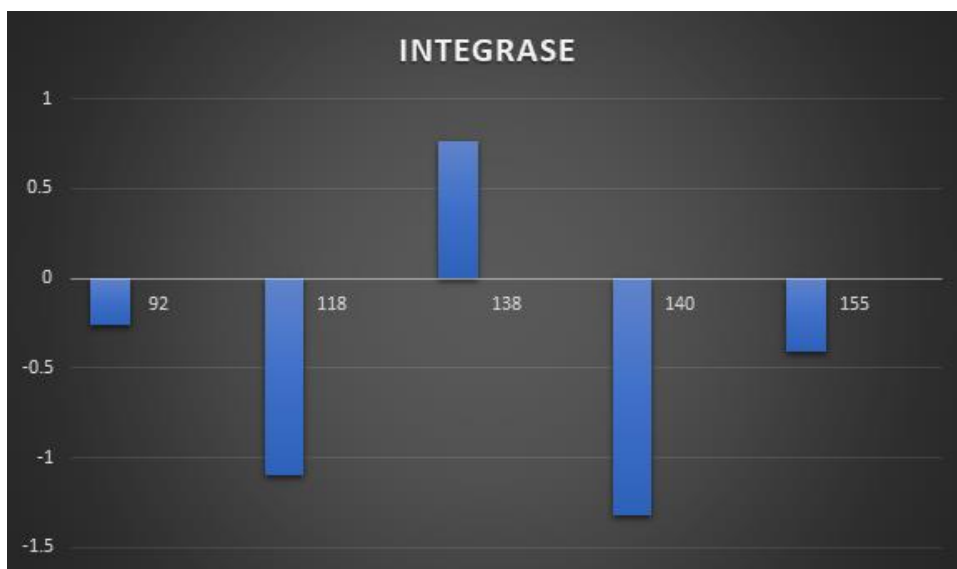


Fig 4.1 Mutation stability analysis of Integrase

Table 4.1.1 Comparison of Depsidone with native and mutated

POSITION	NATIVE	MUTATED
66	-6.76(T)	-6.73(K)
92	-6.76(E)	-5.03(Q)
138	-5.96(E)	10.35(A), -6.74(K), -6.73(T)
140	-3.17(G)	-2.90(A), -6.73(C), -3.43(S)

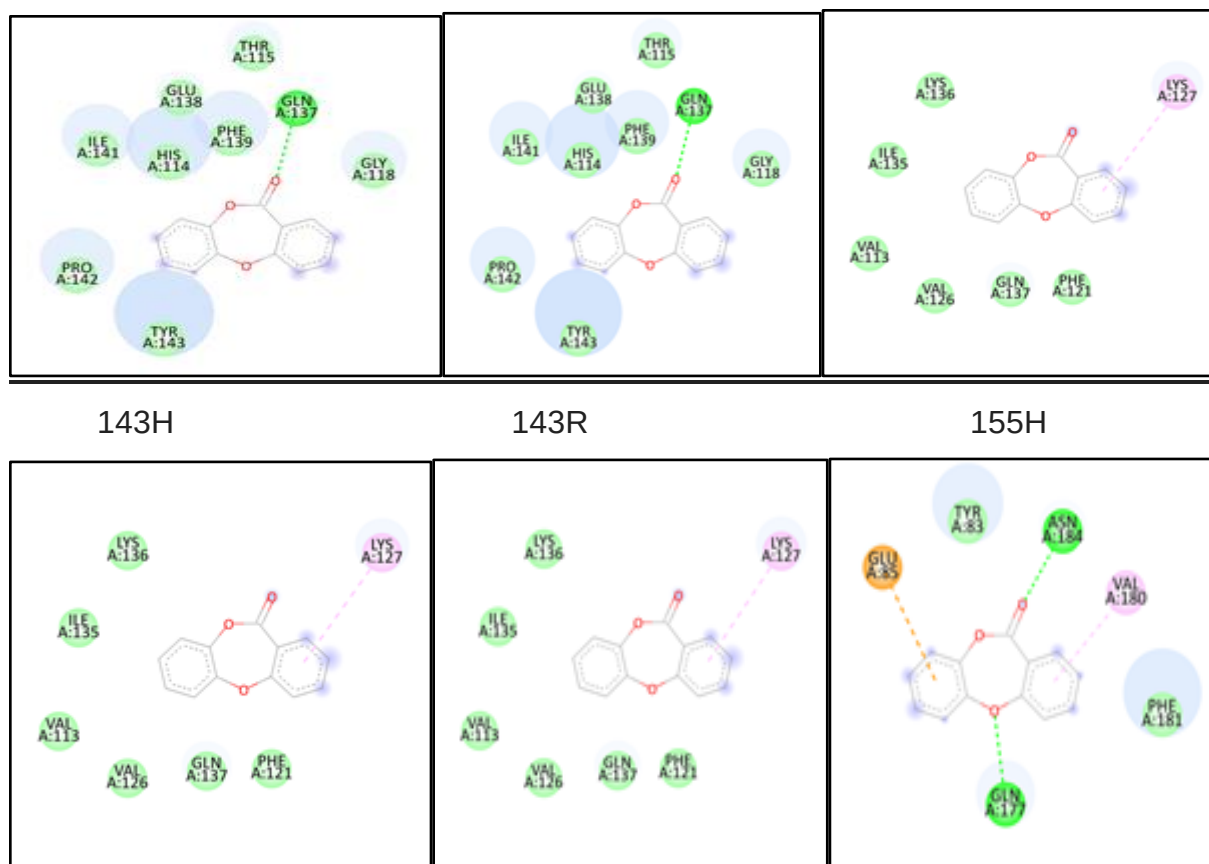


Fig 4.1.1 The fig refers to the two dimensional interaction between the protein(integrase:3lpt) and the ligand depsidone

POSITION	NATIVE	MUTATED
66	-6.90(T)	0.11(K)
92	-3.88(E)	-4.39(Q)

138	47.38(E)	656.67(A), 4.90(K), 450.23(T)
140	56.44(G)	29.16(A), -0.21(S), -6.86(C)
143	5.75(Y)	25.18(C), 1.84(H), -4.80(R)
155	-5.20(N)	-3.37(H)

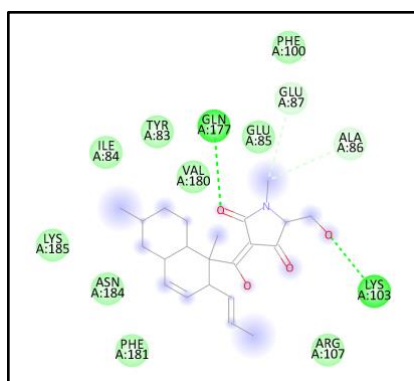
Table 4.1.2 Equisetin

EQUISETIN

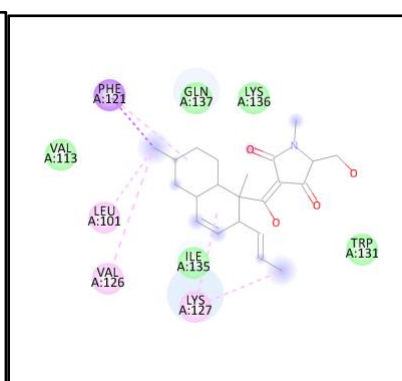
66K

92Q

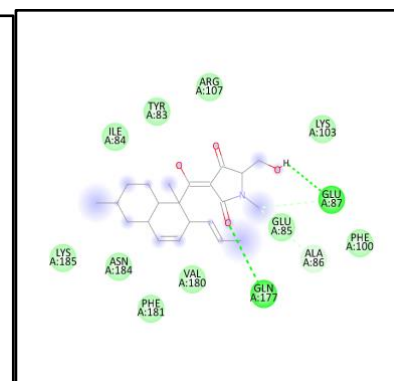
138A



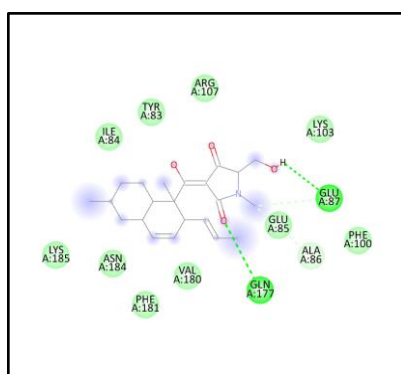
138K



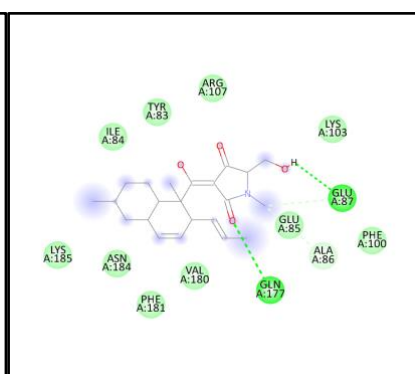
138T



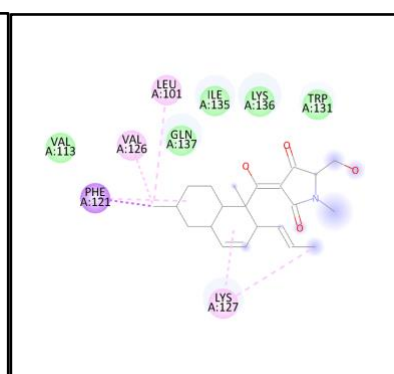
140A



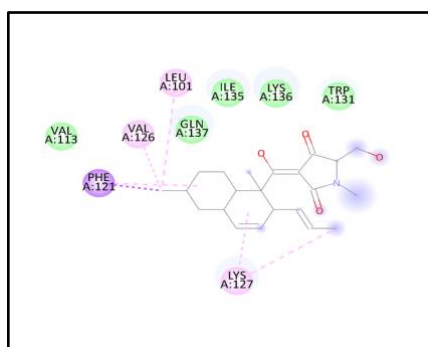
140C



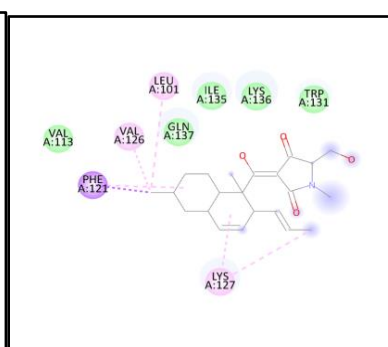
140S



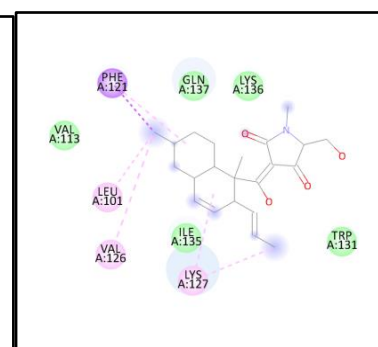
143C



143H



143R



155H

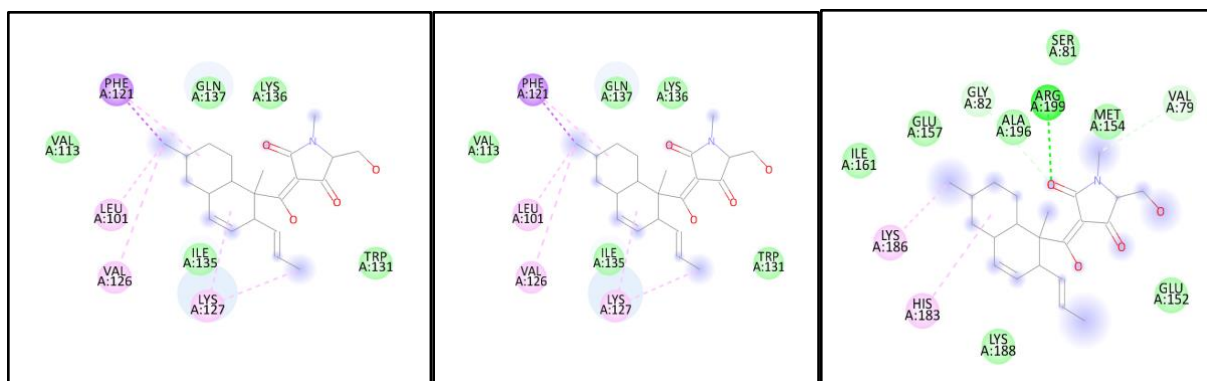


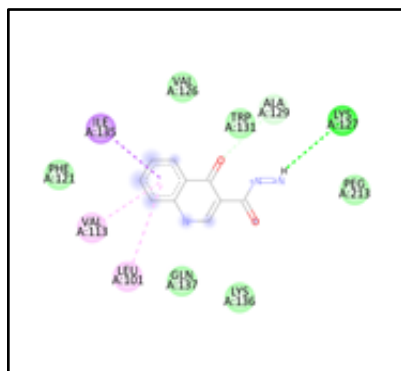
Fig 4.1.2 The fig refers to the two dimensional interaction between the protein(integrase:3lpt) and the ligand equisetin

