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IN-SILICO DOCKING AND MOLECULAR DYNAMIC SIMULATION STUDIES OF NsP2 PROTEASE FROM CHIKUNGUNYA VIRUS

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ABSTRACT

Chikungunya virus (CHIKV) is an Arbovirus, transmitted to humans primarily by *Aedes aegypti* a species of mosquito. Infection due to this pathogen is often associated with fever, rash and arthralgia. The NsP2 protease of Chikunguniya virus has a crucial role in genome replication and hence acts as a promising drug target. We have used computer-aided drug design approach to find out the natural agents that can used in the treatment of Chikunguniya infection. We initially screened 162 natural compounds having antiviral activity. The 3D structures of these compounds were retrieved from PubChem Database. Molecular docking studies of these ligands were performed using PyRx (V 8.0) and, ADMET profiles were obtained by using SWISS ADME and data warrior tools. The obtained results after data analysis demonstrated that the ligand taxifolin has good binding affinity and complies with all the ADME parameters. The Molecular dynamic simulation studies of the taxifolin in complex with the PDB structure of NsP2 Protease of CHIKV (PDB ID: 3TRK) were carried out and the parameters like RMSD, RMSF, and radius of gyration were observed to understand the fluctuations and protein-ligand interaction.

Key Words: Molecular Docking, MD simulation, CHIKV, Protease, VMD and NAMD, RMSD

Introduction

Chikungunya virus (CHIKV) is an arbovirus which belongs to the *alphavirus* genus, family *Togaviridae*. It has been responsible for major outbreak of devastating human arthritis. Chikunguniya fever caused by the virus was first described in 1952 during an epidemic in Newala district of Tanzania. "Chikunguniya" in Makonde language translates to "that which bends up" relating to the stopped posture (Her et al., 2009) developed as a result of rheumatologic inflammation.

CHIKV can be transmitted through an urban cycle, man to mosquito to man, or a sylvatic cycle, animal to mosquito to man (Chhabra et al., 2008). The virus is transmitted to humans by mosquitoes of the *Aedes* genus (*Aedes furcifer* in Africa and *Aedes aegypti* in Asia), similar to the dengue fever causing virus (Schwartz & Albert, 2010).

The symptoms of "Chikunguniya fever" CHIKF infection generally start after 4–7 days of the mosquito bite. Infection presents in two phases, the first being acute, while the second stage is persistent (chronic), causing disabling polyarthritis (Ziegler et al., 2008). Acute infection lasts 1–10 days and is characterized by a painful polyarthralgia, high fever, asthenia (weakness), headache, vomiting, rash, and myalgia (muscle pain).

The CHIKV contains an RNA genome of approximately 11.8 kB that is single stranded and messenger ('positive strand') sense. It consists of two open reading frames (ORFs) viz non-structural and structural. During viral replication, non-structural polyprotein proceeds to four non-structural protein (nsP1, nsP2, nsP3, nsP4) responsible for viral replication, RNA capping and invasion of the host defence mechanism. In addition the structural polyprotein proceeds' into various protein (C, E3, E2, 6K, E1) that are involved in viral particle assembly (Weaver et al., 2012).

Currently, targeting viral enzymes that are crucial for viral replication is believed to be an attractive strategy for development of antiviral therapy. The nsP2 protein is a multifunctional protein. The proteolytic domain has been allocated to its C-terminal section which forms a papain like cysteine protease (also known as thiol protease).The nsP2 proteolytic activity is critical for virus replication and is responsible for cleavage of the non-structural polyprotein complex. The N-terminal having RNA-triphosphatase (RTPase) activity that perform the RNA-capping. It was also found to have the nucleotide triphosphatase (NTPase) activity. Both NTPase and RTPase activities are completely dependent on Mg2+ ions (Karpe et al., 2011). Therefore, the inhibition of NsP2 protein not only inhibits the NTPase and RTPase activity but also inhibits the virus replication.

So we have selected NsP2 protease enzyme as the drug target. The crystal structure PDB ID: 3TRK retrieved from the protein data bank was used here to find suitable ligands against it (Berman et al., 2000).

