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Selected Inhibitors for Dengue Virus NS5 RNA-Dependent RNA Polymers Using a Molecular Docking Approach

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Abstract

The Dengue virus is a mosquito-borne viral disease occurring in tropical and subtropical areas of the world. The dengue virus (DENV) non-structural protein 5 (NS5) which contains two domains N-Terminal domain & C-Terminal domain. The N-Terminal has methyl transferase domain and the C-Terminal has RNA-dependent RNA polymerase domain (RDRP). Both activities are responsible for viral RNA synthesis and replication. The incidence of dengue has increased rapidly and now which is estimated half of the human population at risk. There are no specific drugs to treat DENV infections. The present in silico study which is aimed at looking for new inhibitors of the dengue virus. NS5 RNA-dependent RNA-Polymerase (RdRp) against dengue. Antiviral substances which is obtained from natural products, including phytocompounds and standard drugs. Docking study which is performed by using autodock vina, pymol molecular graphics system, version 1.5.0.4, Schrodinger, LLC phytocompounds with high negative free energy docking score is-8.7 kcal/mol.
Phytocompounds and standard drugs were screened and their interaction with NS5 was identified.

Keywords: Flavivirus, Dengue virus, virtual screening, molecular docking, autodock/vina, Inhibitors, phytocompounds, standard drugs, NS5 RNA- dependent RNA polymerase.

1. Introduction

Dengue virus is a disease caused by a virus. The dengue virus belongs to the members of flaviviridae family. Flavivirus which can be transmitted infections from animals to humans by arthropod vectors species such as mosquitoes and ticks. Dengue virus is a group of 70 virus including yellow fever, thick borne encephalitis (TBEU), west nile virus (WNV) and Japanese encephalitis virus (JEV)(Ref: Vicente Galiano et al., 2016). Dengue virus has grown dramatically in recent decades. It process a majority of cases are asymptomatic or mild and self-managed. Many cases are also misdiagnosed as other febrile illnesses. Recent data indicated that 390 million dengue virus infection per

year (95% credible interval 284-528 million), of which 96 million (67-136 million) manifest clinically (with any severity of disease). Another study on the prevalence of dengue estimates that 3.9 billion people are at risk of infection with dengue viruses. Despite a risk of infection existing in 129 countries, 70% of the actual burden in Asia. There are 4 distinct serotypes of dengue virus (DENV-1, DENV-2, DENV-3 and DENV-4), dengue virus causes a wide range of diseases in humans, from a self-limited dengue fever (DF) to a life-threatening syndrome called dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS)(WHO,2020). The first dengue vaccine, dengvaxia(CYD-TDV) manufacture by snofi pasture (Lyon codex, France) was registered in many countries for its use specially in individuals age between 9 & 45 and leaving in DENV-endemic areas. The DENV virus genome is made up of a single strand of positive-sense RNA. The genome RNA which contains a 5‟ un translated region and 5‟ un translated region. The poly protein is cleaved in the cytoplasm into three structural proteins (capsid protein C, membrane protein M and envelop protein E) and seven non- structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5).

Non-structural protein (NS5) which is the largest viral protein, and comprising with the N- terminal methyltrans-ferase (MTase).

Domain and the C-Terminal which has RNA-dependent RNA polymerase (RdRp).

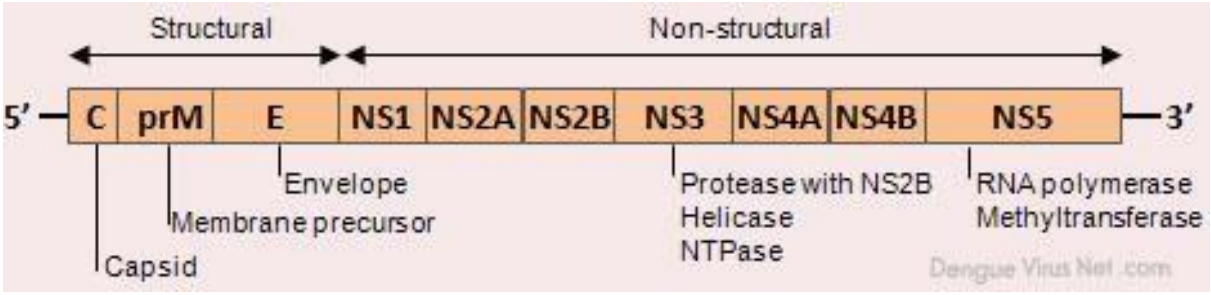


Fig1: Dengue Virus genome structure with the structural and Non-structural Genes. (Ref: Dengue Virus Net.com)

The domain is essential for both the strand positive strand as well as negative strand RNA synthesis during replication. The structure of domain is formed by 3 sub domains called thumb, fingers and palm. There are many inhibitors which binds against DENV near the priming loop. RNA viruses, such as DENV are the only organisms that possess enzymes with RdRp activity which is essential for their replication (Ref: Hideaki Shimizu et al.,2019). Polymerase inhibitors belongs to two types of molecules: first 1, nucleoside\nucleotide analogs (NI‟s) which function has RNA chain terminators and second, non-nucleoside inhibitors which bind to many different sites of the catalytic binding site of the enzyme.