POSITION	NATIVE	MUTATED
66	-2.98(T)	-3.87(K)
92	-4.04(E)	-5.08(Q)
138	-3.85(E)	6.21(A), -6.09(K), -2.54(T)
140	-2.16(G)	-2.86(S), -2.01(A), -6.1100(C)
143	-3.80(Y)	-4.77(R), -2.83(C), -3.58(H)
155	-4.83(N)	-3.47(H)

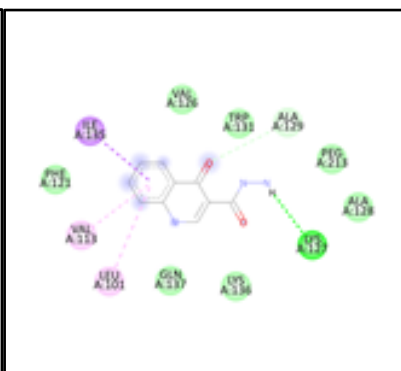
Table 4.1.3 4-hydroxyquinoline-3-carbohydrazide

4-hydroxyquinoline-3-carbohydrazide

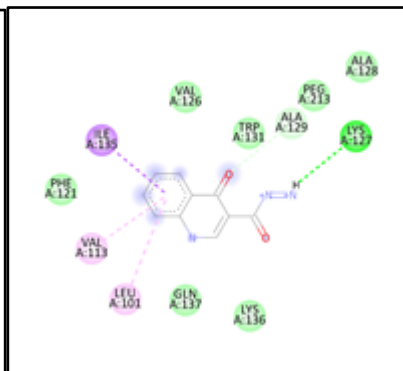
66K



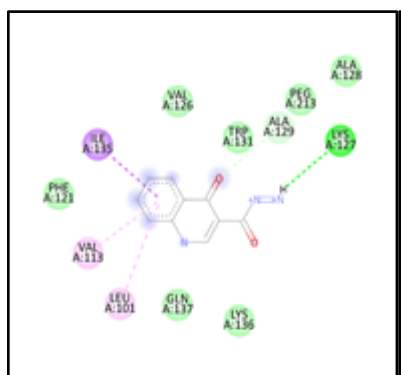
92Q



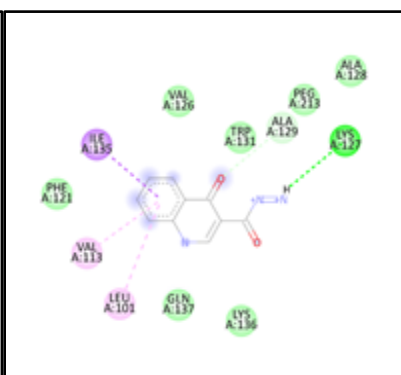
138A



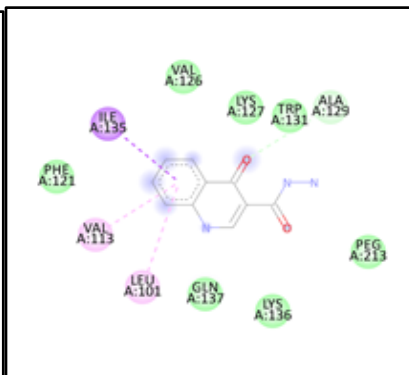
138K



138T



140A



140C

140S

143C

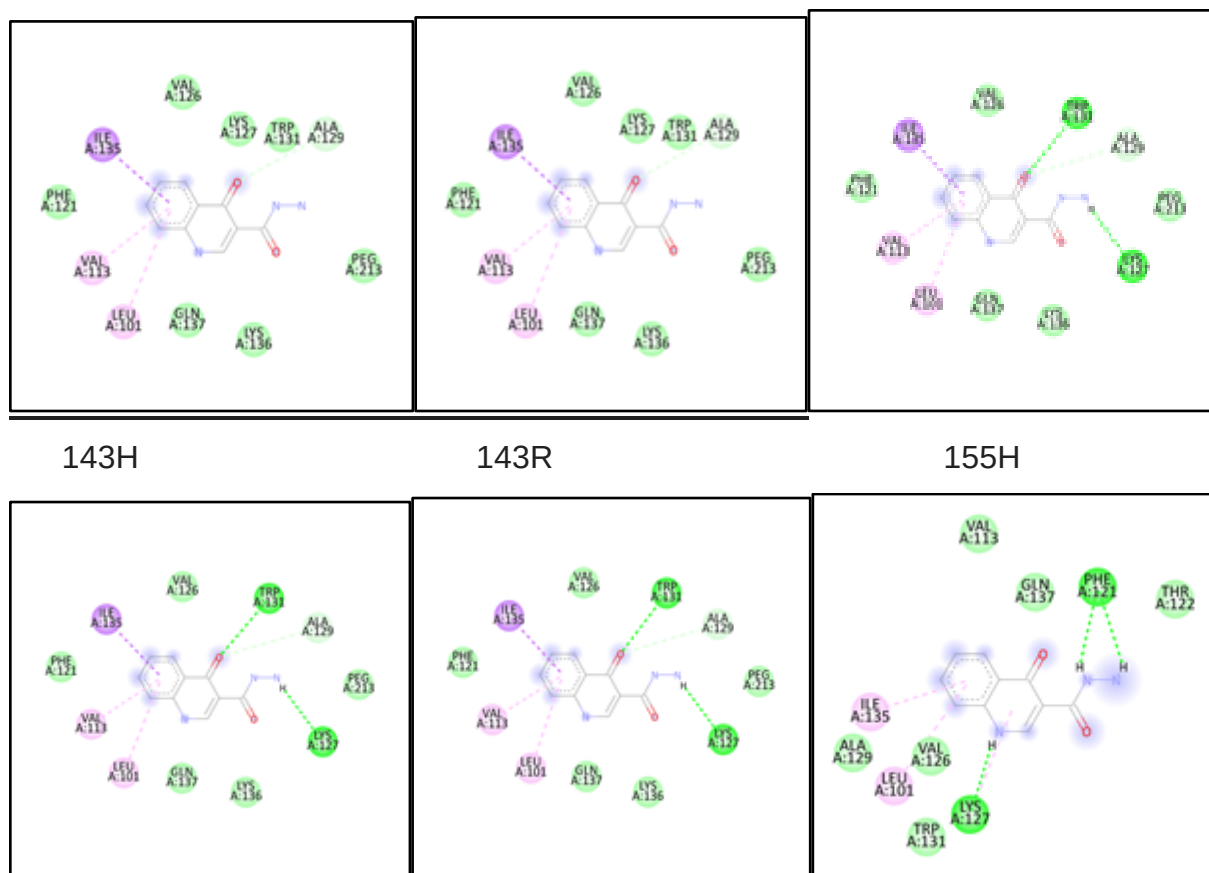


Fig 4.1.3 The fig refers to the two dimensional interaction between the protein(integrase:3lpt) and the ligand 4-hydroxyquinoline-3-carbohydrazide

POSITION	NATIVE	MUTATED
66	1370.00(T)	-7.51(K)
92	-2.49(E)	-2.44(Q)

138	172.56(E)	5050.00(A), 2900.00(T), -8.11(R)
140	280.31(G)	-7.01(C), 689.88(A), 166.84(S)
143	7.67(Y)	1330.00(C), 10.80(H), -2.43(R)
155	7.00(N)	21.51(H)

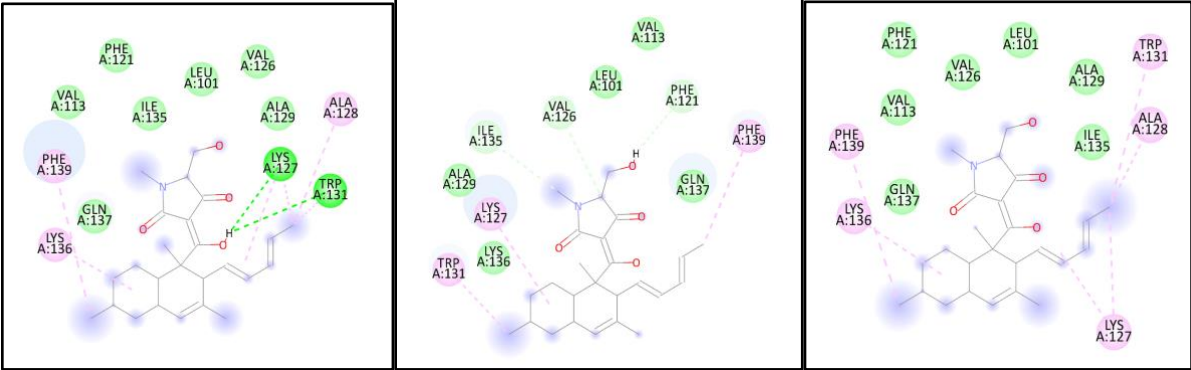
Table 4.1.4 Phomasetin

PHOMASETIN

66K

92Q

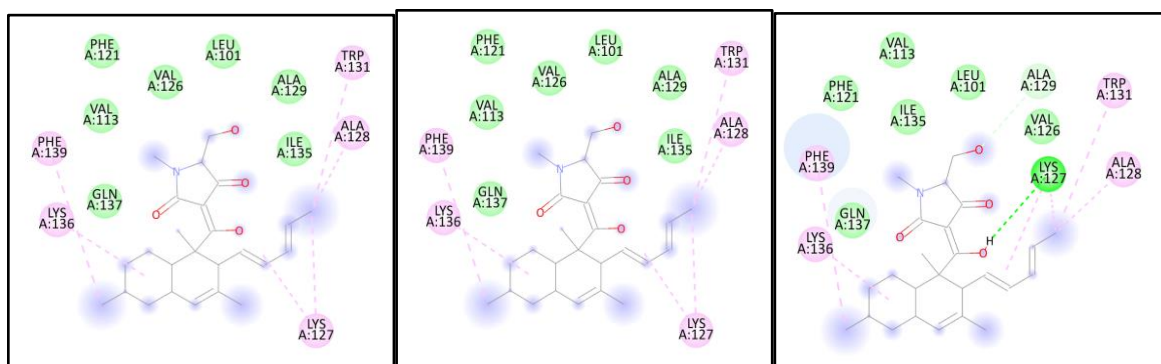
138A



138K

138T

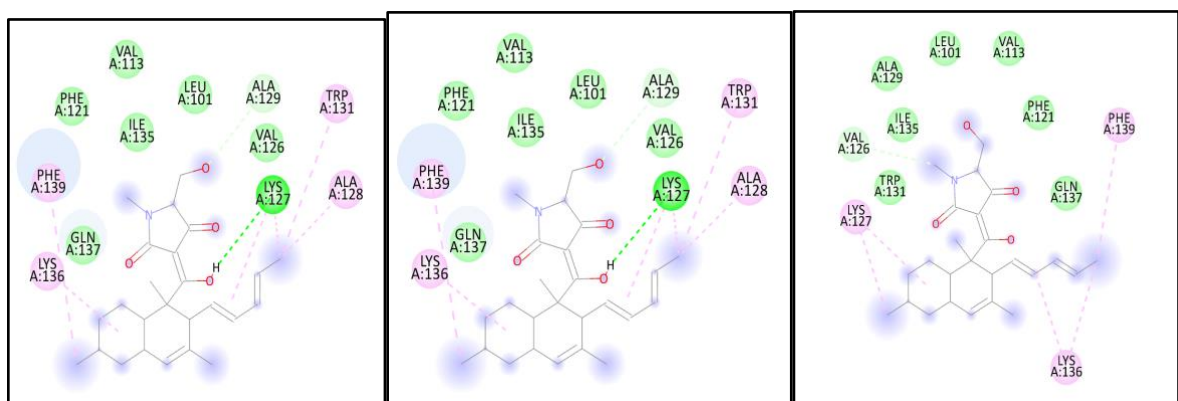
140A



140C

140S

143C



143H

143R

155H

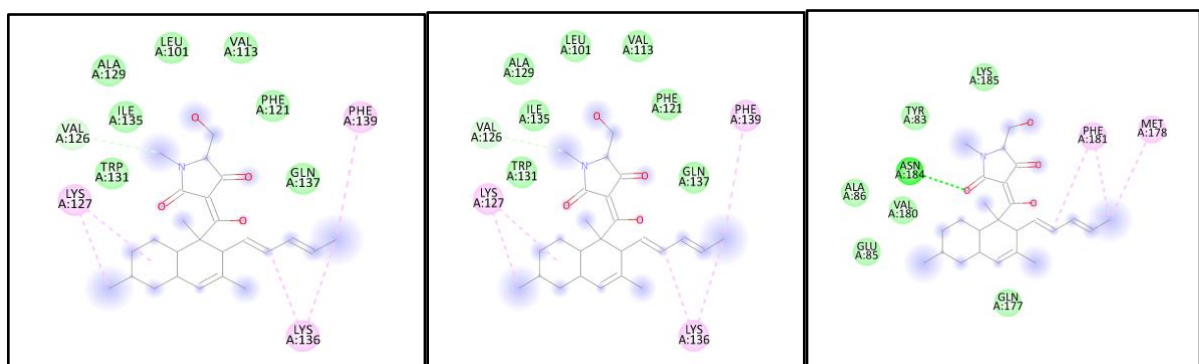
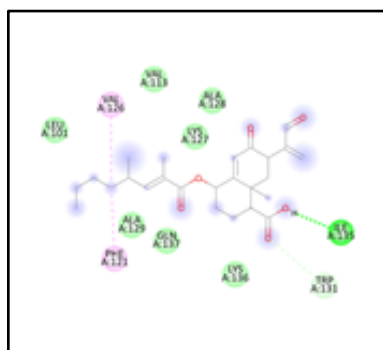