Currently, there is no specific antiviral drug and vaccine available in the market for Chikunguniya virus. Symptomatic treatment is available which include analgesic drug, NSAIDs and

corticosteroids. As *in vitro* and in vivo screening of antiviral is laborious and time consuming, here we have performed insilico approach to find natural alternatives to treat the CHIKV infection. We initially selected natural compounds having antiviral activity, from literary sources and subjected them to molecular docking and molecular dynamic simulation studies to get to the lead natural candidate for treating Chikunguniya virus infection.

Material and Methods

Protein structure preparation

The X-ray diffraction-based crystal structure of the Chikunguniya Virus NsP2 Protease (PDB ID: 3TRK) in a complex with ligand Na^+ (sodium) with a resolution of 2.40 Å was sourced from the protein data bank. The structure was cleaned to ensure maximum quality and reliability (Isa et al., 2019) by removing the bound ligands and water molecules. The missing atoms and residues were added to bridge any gaps in the protein molecule. Steric clashes were minimized by choosing proper orientation and polar hydrogen atoms were added to ascertain hydrogen atoms bonded to electronegative atoms like oxygen and nitrogen. Formal bond orders were determined, side chains were optimized and fixed, charges added using program implemented in chimera, SWISS PDB viewer, and Chiron minimization and refinement tool (Johansson et al., 2012, Porollo & Meller, 2010, Ramachandran et al., 2011).

Computational screening

A thorough literature survey was conducted to find out the natural compounds having said antiviral properties. A total of 162 compounds were identified and the structures of the identified compounds were retrieved from the PubChem database. The compounds were imported in to the PyRx (V 8.0) and energy minimization was done using Open Babel (Version 2.3.1) (O'Boyle et al., 2011) module. Energy minimization was done via the Universal force field (UFF) using the conjugate gradient algorithm. A total number of 200 steps were set and the number of steps to update was set to 1. The minimization was set to stop at an energy difference of less than 0.1 Kcal/mol (Luo et al., 2020).

Docking studies

Molecular docking was performed with PyRx (V 8.0), which is an extension of the python molecular viewer. A Lamarckian genetic algorithm was used to perform the automated molecular docking of the protein with each ligand. The torsion bonds and side chains were kept to rotate freely, while the protein structure was kept rigid. Gasteiger charges were computed, and all the charges of non-polar hydrogens were assigned (Morris et al., 1998). The grid map was set at 60×60×60× and the grid was spaced at 0.375Å. Both selected ligand and protein were converted in to pdbqt structure format. Protein and ligands were loaded in to PyRx as macromolecule and ligand, respectively. All the compounds were docked and affinity was calculated in kilocalories per mole (Gasteiger & Marsili, 1980).

Table 1. Molecular Properties & Drug likeness of Selected Ligands.

Molecule Name	PubChem ID	Number of HBA	Number of HBD	Molecular weight	cLog P	Drug likeness
73659	73659	4	3	472.7	5.26	-2.0276
36462	36462	13	3	588.56	1.13	-1.9839
357293	357293	4	0	310.34	2.02	-0.56695
5271805	5271805	10	4	566.51	4.34	0.40331
5281627	5281627	10	5	538.46	3.98	0.28194
3663	3663	8	6	504.44	4.26	-1.9057
107876	107876	13	10	594.52	1.14	0.1505
439501	439501	12	8	584.65	-0.52	0.43549
4978	4978	9	7	520.44	3.5	-2.0279
10097848	10097848	8	6	510.49	3.9	-0.66701
10621	10621	15	8	610.56	-1.06	2.0396
5154	5154	4	0	332.33	2.88	-2.4707
5213	5213	10	5	482.44	1.59	0.22481
1.3E+08	1.3E+08	19	12	651.48	-1.13	0.62055
222154	222154	8	4	530.65	2.6	0.14199
441688	441688	16	11	611.53	-2.67	-8.6993
5280637	5280637	11	7	448.38	0.15	-3.2535
5282160	5282160	12	8	464.38	-0.24	-3.7091
6473766	6473766	4	2	338.4	2.38	-0.41
46173996	46173996	11	9	562.52	2.34	0.43168
471393	471393	10	7	442.37	1.24	0.37426
5320826	5320826	13	9	480.38	-0.72	-3.7091
64945	64945	3	2	456.7	5.93	-3.658
5280805	5280805	16	10	610.52	-1.51	1.9337
10494	10494	3	2	456.7	6.07	-1.782
5280343	5280343	7	5	302.24	1.23	-0.082832
5280804	5280804	12	8	464.38	-0.48	-3.6679
5280863	5280863	6	4	286.24	1.58	-0.082832
5281670	5281670	7	5	302.24	1.2	-0.082832
5281718	5281718	8	6	390.38	0.64	-4.3466
5318585	5318585	6	4	354.35	3.09	-0.33653