Phytocompounds are found abundantly in medicinal plants. These phytocompounds act as strong defence mechanism for plants and also safeguard humans and animals against various viruses and epidemics. (Ref: Sajin A.K et al., 2015)

Phyto compounds can be traced in medicinal plants including limonoids, alkaloids,terpenoids and saponins. Treatment of dengue virus with medicinal plant costs very less compare to old traditional methods. It is also preferred because of the multiple target activities.

2. REVIEW OF LITERATURE

Dengue is a mosquito borne viral infection, dengue virus is transmitted by female mosquitoes mainly of the species Aedes aegypti and to a lesser extent, Ae.albopictus (WHO, 2nd march 2020). There are four distinct serotypes of the dengue virus which cause dengue (DENV-1, DENV-2, DENV-3and DENV-4). They have multiple genotype and antigenic differences among them.(simmons,farrar,nguyen and wills 2012) recovery from infection is believed to provide lifelong immunity towards that particular serotypes. However, cross immunity to the other serotypes, there is a risk of developing severe dengue. Dengue has an alarming impact on both human human health and the global and national economics (WHO, 2020). Dengue virus which can be frequently transported from one place to another by infected travellers. Dengue virus (DENV) non-structural protein 5 (NS5) contains two domains, N-terminal which has methyl transferase activity and a C-terminal which has RNA- dependent RNA polymerase activity (Vicente galiano, Pablo gracia-valtanen, Vicente mico, jose Antonio encinar, 11 October 2016).

Distribution and outbreaks of Dengue:

Before 1970, only 9 countries had experienced severe dengue epidemics. The disease is now endemic, which has spreaded more than 100 countries in the regions of Africa, the eastern Mediterranean, the Americas, South-east Asia and the western pacific. The largest numbers of dengue cases were reported globally in 2019. All regions were affected; Afghanistan was received for the first time dengue transmission. High numbers of cases were reported in Bangladesh (101,000), Malaysia (131,000), Philippines (420,000), Vietnam
(320,000) in Asia (WHO, 2020).

Socio-economic status and dengue fever: Most of the dengue cases occur in low to middle income countries, thus understanding their social economic capacity is vital especially drawing conclusion in regarding for future dengue vaccination programme (Wanrozital WM, 2006). This study was done in south asia countries which shows strong correlation between high socio-economic status and dengue prevention practices and attitude (Dhmal et al.,2014; Itrat et al., 2008, syed et al., 2010). In study which has done analyse the dengue vaccine acceptance in Bandung and Aceh, Indonesia, this is concluded that high socio-economic status is one of the major factor that correlated to dengue vaccine acceptance (Harpan, Fajar, Sasmono and Kuch, 2017).

Transmission: The virus which can be transmitted to humans through the bites of infected female mosquitoes. Human to mosquitoes transmission can occur upto 2 days before someone shows symptoms of the illness (Nguyen et al,2013 PNAS). The primary mode of transmission of DENV between humans which involves mosquito vectors. There is an evidence of the possibility of material transmission (from a pregnant mother to her baby).

Signs and Symptoms:

Symptoms, which is usually begin four to six days after infection and last for upto 10 days, It may include:

- Severe Headaches
- Pain behind the eyes

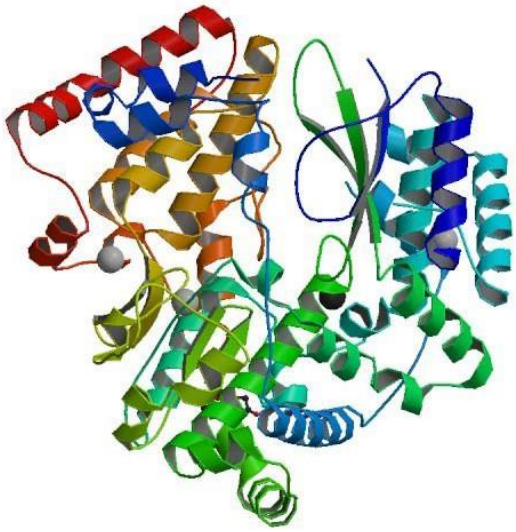
- Sudden, High fever
- Fatigue
- Nausea
- Severe joint and muscle pain
- Vomiting
- Mild Bleading (Such as nose bleed, Bleeding Gums or easy bruising)
- Skin Rash, which appears 2 to 5 days after the oncent of fever (Ref:webmd.com) **Diagnostics:** There are several methods which can be used for diagnosis of Dengue virus infection. First one is virological tests(Which directly detect elements of virus) and second one is serological tests, which detect human-derived immune components that are produced in response to the virus (Ref:WHO,2020).

Vaccination Against Dengue: The first dengue vaccine, Dengvaxia(CYD-TDV) developed by Snofi Pasteur was licenced in December-2015 and have been approved by regulatory authorities in more than 20 countries. Use of the vaccine was targeted for persons living in endemic areas, ranging from aged between 9 & 45 years (Vicente Galiano et al., 2016).