Fig 4.1.4 The fig refers to the two dimensional interaction between the protein(integrase:3lpt) and the ligand phomsetin

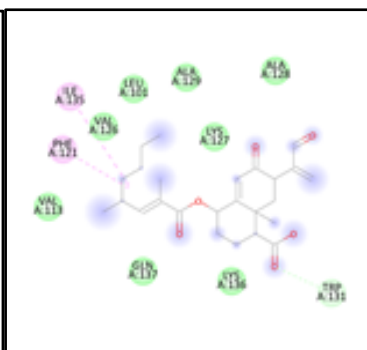
POSITION	NATIVE	MUTATED
66	126.11(T)	0.10(K)
92	-2.85(E)	-4.70(Q)
138	-5.93(E)	609.37(A), 269.80(T), 8.39(K)
140	24.19(G)	8.27(A), 2.65(S), -7.88(c)
143	0.11(Y)	-4.42(R), 25.41(C), -0.1(H)
155	-1.55(N)	7.48(H)

Table 4.1.5 Integric acid

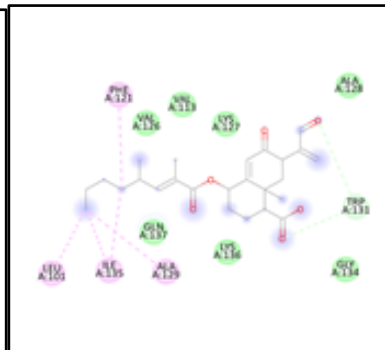
66K

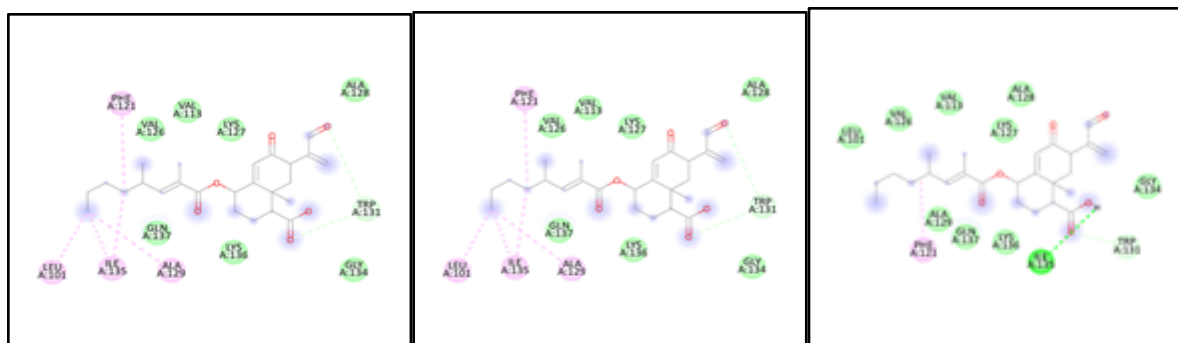


92Q



138A

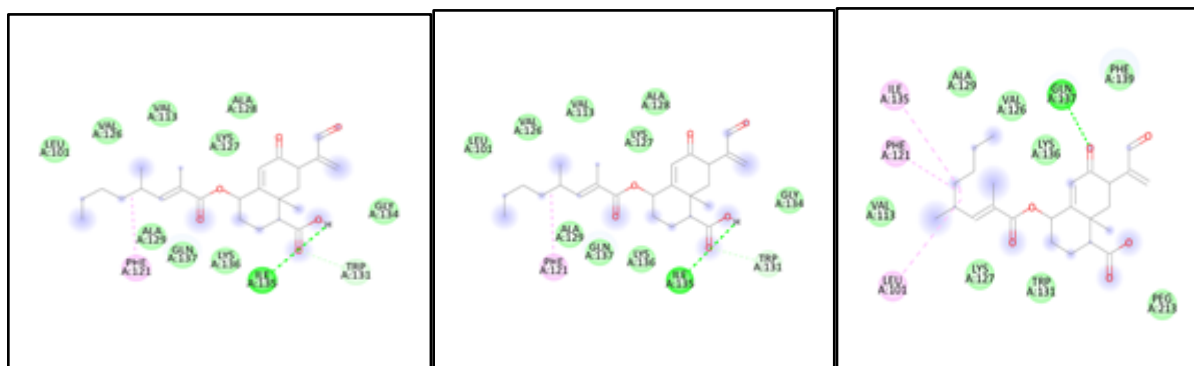




140C

140S

143C



143H

143R

155H

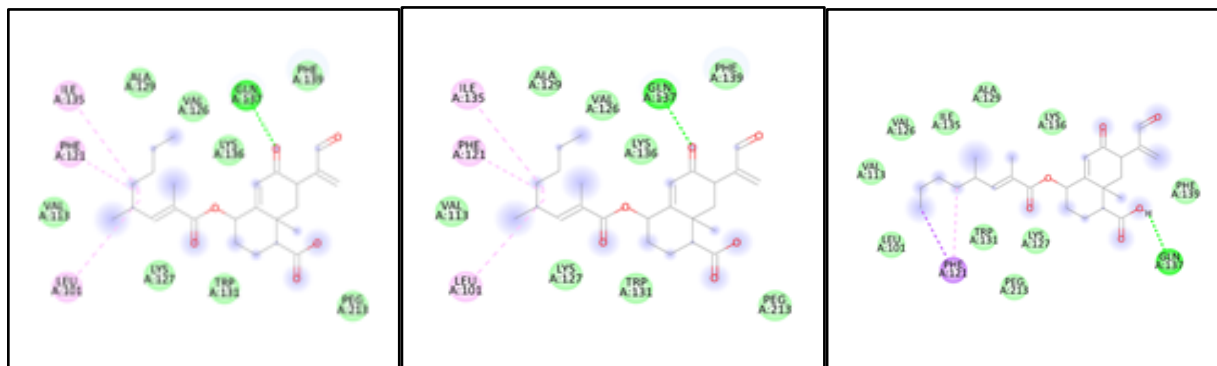


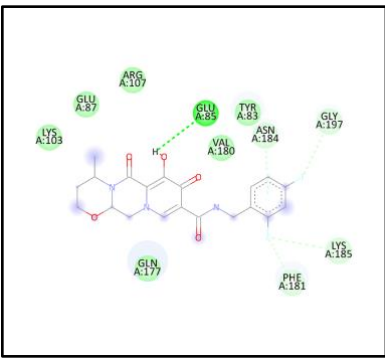
Fig 4.1.5 The fig refers to the two dimensional interaction between the protein(integrase:3lpt) and the ligand integric acid

POSITION	NATIVE	MUTATED
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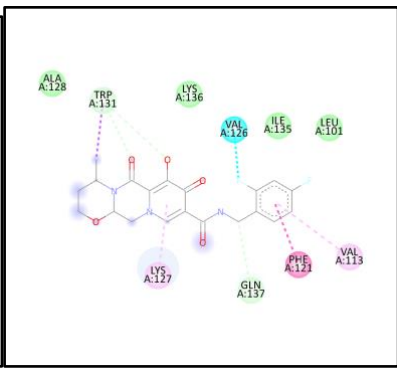
66	25100.00(T)	396.87(K)
92	-3.19(E)	-6.82(Q)
138	4520.00(E)	73900.00(A), 2290.00(K), 65600.00(T)
140	106.00(G)	102000.00(A), -6.81(C), 39700.00(S)
143	-2.98()	69700.00(C), -1.95(H), -4.88(R)
155	26.09	17.14(H)

Table 4.1.6 Dolutegravir(fda)

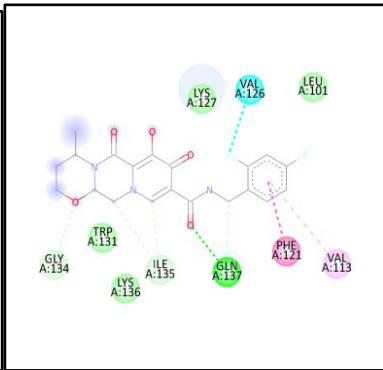
66K



92Q



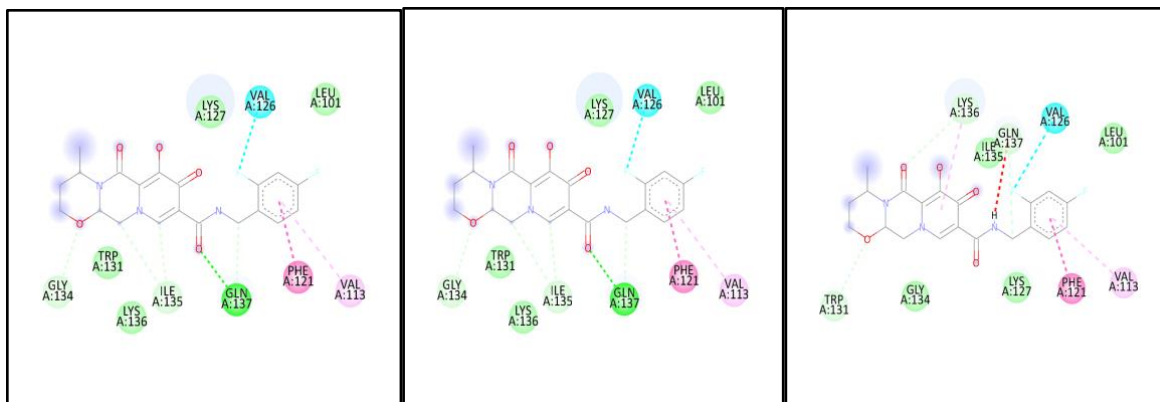
138A



138K

138T

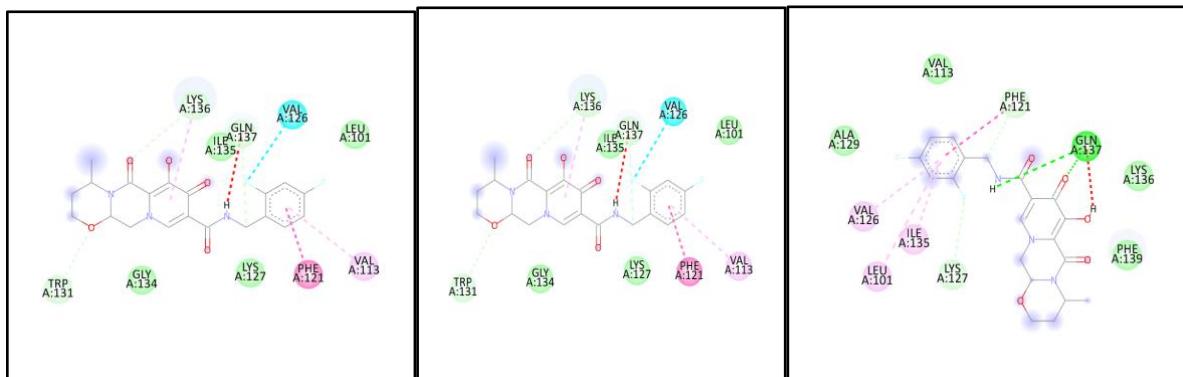
140A



140C

140S

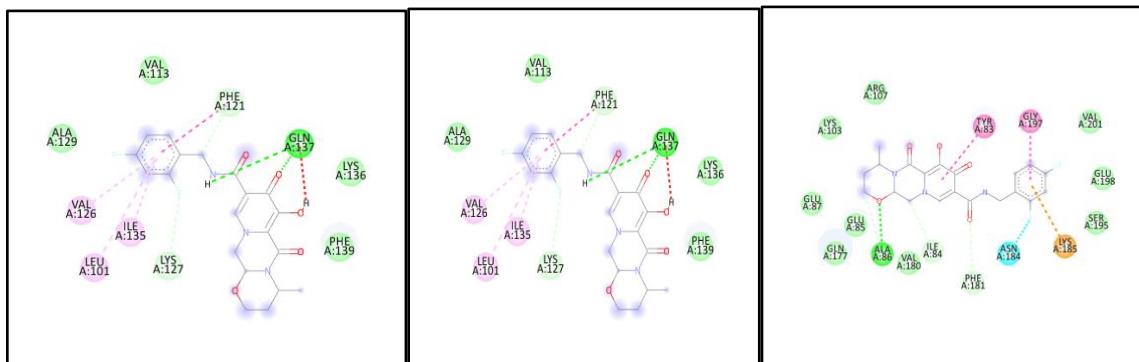
143C



143H

143R

155H



Interactions

	van der Waals		Pi-Cation
	Conventional Hydrogen Bond		Pi-Pi T-shaped
	Carbon Hydrogen Bond		Amide-Pi Stacked
	Halogen (Fluorine)		