10168	10168	6	3	284.22	1.48	-1.1733
16203170	16203170	12	11	580.54	1.68	0.31525
439533	439533	7	5	304.25	0.51	0.44477
5280794	5280794	1	1	412.69	6.98	1.2217
5281614	5281614	6	4	286.24	1.55	-0.082832
5281616	5281616	5	3	270.24	1.99	-0.082832
246330	246330	6	4	288.25	0.91	0.44477
275196	275196	8	0	413.42	2.61	4.3358
440832	440832	5	4	271.24	0.73	-6.14
442428	442428	14	8	580.53	-0.87	0.64246
5317435	5317435	6	4	288.25	0.89	0.44477
10607	10607	8	1	414.41	2.33	0.17227
345501	345501	7	0	398.41	3.08	0.17601
3503	3503	8	6	518.55	5.04	-4.3442
444170	444170	18	8	663.43	-3.77	-29.561
5281612	5281612	6	3	300.26	2.19	0.40331
5281665	5281665	6	4	286.24	1.72	0.28194
5281672	5281672	8	6	318.24	0.79	-0.082832
9890209	9890209	3	1	454.68	5.95	-5.7793
11385155	11385155	5	2	440.57	4.36	-1.4045
11729855	11729855	5	1	438.56	4.54	-2.7591
12315393	12315393	4	2	384.51	3.68	-0.95205
12315515	12315515	4	2	474.72	5.94	-20.119
16760705	16760705	6	2	470.6	3.42	1.6889
5280445	5280445	6	4	286.24	1.73	0.28194
5281697	5281697	6	4	286.24	1.81	0.28194
72281	72281	6	3	302.28	1.91	-0.0783
72435	72435	8	1	414.41	2.32	0.17227
1.02E+08	1.02E+08	3	3	460.73	5.77	-2.0603
10219	10219	6	1	480.64	4.24	3.8313
12315516	12315516	4	2	474.72	5.98	-20.119
5280459	5280459	11	7	448.38	0.22	1.9289
5281855	5281855	8	4	302.19	1	-1.5983
932	932	5	3	272.25	1.84	-0.22006
21582894	21582894	2	1	442.72	6.5	-4.2965
5281607	5281607	4	2	254.24	2.55	0.28194
5281643	5281643	12	8	464.38	-0.38	-3.6679
5281647	5281647	11	8	422.34	-0.77	-3.0467
5282102	5282102	11	7	448.38	-0.09	-3.6679
92765	92765	4	0	377.48	4.34	0.52242
24360	24360	5	1	348.35	2.2	5.3292
3220	3220	5	3	270.24	1.87	-1.1275
443013	443013	8	1	414.41	2.32	0.17227

13893946	13893946	1	1	426.72	7.51	-5.3696
215159	215159	7	0	369.37	2.89	2.7556
40305	40305	4	1	350.39	2.69	-2.1697
442835	442835	7	0	400.42	3.3	0.34682
5280961	5280961	5	3	270.24	2.04	-0.093853
72276	72276	6	5	290.27	0.85	0.31525
259846	259846	1	1	426.72	7.28	-22.172
3884	3884	3	1	242.27	2.54	-3.8914
44584517	44584517	2	1	442.72	6.81	-2.8027
11012233	11012233	2	2	306.48	3.88	-0.37188
5281677	5281677	7	2	344.32	2.61	-0.10513
5487772	5487772	5	2	301.29	2.04	1.1795

ADME and Toxicity predictions

The compounds with good binding energies were further studied for their adsorption, distribution, excretion, metabolism, and toxicity profile using SWISS ADME(Daina et al., 2017) and data warrior tools (Sander et al.,2009, Sander et al., 2015). The predicted properties considered were blood-brain barrier penetration properties, human intestinal absorption, inhibition to cytochrome P450 enzyme and PGP substrate binding. Compounds showing satisfactory properties were further studied for their toxicity profile using data warrior tools. Toxicity profiles included were mutagenicity, tumorigenicity, irritability, reproducibility, Ames toxicity and carcinogenesis.