3. MATERIALS AND METHODS

Protein preparation:

The Protein 2J7U (Crystal structure of the Dengue virus NS5 RNA-Dependent RNA polymerase domain) was retrieved from Protein Data bank website and the active site residues of the protein are resided in the palm region of the protein from 497 to 542 , Asp663, Asp664, Arg729 and Arg737, *these active site residues are predicted based on literature review (***REFERERNCE IS PDB ARTICLE OF 2J7U: 10.1128/JVI.02283-06***), The protein structure is prepared for in Autodock and Pymol software package.*



2J7U-3D Structure

Ligand prepartion

The structures of Phytocompounds and standard drug compounds against 2J7U (Crystal structure of the Dengue virus NS5 RNA-Dependent RNA polymerase domain) protein (**PMID:17301146**) retrieved from PubChem website, and all these lead molecules are prepared for docking using Openbabel software (https://doi.org/10.1186/1758-2946-3-33).

ocking studies: *In silico* Docking studies are carried out to check the inhibiting potential of lead molecules against 2J7U the dengue virus NS5 RNA-dependent RNA polymerease Domain. Docking study performed using Autodock vina (O. Trott, A. J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, Journal of Computational Chemistry 31 (2010) 455-461*),* The protein structures were prepared for docking by removing water, bound inhibitors and by adding polar hydrogen in Autodock. Grid box (2J7U, Box size 36 x 42 x 40, box center 23.606 x 48.64 x 24.615 for x, y, and z, respectively) , was designed inorder to obtain favourable binding with active residues, followed by docking the protein-ligand complexes are prepared using PyMOL (The PyMOL Molecular Graphics System, Version 1.5.0.4, Schrödinger, LLC). The Ligand-target complexes after docking were analyzed to identify the binding affinities. The docking score and the best conformation were saved for reference.

The bonded and non bonded interactions are visualized via Ligplot software ([PubMed id: **7630882**]).

4. RESULTS AND DISCUSSION

Molecular docking studies: The docking of protein and ligands were done using Autodock vina in Linux enviroment, among 12 phytocompounds and 5 standard drugs 3 top docked compounds from phytocompounds and 2 standard drug were screened based on the Docking score. Scopadulcic acid B , Oleanane and 1,4-Dicaffeoylquinic acid are phytocompounds having docking score -8.7 kcal/mol, -8.7 kcal/mol and -8.6 kcal/mol .

The bonded residues are:

1. **Scopadulcic acid B:** There are 4 hydrogen bonds and bonded interactions ASN609(3.12), ASP663(3.04), SER661(3.00, 2.71).
2. **Oleanane:** There is no hydrogen bond and bonded interactions.
3. **1,4- Dicaffeoylquinic Acid:** There are 8 hydrogen bonds and bonded interactions ASN609(3.01), ASP663(3.08, 2.79), GLN602(3.34, 3.15), ASN492(3.13, 3.12)

The Non-bonded residues are:

1. **Scopadulcic acid B:** Non-Bonded interactions ASP538, TRP537, THR605, ILE797.
2. **Oleanane:** TYR606, GLN602, LEU478, PHE398.
3. **1,4- Dicaffeoylquinic Acid:** GLY662, TYR606, VAL603, LEU478, LYS401, PHE485, THR605.

LOVASTATIN and **PREDNISOLONE** is the top docked compounds among the standard drug and it is having docking score -7.8 kcal/mol and -7.6 kcal/mol.

The bonded residues are:

1. **Lovastatin:** There are 4 hydrogen bonds and bonded interactions are ARG499(3.06), LYS656(3.00), GLU500(3.14, 2.92).
2. **Prednisolone:** There are 6 hydrogen bonds and bonded interactions are THR383(2.69, 2.83), TRP379(3.17), GLU648(3.02, 2.70), GLU653(3.05)

The Non-bonded residues are:

1. **Lovastatin:** Non-bonded interactions are ASP520, ILE517, TRP823, ILE524.
2. **Prednisolone:** Non-bonded interactions are THR649, VAL652, ARG383

The docked compounds are abbreviated as Phytocompounds – P9: Scopadulcic acid B, P6: Oleanane, P1: 1,4-Dicaffeoylquinic acid and Standard drug: STD5: LOVASTATIN, STD6: PREDNISOLONE

Discussions:

On the basis of binding interactions of the 12 compounds, one lead compound was discovered. The information in the table no showed that scopadulcic acid and prednisolone had good hydrogen bond some common reported amino acids were ASN609, ASP663, SER661, ASN609, ASP663, GLN602, ASN492, ARG449, LYS656, THR383 with the distance $3.12A^0$, $3.04A^0$, $3.00A^0$, $3.08A^0$, $3.34A^0$, $3.13A^0$, $3.14A^0$, $2.69A^0$ and $3.05A^0$ respectively.

Including both phytocompounds and standard drugs a total 98 ligands have been used for docking and out of those 18 ligands only 5 have been highlighted. Phytochemilals have the docking score of scopadulcic acid (-8.7 kcal/mol), 1,4- Dicaffeoylquinic Acid (-8.6kcal/mol) and standard drugs have the docking score, lovastatin (-7.8kcal/mol), prednisolone (- 7.6kcal/mol) other than these ligands, few other ligands have also shown a very good binding energy, and can be considered useful against dengue virus.