Fig 4.1.6 The fig refers to the two dimensional interaction between the protein(integrase:3lpt) and the ligand Dolutegravir

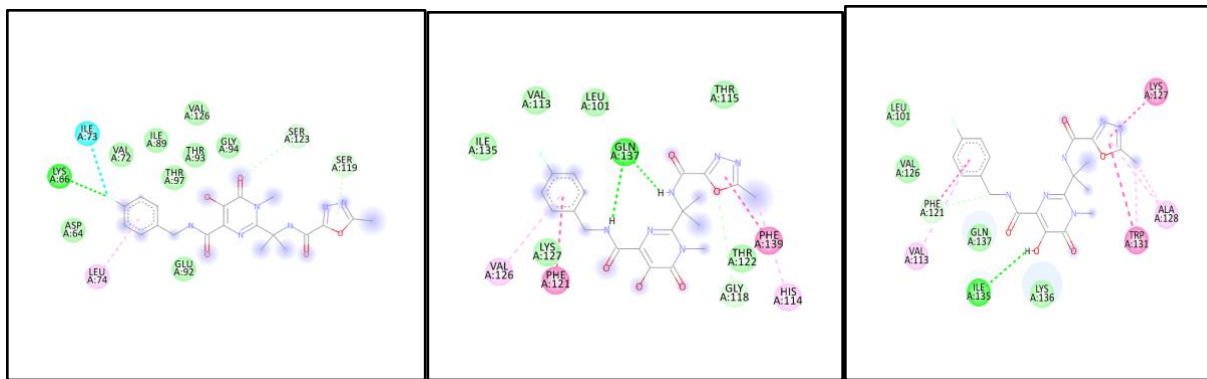
POSITION	NATIVE	MUTATED
66	98.70(T)	-0.69(K)
92	-5.37(E)	-5.15(Q)
138	78.74(E)	111.04(A), 11.65(K), 310.55(T)
140	-1.12(G)	-1.90(A), -6.26(S), -6.10(C)
143	-3.27(Y)	-4.66(R), -1.58(C), -3,23(H)
155	-7.7(N)	-6.00(H)

Table 4.1.7 Raltegravir (fda)

66K

92Q

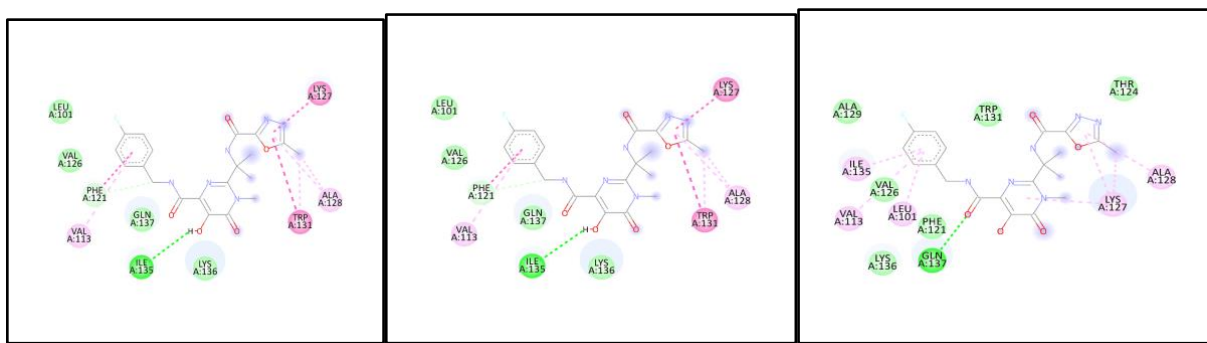
138A



138K

138T

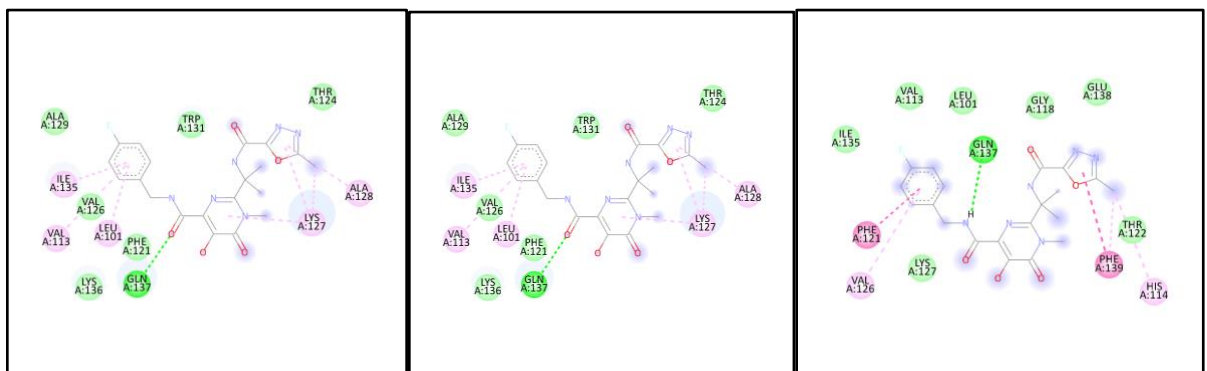
140A



140C

140S

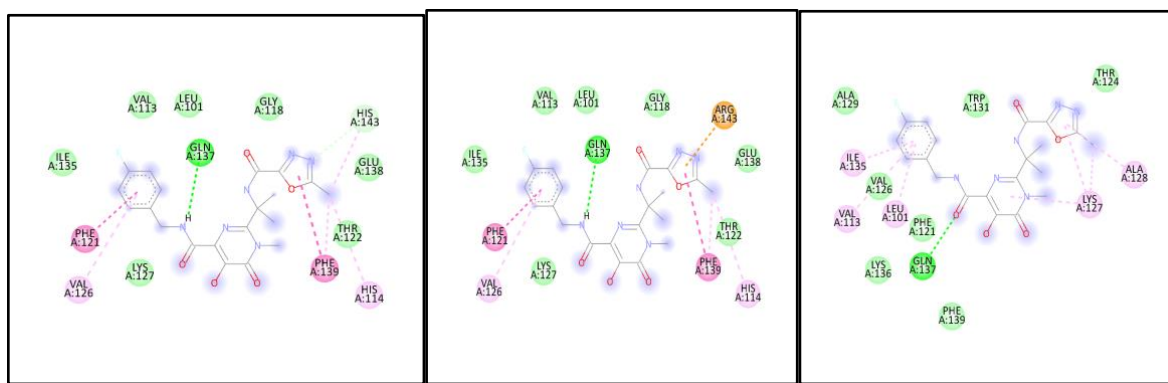
143C



143H

143R

155H



Interactions

	van der Waals		Pi-Cation
	Conventional Hydrogen Bond		Pi-Pi T-shaped
	Carbon Hydrogen Bond		Amide-Pi Stacked
	Halogen (Fluorine)		

Fig 4.1.7 The fig refers to the two dimensional interaction between the protein(integrase:3lpt) and the ligand Raltegravir (fda)

Table 4.2 Mutation stability analysis of Reverse Transcriptase

MUTATED AA	SITE	MUTATION STABILITY NEUTRAL	DESTABILIZING	STABILIZING	MUTATION STABILITY ANALYSIS GRAPH
Leu	100	-	-	-0.54(Ile)	

Lys	101	0.32(Glu)	-	-1.02(Pro)	
Lys	103	0.22(Asn)	3.20(Ser)	-	
Val	106	-	0.6(Met) 2.06(Ala)	-	
Tyr	181	-	0.55(Ile) 0.73(Cys) 0.99(Val)	-	

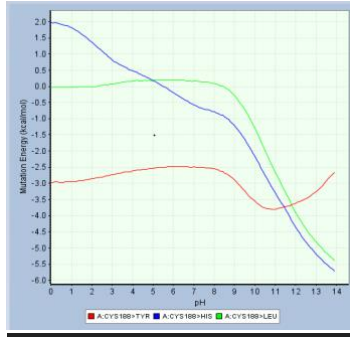
Cys	188	0.14(Leu)	-	-2.51(Tyr) -0.68(His)	
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Table 4.2 REVERSE TRANSCRIPTASE

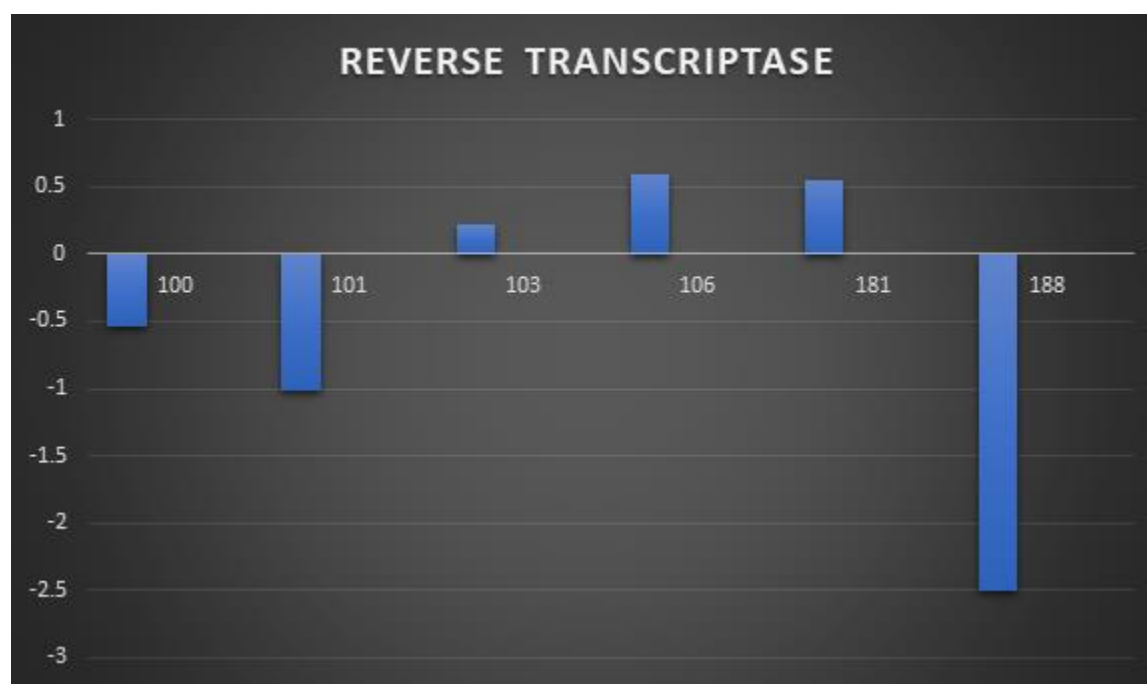
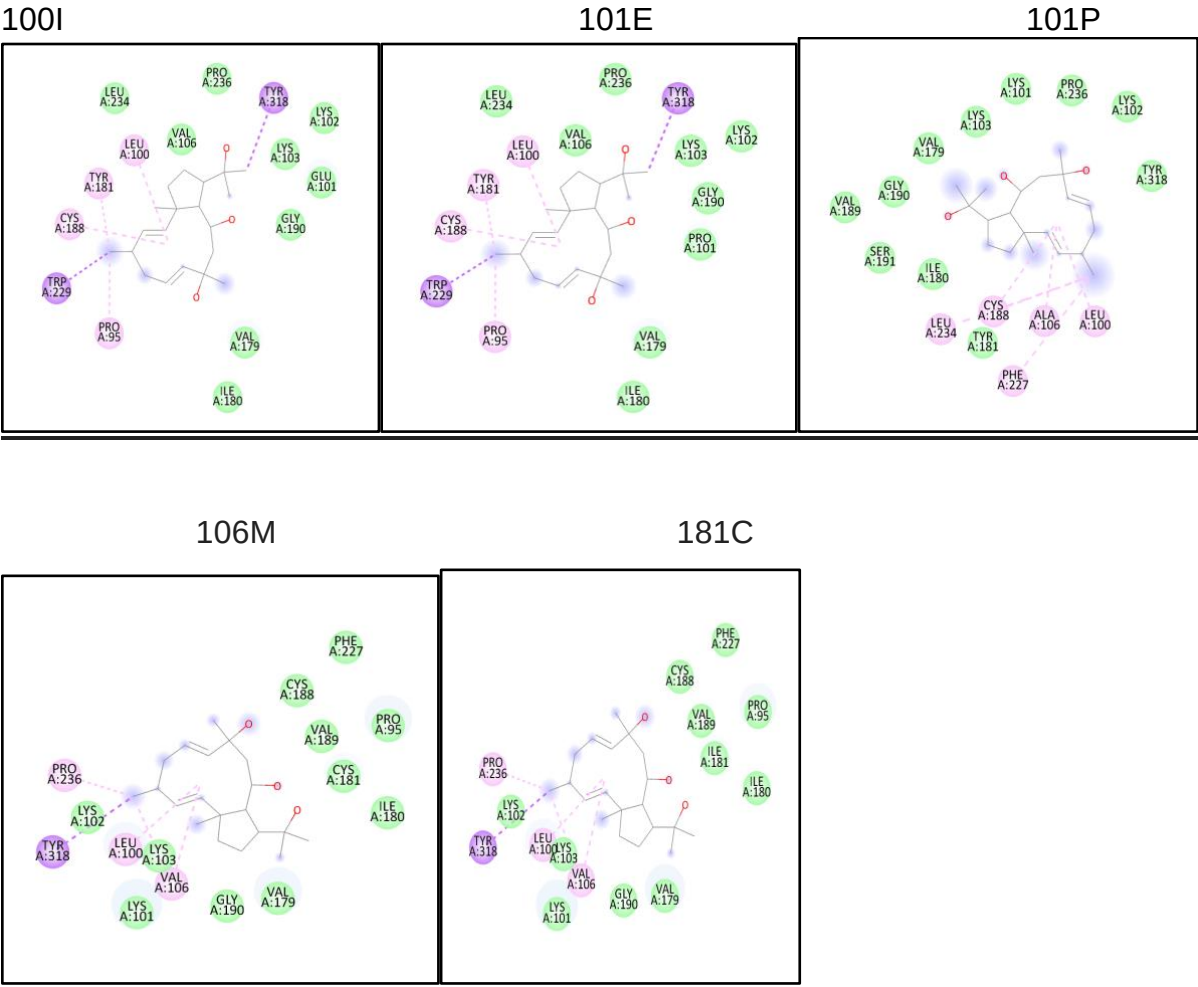


Fig 4.2 Mutation stability analysis of Reverse Transcriptase

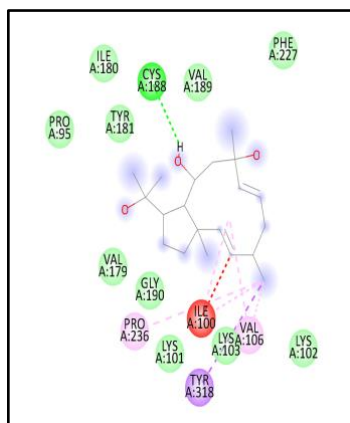
POSITION	NATIVE	MUTATED
100	-7.26(K)	13.38(I)
101	2.76(K)	-3.61(E),

		28.06(P)
106	282.71(V)	208.16(M)
181	128.82(Y)	103.98(I)
188	-5.51(Y)	-7.16(C) , -7.45(C)

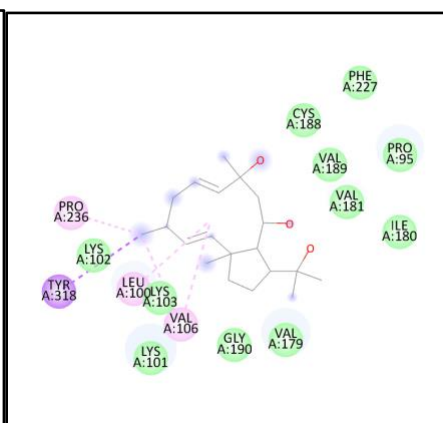
Table 4.2.1 Dolabelladienetriol



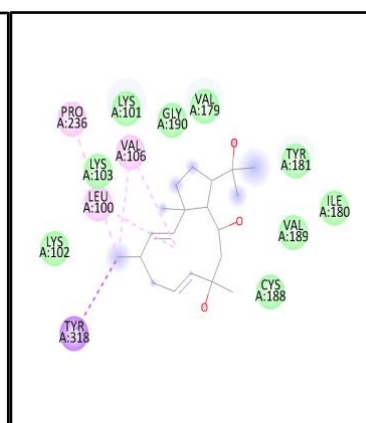
181I



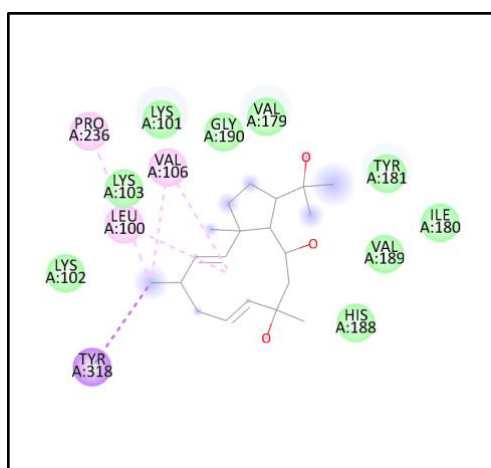
181V



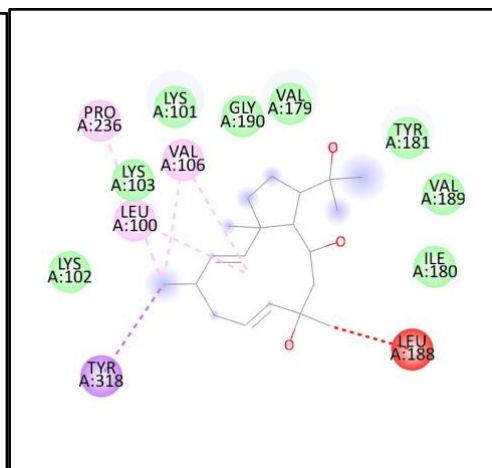
188C



188H



188L



Interactions

- van der Waals
- Unfavorable Bump
- Pi-Sigma

- Pi-Pi T-shaped
- Alkyl
- Pi-Alkyl

Interactions

- van der Waals
- Conventional Hydrogen Bond
- Carbon Hydrogen Bond
- Halogen (Fluorine)

- Pi-Cation
- Pi-Pi T-shaped
- Amide-Pi Stacked

Fig 4.2.1 The fig refers to the two dimensional interaction between the protein(reverse transcriptase:1jlf) and the ligand Dolabelladienetriol

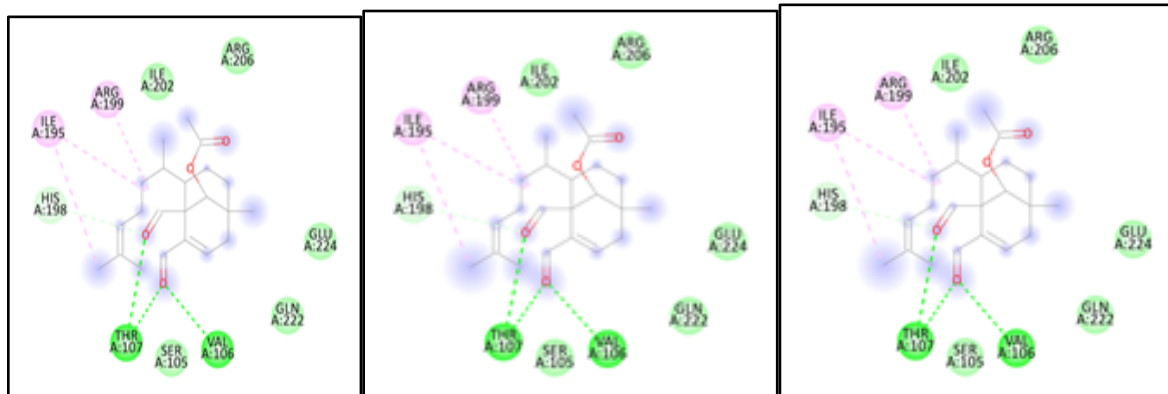
POSITION	NATIVE	MUTATED
100	-5.49(K)	9.56(I)
101	-2.04(K)	-4.81 (E), 94.78(P)
106	544.01(V)	97.74(A), 127.65(M)
181	16.16(Y)	-5.96(C), 23.58(I), 130.62(V)
188	-4.44(Y)	27.28(C), 8.63(H), 10.64(L)