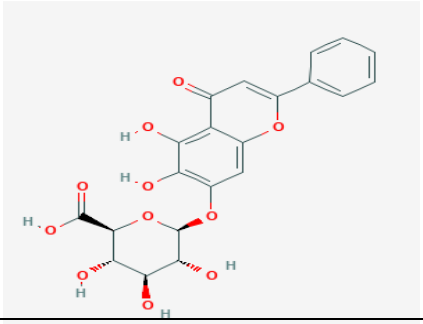
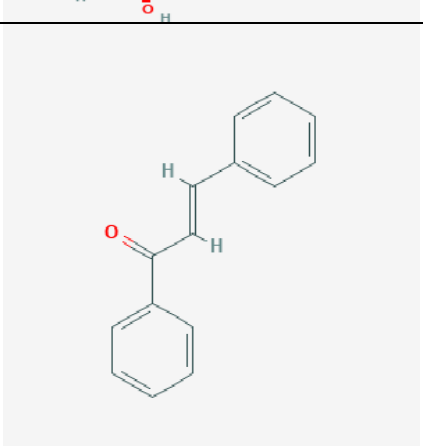
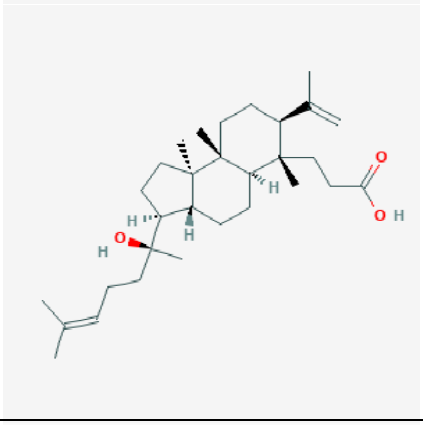
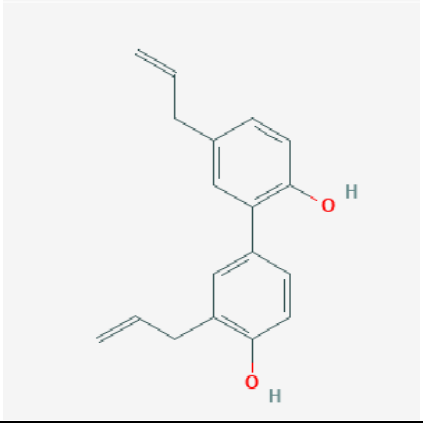
5. CONCLUSIONS

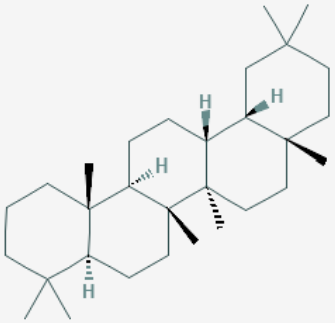
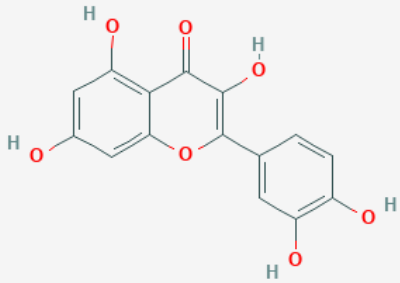
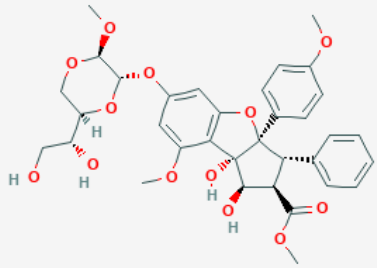
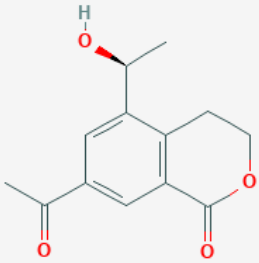
The study was intended to find novel drug molecule as anti-dengue compounds using the structure based drug design technique 2J7U protein was used as target for the purpose of finding novel drugs. About 12 phyto compounds and 6 standard drugs were selected as ligands for the study that showed activity against the dengue virus.

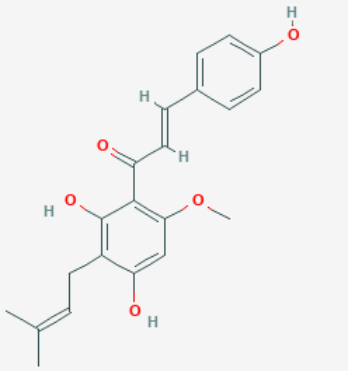
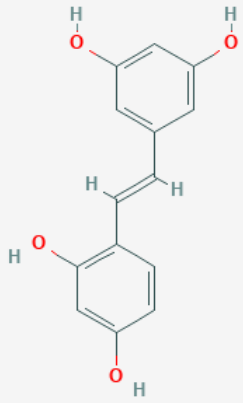
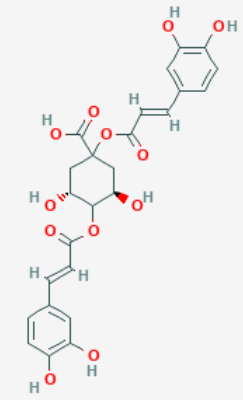
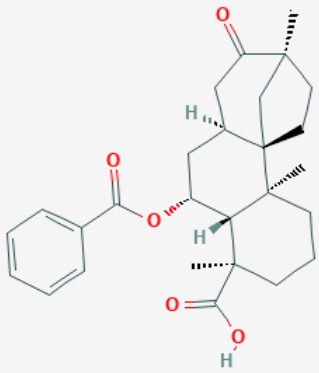
Protein docked with phyto compounds and standard FDA drugs, and phytocompound having best docking score among them scopadulcic acid B (-8.7 kcal/mol) and oleanane (-8.7 kcal/mol) in particular have shown higher binding energy than other standard drugs and phyto compounds.No of bonded interactions are ASN609, ASP663, SER661.Standard drugs is not having much docking score, lovastatin (-7.8kcal/mol) and prednisolone (-7.6 kcal/mol) in particular have shown lower binding energy compare to phyto compounds. No of bonded interactions are ARG499, LYS656, GLU500, THR383, GLU648, TRP379, GLU653. Phyto