Table 4.2.2 AcDa-1

100I

101E

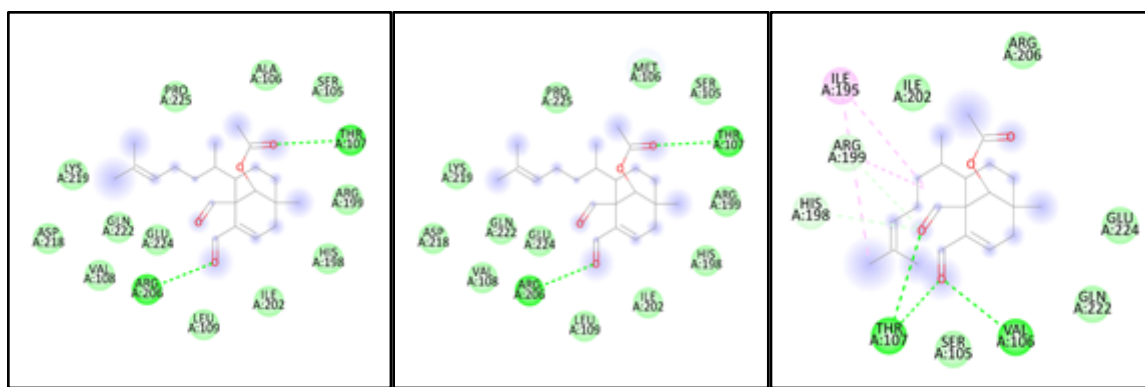
101P



106A

106M

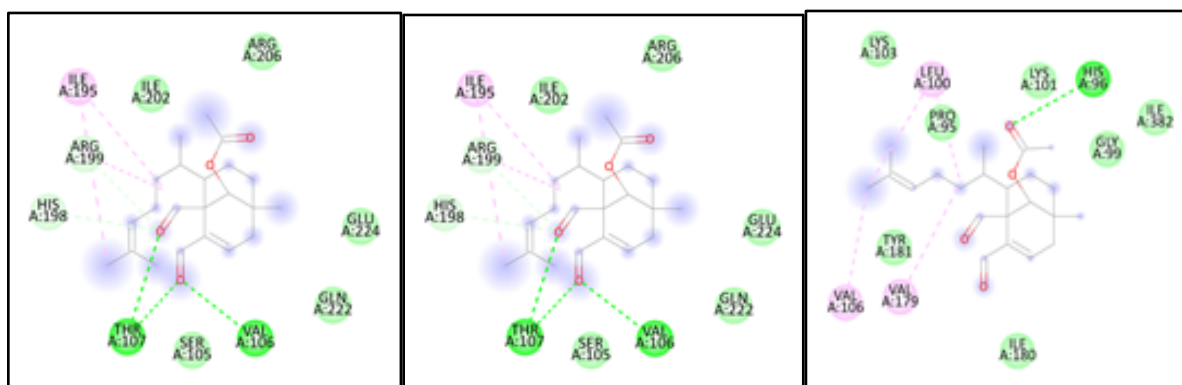
181C



181I

181V

188C



188H

188L

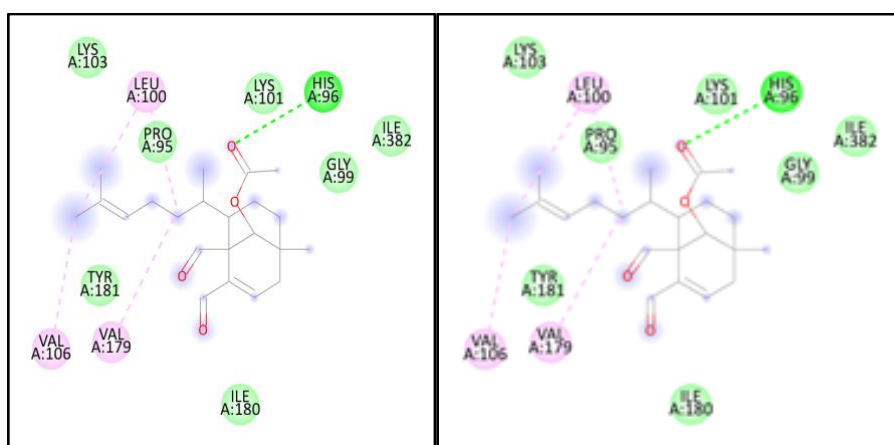
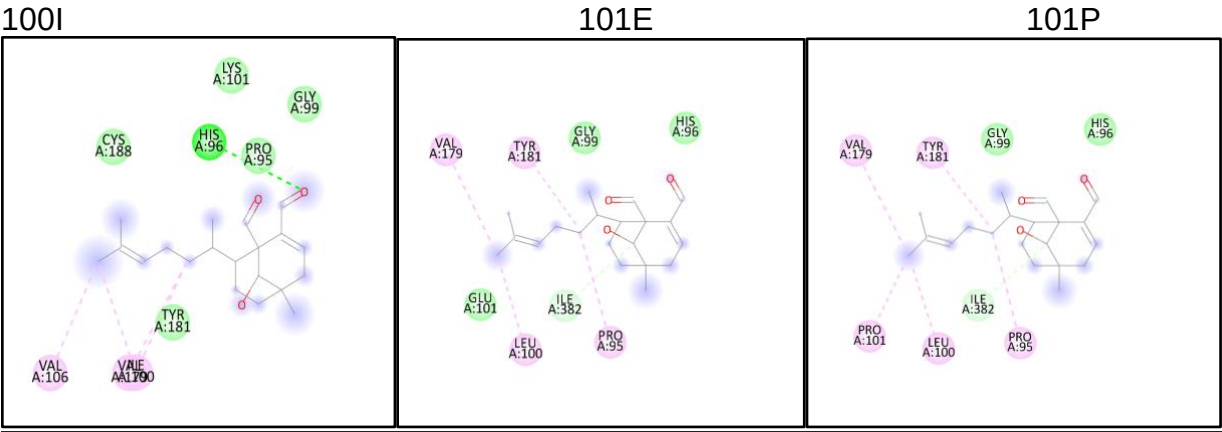


Fig 4.2.2 The fig refers to the two dimensional interaction between the protein(reverse transcriptase:1jlf) and the ligand AcDa 1

POSITION	NATIVE	MUTATED
100	-6.71(K)	11.41(I)
101	-5.07(K)	-5.43, (E), 22.90(P)
106	143.79(V)	-5.96(A), -5.96(M)
181	11.80(Y)	86.05(C), 8.48(I), 48.27V)
188	-4.88(Y)	16.14(C), -4.58(H), 7.21(L)

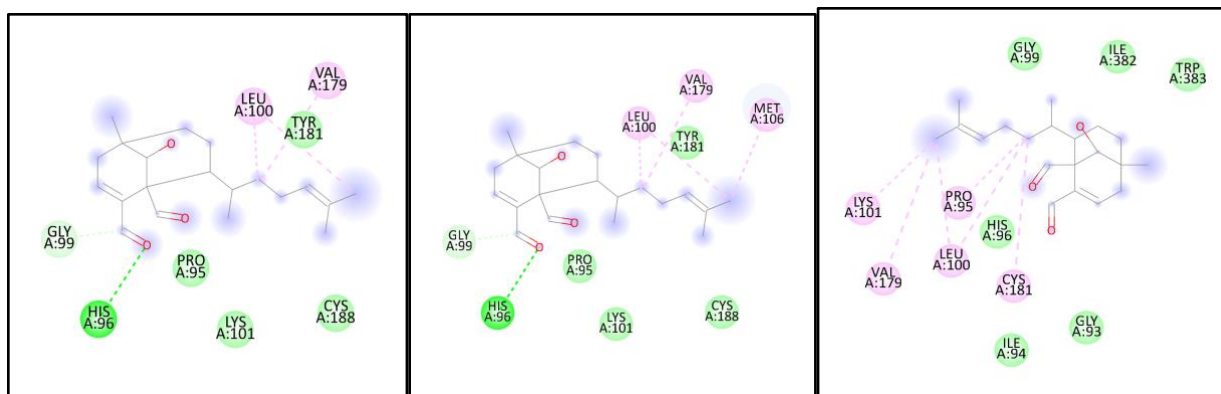
Table 4.2.3 Da 1



106A

106M

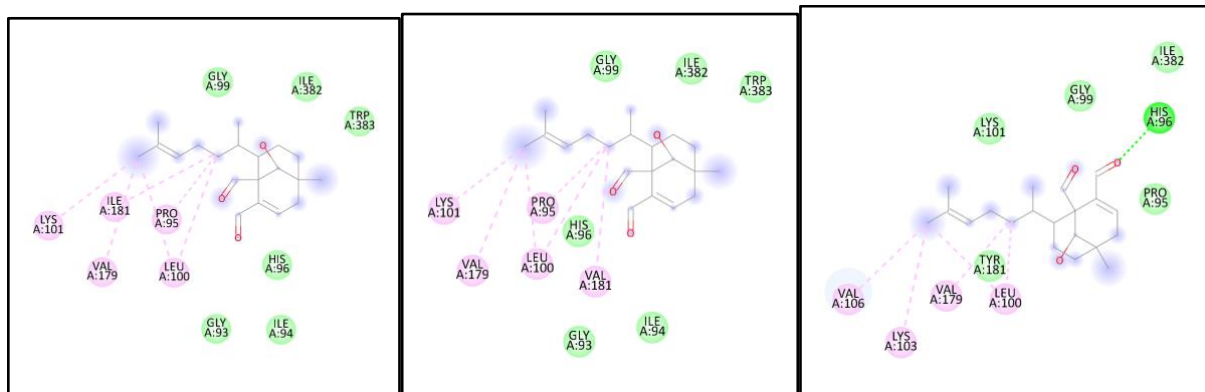
181C



181I

181V

188C



188H

188L

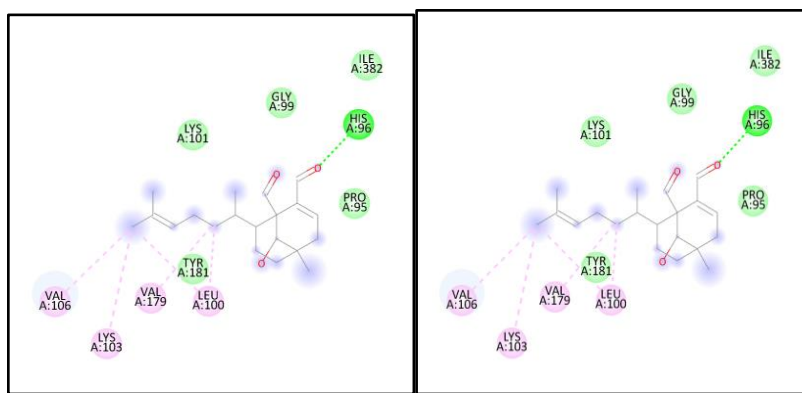
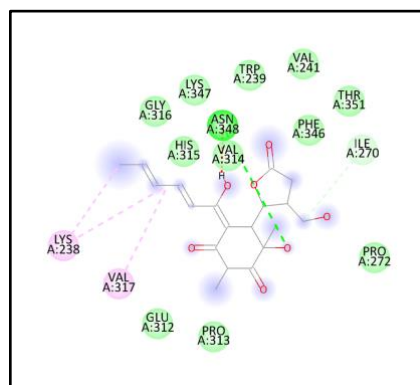


Fig 4.2.3 The fig refers to the two dimensional interaction between the protein(reverse transcriptase:1jlf) and the ligand Da 1

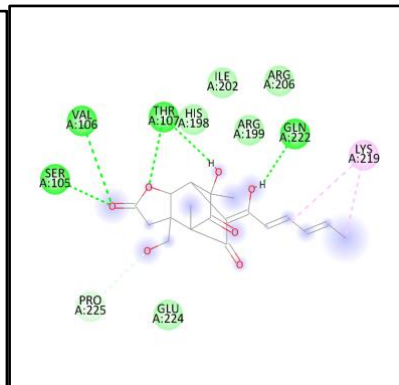
POSITION	NATIVE	MUTATED
100	-7.03(K)	11.08(I)
101	-7.27(K)	-6.32(E), 85.81(P)
106	920.15(V)	551.15(A), 142.56(M)
181	15.67(Y)	210.90(C), 121.12(V), -6.66(I)
188	-6.66(Y)	28.46C), 4.36(L), -5.97(H)

Table 4.2.4 Rezishanone

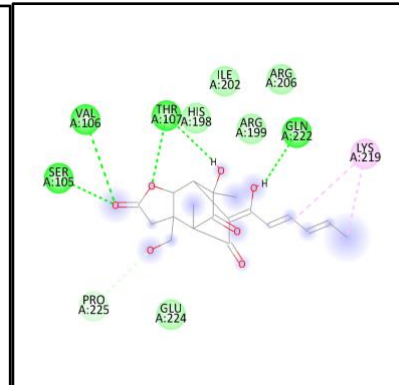
100I



101E



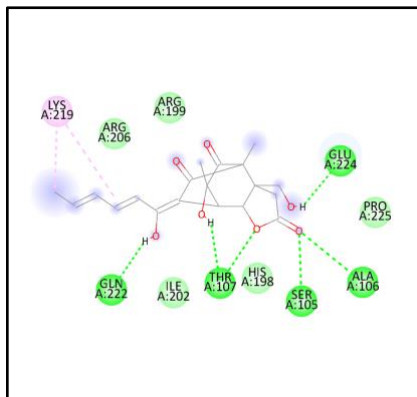
101P



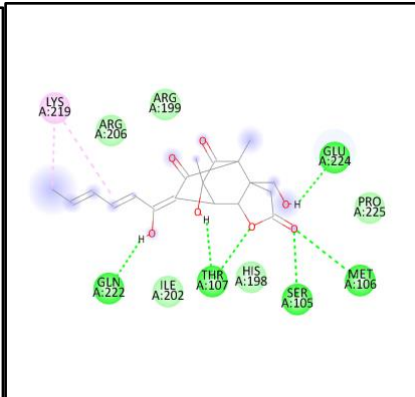
106A

106M

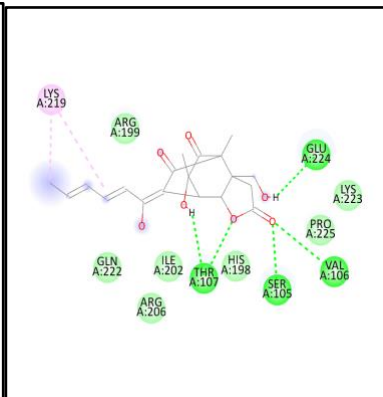
181C



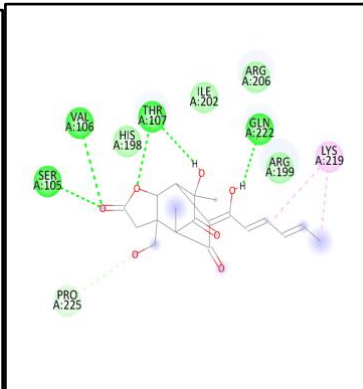
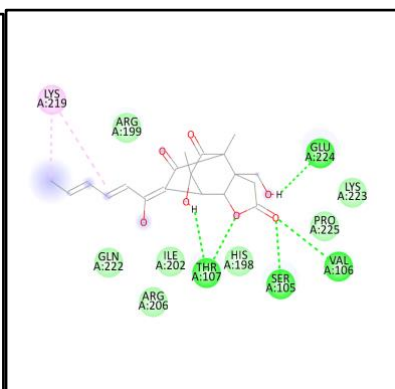
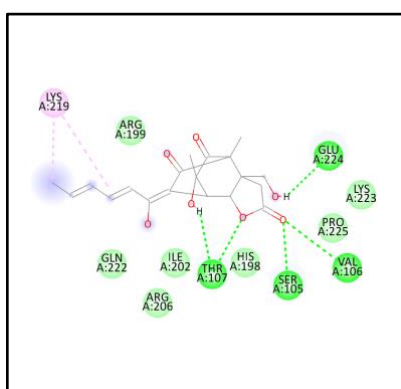
181I



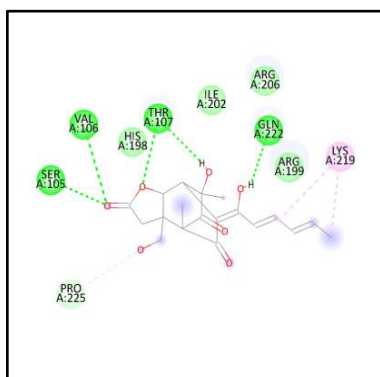
181V



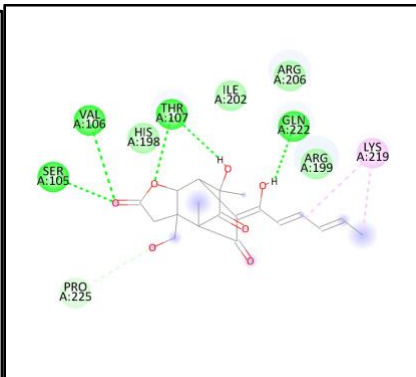
188CYS



188H



188L



Interactions

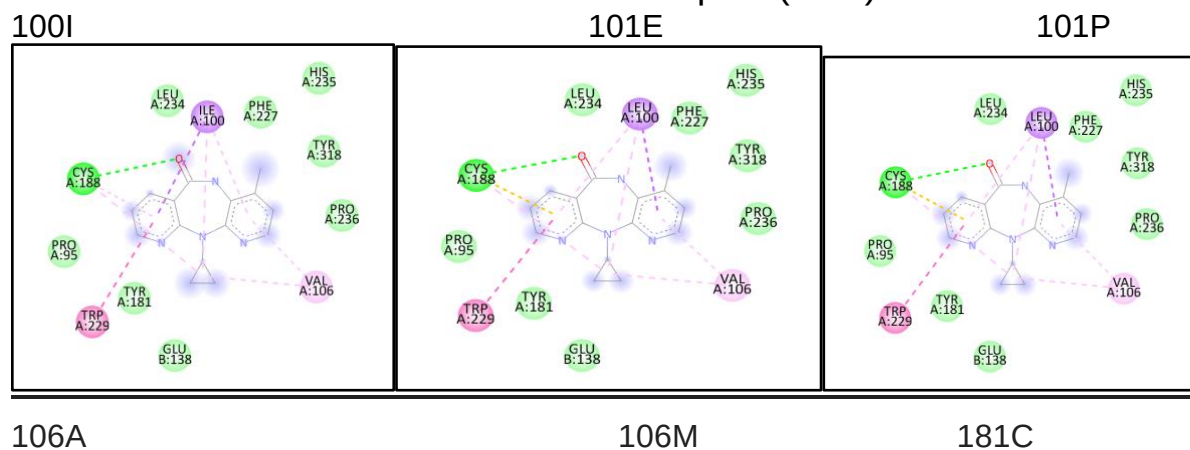
- van der Waals
- Conventional Hydrogen Bond
- Carbon Hydrogen Bond
- Halogen (Fluorine)

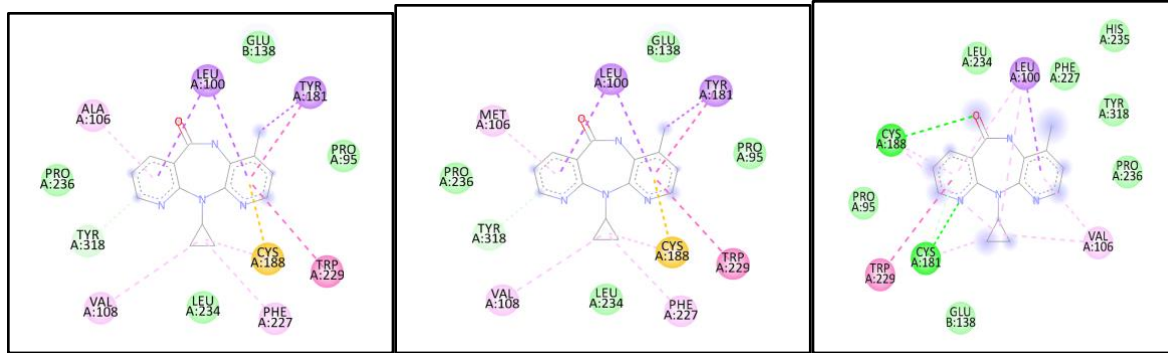
- Pi-Cation
- Pi-Pi T-shaped
- Amide-Pi Stacked

Fig 4.2.4 The fig refers to the two dimensional interaction between the protein(reverse transcriptase:1jlf) and the ligand Rezishanone

POSITION	NATIVE	MUTATED
100	-7.66(K)	-5.91(I)
101	-5.10(K)	-6.29(Q), 8.85(P)
106	44.62(V)	-5.94(A), 1.11(M)
181	9.90(Y)	9.74(C), -6.03(I), 18.56(V)
188	-6.03(Y)	-7.5(L), -4.95(C), -6.85(H)

Table 4.2.5 Nevirapine (FDA)

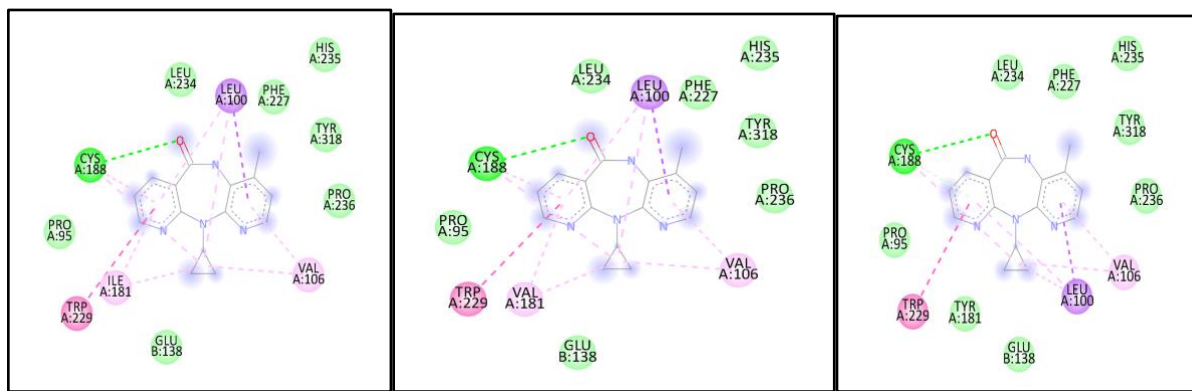




181I

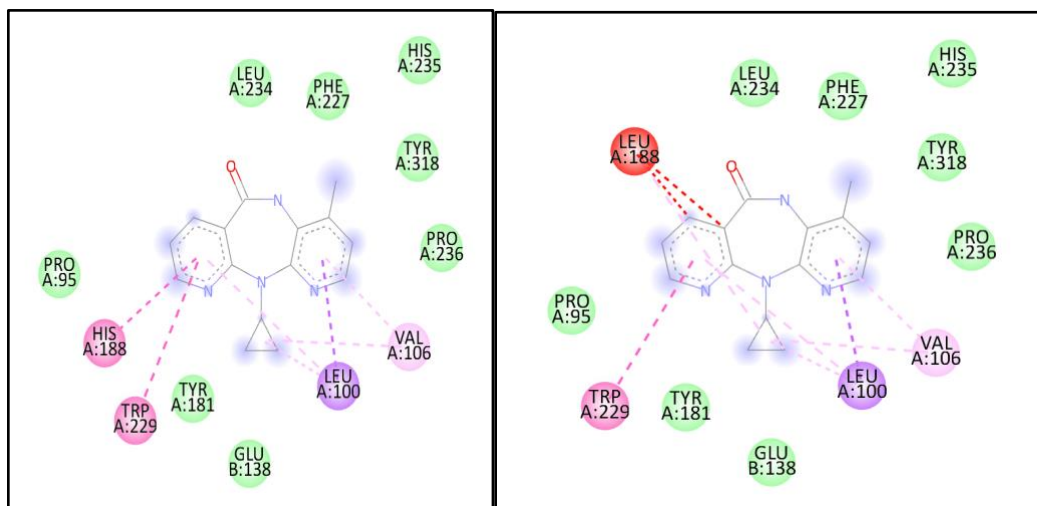
181V

188C

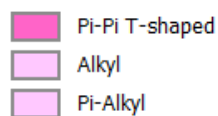
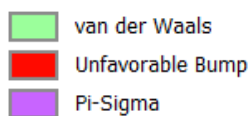


188H

188L



Interactions



Interactions

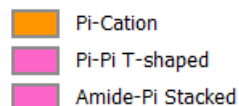
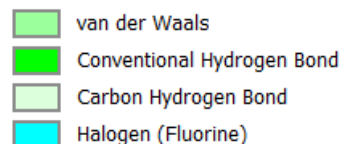


Fig 4.2.5 The fig refers to the two dimensional interaction between the protein(reverse transcriptase:1jlf) and the ligand Nevirapine

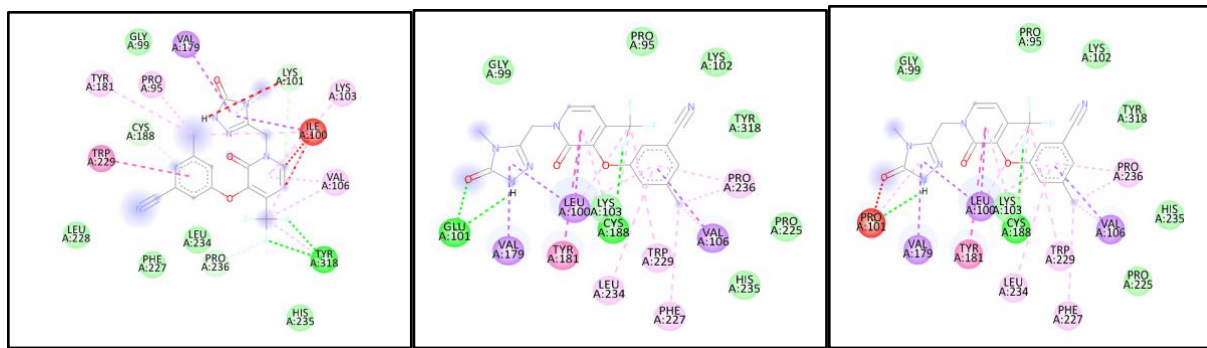
POSITION	NATIVE	MUTATED
100	-6.97(K)	769.77(I)
101	72.21(K)	3.42(E), -6.14(P)
106	-7.84(V)	4490.00(A), 1060.00(M)
181	1390.00(Y)	2370.00(V), 712.80(I), 3260.00(C)
188	-6.960(Y)	-6.80(H), 1710.00(C), 151.11(L)

Table 4.2.6 Doravirine (FDA)