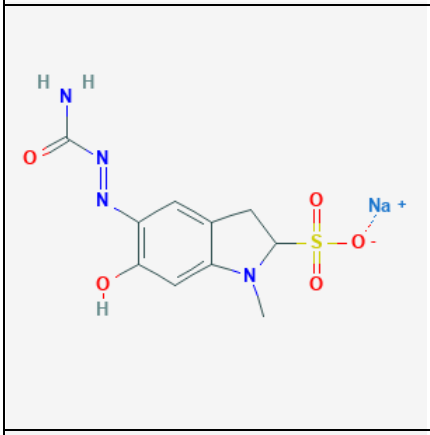
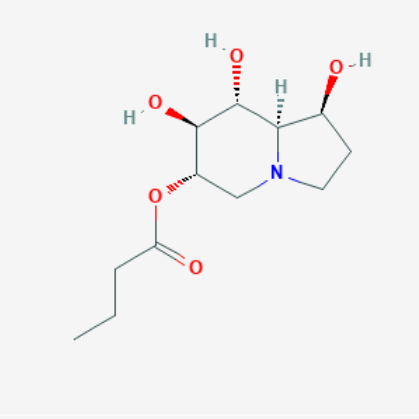
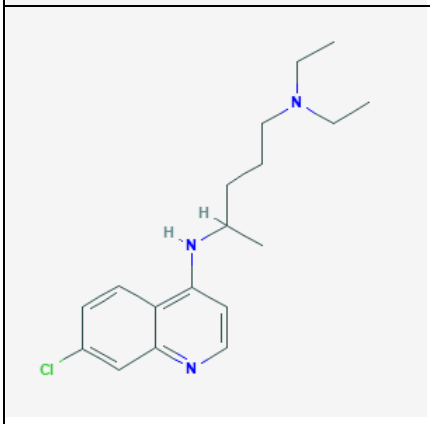
compounds are better than standard drugs. The binding affinities of ligand can be observed over a period of time so simulation studies should be carried out further for this data.

2D IMAGES OF PHYTOCOMPOUND AND STANDARD DRUGS

	1. Baicalin: It has a role as a non-steroidal anti-inflammatory drug, and inhibitor a prodrug and plant metabolite.
	2. Chalcone: It is an aromatic ketone that forms the central core for a variety of important biological compounds.
	3. Dammarenolic Acid: It is a dammarame triterpene that shows antiviral activity with relatively low cytotoxicity.
	4. Honokiol: It is a poly-phenalic compound that exterts nuroprotective properties through a variety of mechanisms

	5. Oleanane: It is a terpenoid fundamental parent and a triterpene.
	6. Quercetin: It is a polyphenolic flavonoid with potential chemopreventive activity.
	7. Silvestrol: It is an organic heterotricyclic compound, a member of dioxanes, an ether and a methyl ester.
	8. Swerilactone M: This is a 2-benzopyran. It has a role as a metabolide.

	9. Xanthohumol: It is a member of chalcones, a polyphenol and an aromatic ether.
	10. Oxyresveratrol: This is a Stilbenoid. It is found in the heart wood of artocorpus lakoocha and the traditional drug „Puag-Haad“ made from it.
	11. 1,4-Dicaffeoylquinic acid: It is a naturally occurring polyphenolic compound found in plants like fennel and coffee.
	12. Scopadulcic acid B: It is a new diterpenoids a novel skeleton, from a Paraguayan crude drug.

	13. Balapiravir: It is an orally administered prodrug with activity against the hepatitis C virus (HCV).
	Carbazochrome sodium sulfonate: It is an antiheamorrhagic, that will cease blood flow by causing the aggregation.
	15. Celgosivir: It is used as a treatment for acute Dengue fever.
	16. Chloroquine: It is an aminoquinoline used for the prevention and therapy of malaria.

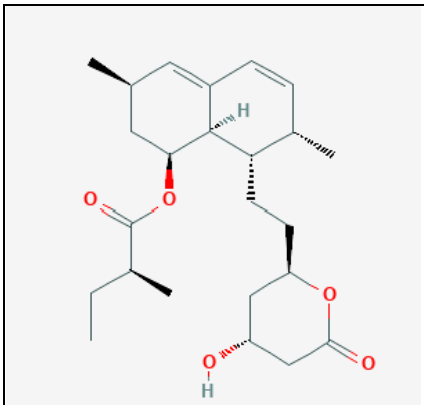
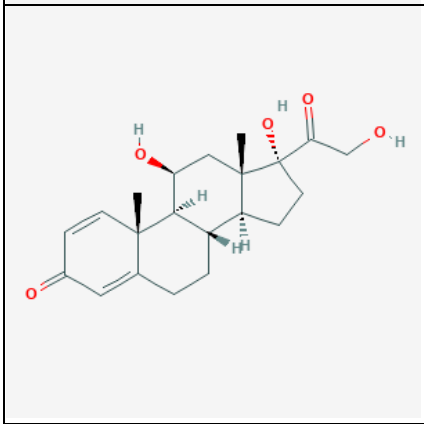
	17. Lovastatin: This is a cholesterol-lowering agent that belongs to the class of medications called Statins.
	18. Prednisolone: It is a synthetic glucocorticoid with anti-inflammatory properties.

Table 02- Top 5 ligands with docking score and their interactions

Ligand	Docking score	Number of H bonds	Bonded interactions	Non bonded interactions
Scopadulcic acid B	-8.7	4	ASN609(3.12), ASP663(3.04), SER661(3.00,2.71)	ASP538,TRP537,THR605, ILE797, TYR606,ASP664
Oleanane	-8.7	0	-	TYR606,GLN602,LEU478,PHE398,LYS401,VAL402, PHE485,VAL603
1,4-Dicaffeoylquinic acid	-8.6	8	ASN609(3.01), ASP663(3.08,3.08,2.79), GLN602(3.34,3.15), ASN492(3.13, 3.12)	GLY662,TYR606,VAL603, LEU478,ARG481, PHE398, VAL402, LYS401, PHE485, THR605
LOVASTATIN	-7.8	4	ARG499(3.06), LYS656(3.00), GLU500 (3.14, 2.92)	ASP520, ILE517, TRP823, ILE524, TYR503

PREDNISOLONE	-7.6	6	THR383(2.69,2.83), TRP(3.17), GLU648(3.02, 2.70) , GLU653(3.05)	THR649,VAL652,ARG383

Supplimentary information

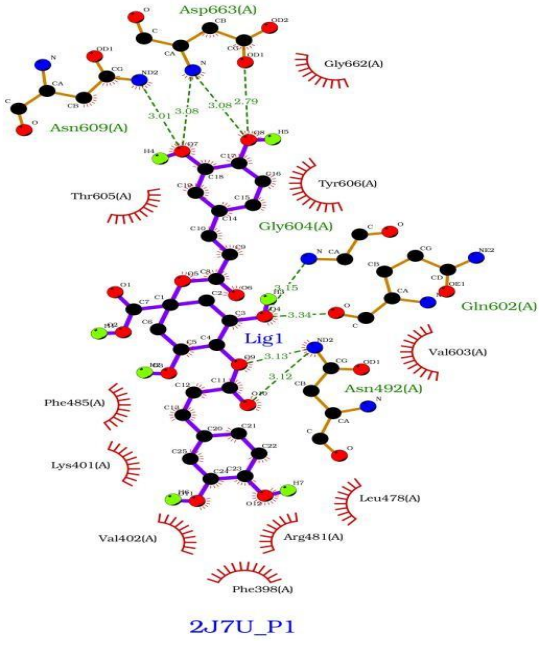
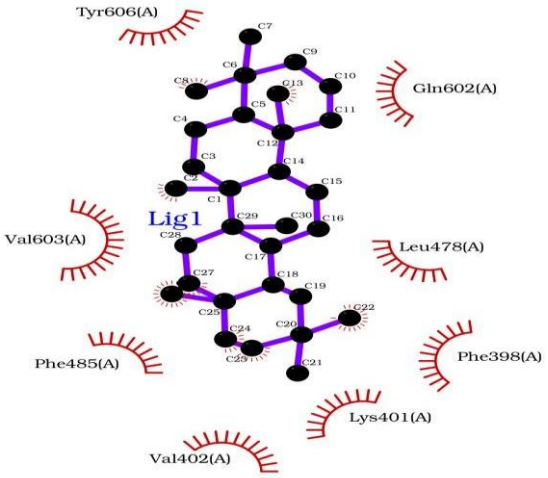
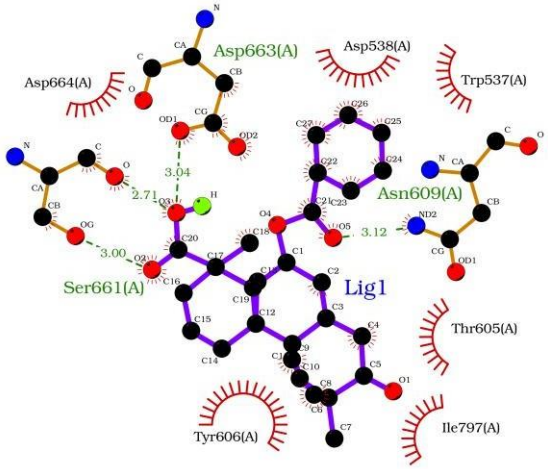
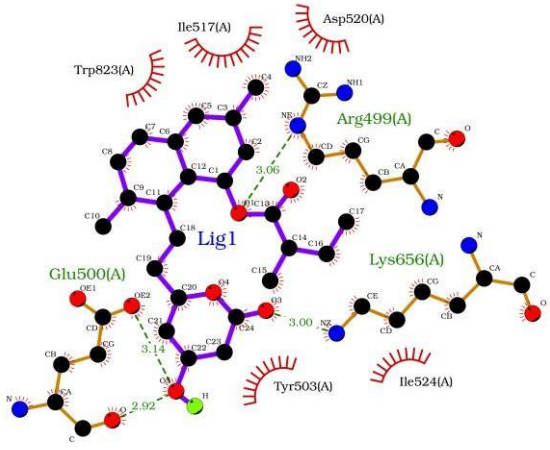
Phytocompounds