100I

101E

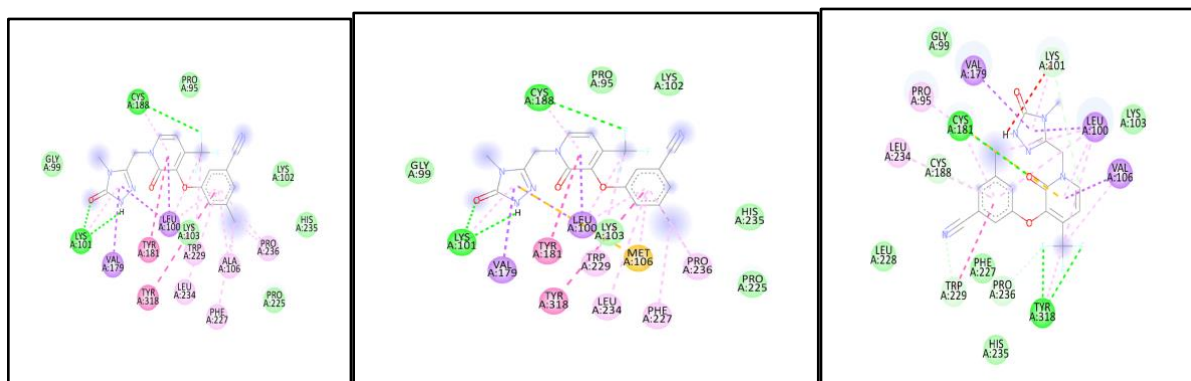
101P



106A

106M

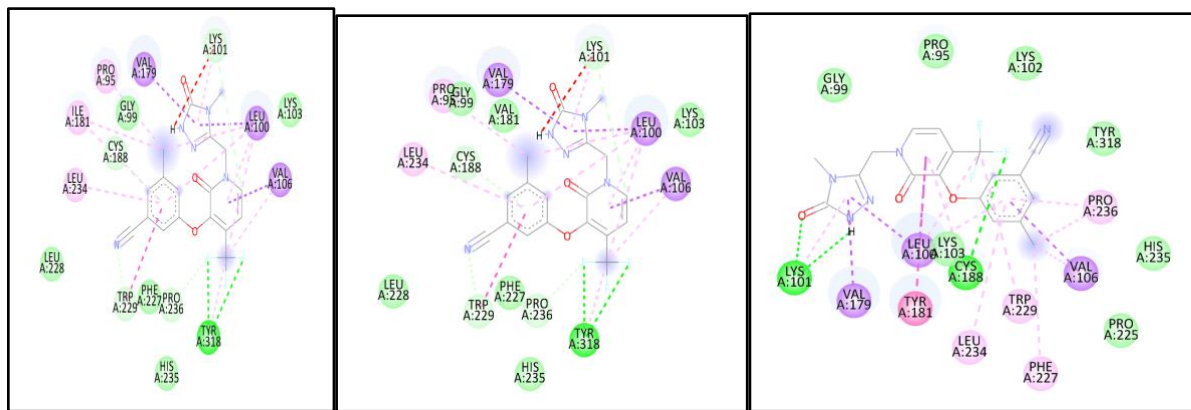
181C



181I

181V

188C



188H

188L

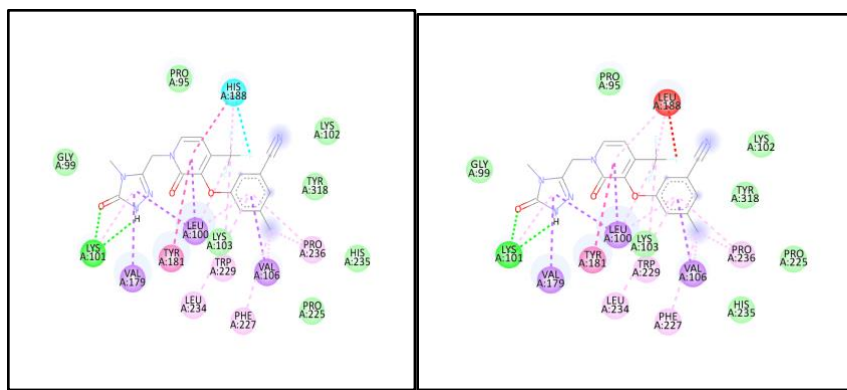


Fig 4.2.6 The fig refers to the two dimensional interaction between the protein(reverse transcriptase:1jlf) and the ligand Doravirine

Interactions

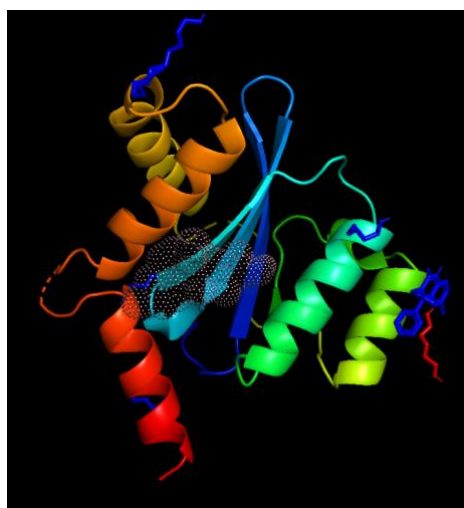
	van der Waals		Pi-Cation
	Conventional Hydrogen Bond		Pi-Pi T-shaped
	Carbon Hydrogen Bond		Amide-Pi Stacked
	Halogen (Fluorine)		

Interactions

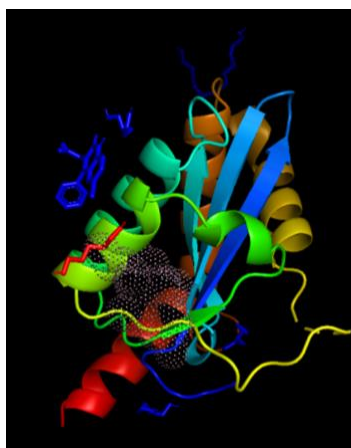
	van der Waals		Pi-Pi T-shaped
	Unfavorable Bump		Alkyl
	Pi-Sigma		Pi-Alkyl

Fig 4.3 3D-Interactions of Integrase and Reverse Transcriptase

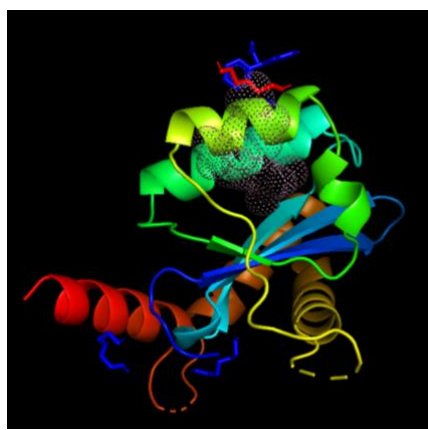
a. Equisetin(66)



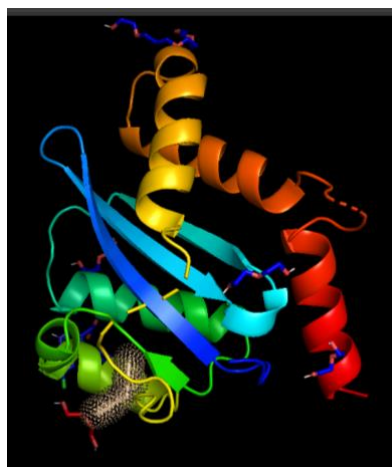
b. Phomasetin



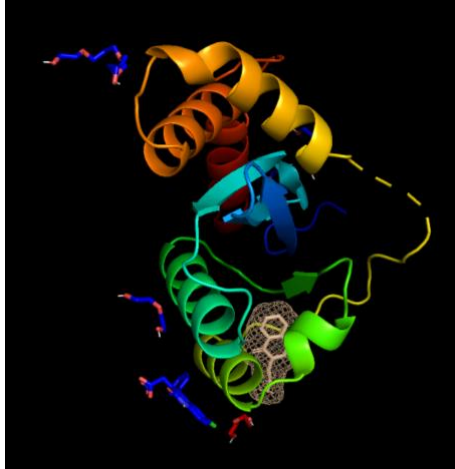
c. Integric acid



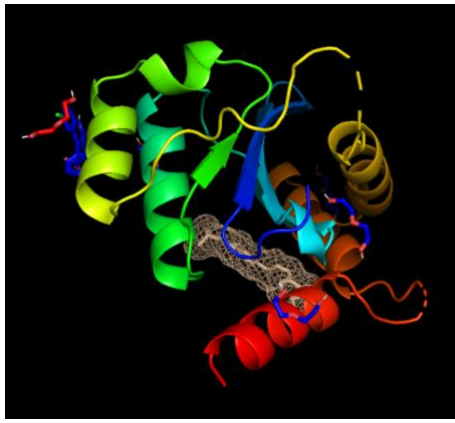
d. Depsidone



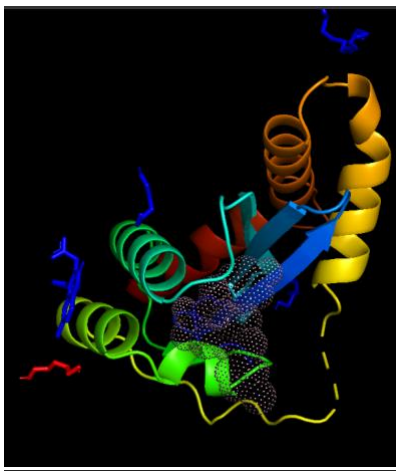
e. 4-hydroxyquinoline-3-carbohydrazide



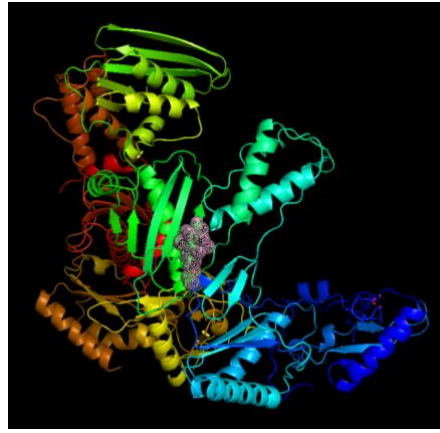
f. Dolutegravir



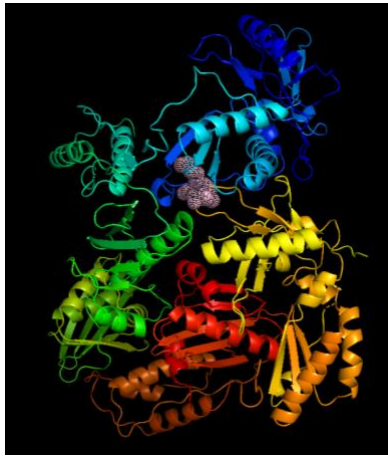
g. Raltegravir



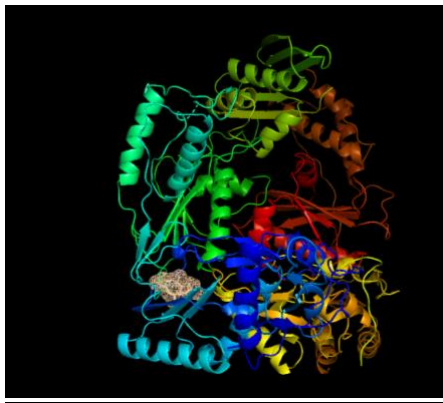
h. Rezishanone



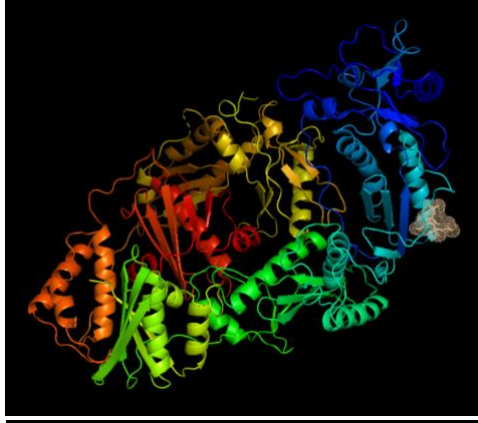
i. Da-1



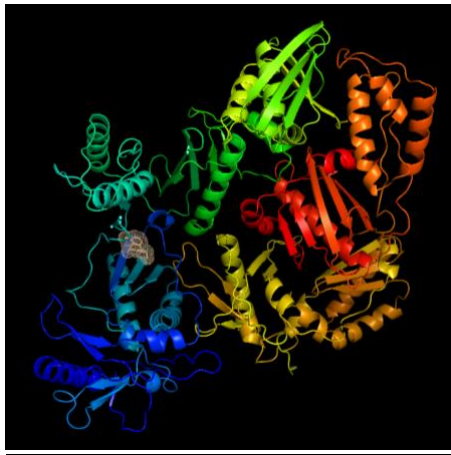
j. Dolabelladienetriol



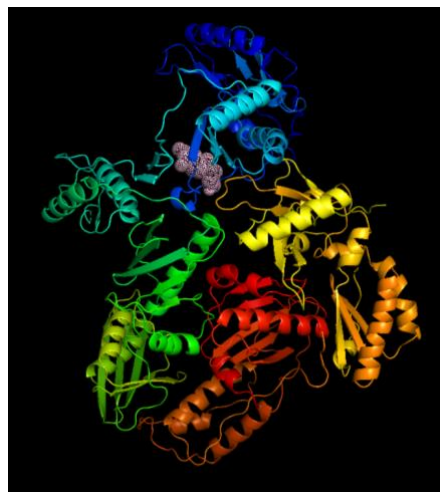
k. AcDa-1



I. Nevirapine



m. Doravirine



5. CONCLUSION:

- Objective of our study involves the comparative study of FDA approved

compounds and MNP's binding efficacy towards the reported resistance mutants.

- Metabolites from marine source is considered as promising agents for drug discovery. Marine natural compounds were assessed for their binding efficacy on both native and mutant models of HIV-int and rt
- Comprehensive In-silico studies involved pharmacokinetics, drug-likeness and medicinal chemistry friendliness evaluation of proposed compounds and molecular docking employed to rationalize ligands activity towards the HIV integrase and rt target protein.
- Binding energy and cumulative dock scores were propitious and can be considered as lead molecules for designing future class of INSTI's and NNRTI's to overcome the drug resistance.
- The efficiency check between the MNPs and FDAs resulted to the conclusion that MNP's are more efficient for the treatment of HIV compared to currently used FDAs.

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