1	1,4-Dicaffeoylquinic acid	-8.6
2	baicalin	-8.6
3	Chalcone	-6.3
4	Dammarenolic acid	-7.2
5	Honokiol	-6.8
6	Oleanane	-8.7
7	Oxyresveratrol	-6.7
8	quercetin	-7.4
9	Scopadulcic acid B	-8.7
10	Silvestrol	-7.8
11	Swerilactone M	-6.6
12	Xanthohumol	-7.1

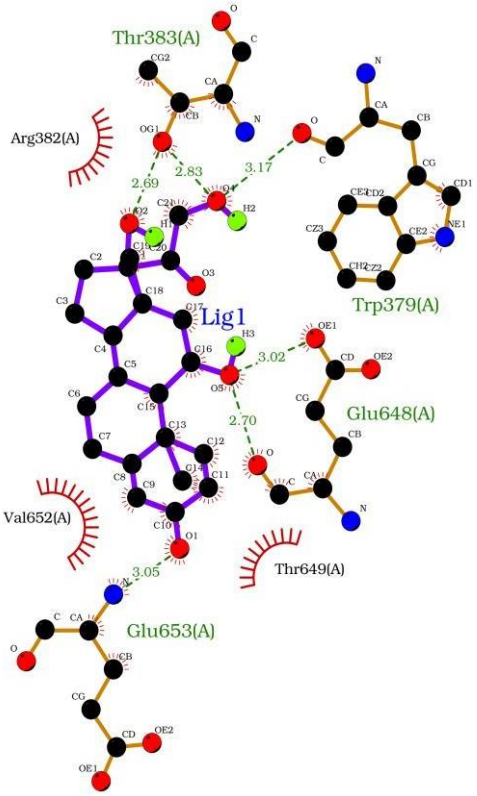
Standard Drugs

1	BALAPIRAVIR	-6.5
2	Carbazochrome sodium sulfonate	-6.8
3	CELGOSIVIR	-6.2
4	CHLOROQUINE	-5.5
5	LOVASTATIN	-7.8
6	PREDNISOLONE	-7.6

TABLE 03- LIGPLOT IMAGES OF LIGANDS SHOWING INTERACTION WITH THE SPECIFIC AMINO ACID RESIDUES OF PROTEIN

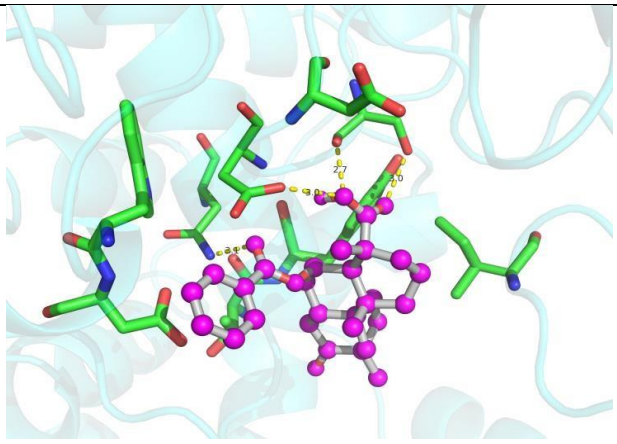
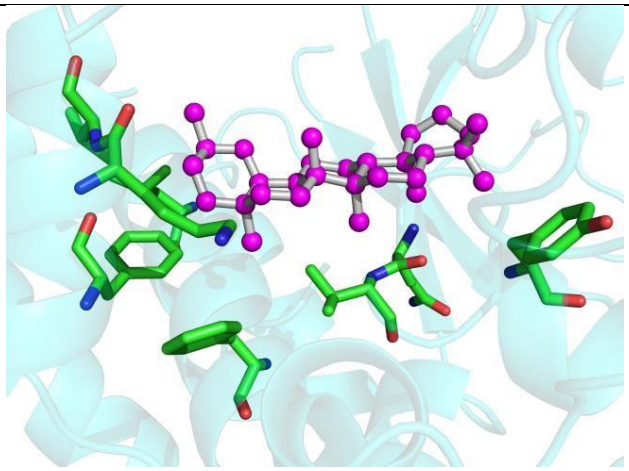
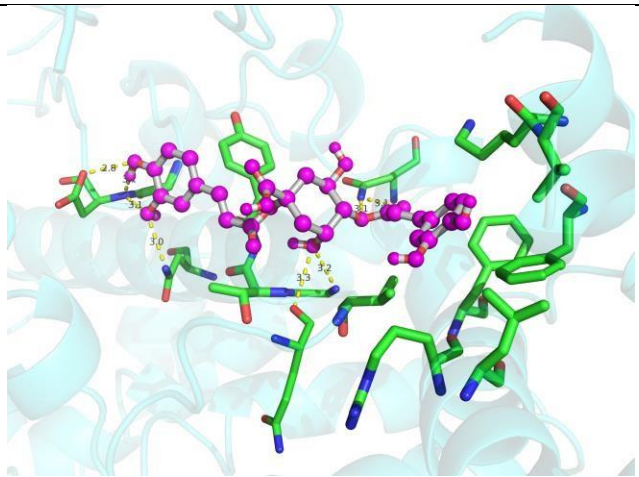
1,4-Dicaffeoylquinic acid Docking score is -8.6  2J7U_P1	**Oleanane** Docking score is -8.7  2J7U_P6
Scopadulcic acid B Docking Score is -8.7  2J7U_P9	**Lovastatin** Docking Score is -7.8  2J7U_STD5

Prednisolone
Docking Score is -7.6



2J7U_STD6

TABLE 04- 3D IMAGES OF TOP 5 LIGANDS INTERACTION WITH PROTEIN

Sl NO	LIGANDS	3D IMAGE OF INTERACTION
1.	**(2J7U-P9) Scopadulcic Acid B** -8.7 kcal/mol	
2.	**(2J7U-P6) Oleanane** -8.7 kcal/mol	
3.	**(2J7U-P1) 1,4-Dicaffeoylquinic acid B** -8.6 kcal/mol	

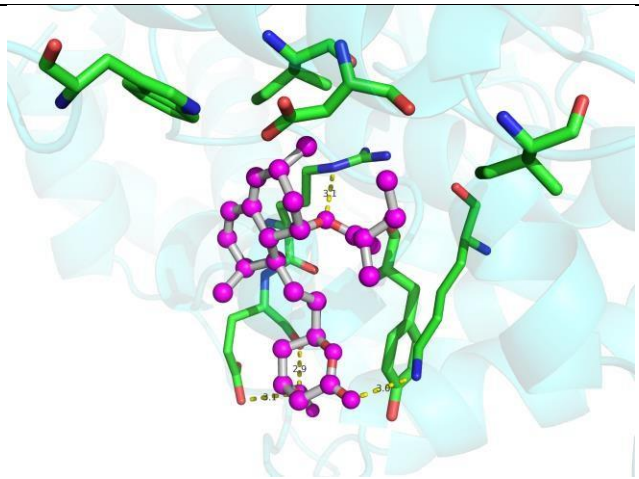
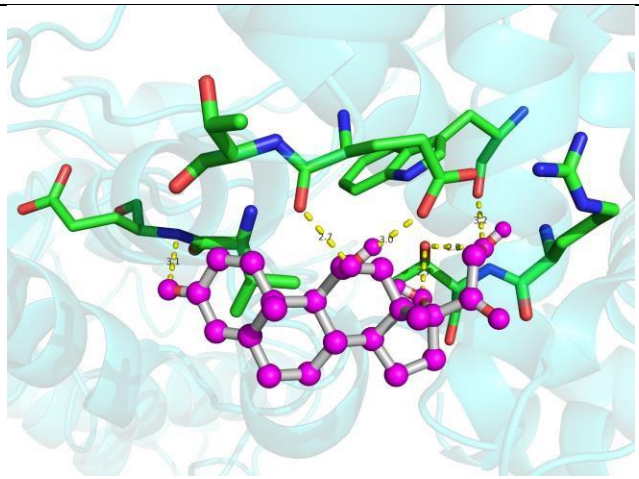
4.	**(2J7U-STD5)** **Lovastatin** -7.8 kcal/mol	
5.	**(2J7U-STD6)** **Prednisolone** -7.6 kcal/mol	

Figure XX: The docking images are taken using PyMol software (The PyMOL Molecular Graphics System, Version 1.5.0.4, Schrödinger, LLC).
The magenta color ball and stick model in theimages represents the respective drug molecule and green color sticks are the interacting residues and the bonded interactions are shown in yellow broken lines with the distance mentioned in Angstromes.

a) 2J7U_P9: Scopadulcic acid B
b) 2J7U_P6: Oleanane
c) 2J7U_P1: 1,4-Dicaffeoylquinic acid B
d) 2J7U_STD5: Lovastatin
e) 2J7U_STD6: Prednisolone

Phytochemical Ligands And Standard Drugs Docking score with 2J7U Protein

Sl no	Name	Docking Score	Molecular Formula	CID
1	1,4-Dicaffeoylquinic acid	-8.6	$C_{25}H_{24}O_{12}$	12358846
2	baicalin	-8.6	$C_{21}H_{18}O_{11}$	64982
3	Chalcone	-6.3	$C_{15}H_{12}O$	637760
4	Dammarenolic acid	-7.2	$C_{30}H_{50}O_3$	22215841
5	Honokiol	-6.8	$C_{18}H_{18}O_2$	72303
6	Oleanane	-8.7	$C_{30}H_{52}$	9548717
7	Oxyresveratrol	-6.7	$C_{14}H_{12}O_4$	5281717
8	quercetin	-7.4	$C_{15}H_{10}O_7$	5280343
9	Scopadulcic acid B	-8.7	$C_{27}H_{34}O_5$	11729855
10	Silvestrol	-7.8	$C_{34}H_{38}O_{13}$	11787114
11	Swerilactone M	-6.6	$C_{13}H_{14}O_4$	53483971
12	Xanthohumol	-7.1	$C_{21}H_{22}O_5$	639665

Standard Drugs

1	BALAPIRAVIR	-6.5	$C_{21}H_{30}N_6O_8$	11691726
2	Carbazochrome sodium sulfonate	-6.8	$C_{10}H_{11}N_4NAO_5S$	135705561
3	CELGOSIVIR	-6.2	$C_{12}H_{21}NO_5$	60734
4	CHLOROQUINE	-5.5	$C_{18}H_{26}CIN_3$	2719
5	LOVASTATIN	-7.8	$C_{24}H_{36}O_5$	53232
6	PREDNISOLONE	-7.6	$C_{21}H_{28}O_5$	5755

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