Table 2. ADMET analysis with the Lowest Binding affinity.

PubChem ID	BBB Penetration	HIA	CYP 2D6 Inhibitor	PGP substrate binding	Mutagenic	Tumorigenic	Reproductive effect	Irritant	Binding affinity
73659	No	High	No	Yes	none	None	none	None	-13.2
36462	No	Low	Yes	Yes	none	None	none	None	-12.3
357293	Yes	High	Yes	No	none	None	none	High	-10
5271805	No	Low	No	No	none	None	none	None	-9.6
5281627	No	Low	No	No	none	None	high	None	-9.4
3663	No	Low	No	No	low	High	none	High	-9.3
107876	No	Low	No	No	none	None	none	None	-8.8
439501	No	Low	No	No	none	None	none	None	-8.8
4978	No	Low	No	No	high	High	none	High	-8.8
10097848	No	Low	No	No	none	None	none	None	-8.7
10621	No	Low	No	Yes	none	None	none	None	-8.7
5154	Yes	High	No	Yes	none	None	none	None	-8.7
5213	No	Low	No	No	none	None	none	None	-8.6
129693153	No	Low	No	Yes	none	None	none	None	-8.5
222154	No	High	Yes	Yes	none	None	high	None	-8.5

441688	No	Low	No	No	none	None	none	None	-8.5
5280637	No	Low	No	Yes	none	None	none	None	-8.5
5282160	No	Low	No	Yes	high	None	none	None	-8.5
6473766	Yes	High	Yes	Yes	none	None	none	None	-8.5
46173996	No	Low	No	No	none	None	none	None	-8.4
471393	No	Low	No	No	none	None	none	None	-8.4
5320826	No	Low	No	No	high	None	none	None	-8.2
64945	No	Low	No	No	none	None	none	None	-8.2
5280805	No	Low	No	Yes	none	None	none	None	-8.1
10494	No	Low	No	No	none	None	none	None	-8
5280343	No	High	Yes	No	high	High	none	None	-8
5280804	No	Low	No	No	none	None	none	None	-8
5280863	No	High	Yes	No	high	None	none	None	-8
5281670	No	High	Yes	No	high	None	none	None	-8
5281718	No	High	No	Yes	none	None	high	None	-8
5318585	No	High	Yes	No	high	None	none	None	-8
10168	No	High	No	No	none	None	none	High	-7.9
16203170	No	Low	No	No	none	None	none	None	-7.9
439533	No	High	No	No	none	None	none	None	-7.9
5280794	No	Low	No	No	none	None	none	None	-7.9
5281614	No	High	Yes	No	high	None	none	None	-7.9
5281616	No	High	Yes	No	high	None	none	None	-7.9
246330	No	High	No	No	none	None	none	None	-7.8
275196	No	High	Yes	No	none	None	none	None	-7.8
440832	No	High	No	Yes	none	None	none	None	-7.8
442428	No	Low	No	Yes	none	None	none	None	-7.8
5317435	No	High	No	No	none	None	none	None	-7.8
10607	No	High	Yes	No	none	None	high	None	-7.7
345501	Yes	High	Yes	No	none	None	high	None	-7.7
3503	No	Low	No	No	low	High	none	None	-7.7
444170	No	Low	No	No	none	None	none	None	-7.7
5281612	No	High	Yes	No	none	None	none	None	-7.7
5281665	No	High	Yes	No	high	None	none	None	-7.7
5281672	No	Low	No	No	high	None	none	None	-7.7
9890209	No	Low	No	No	none	None	none	None	-7.7
11385155	No	High	No	Yes	none	None	none	None	-7.6
11729855	No	High	No	Yes	none	None	none	None	-7.6
12315393	Yes	High	No	Yes	none	None	high	None	-7.6
12315515	No	Low	No	No	none	None	none	None	-7.6
16760705	No	High	No	Yes	none	None	low	None	-7.6
5280445	No	High	Yes	No	none	None	none	None	-7.6
5281697	No	High	Yes	No	none	None	none	None	-7.6
72281	No	High	No	Yes	none	None	none	None	-7.6

72435	No	High	Yes	No	none	None	high	None	-7.6
101974031	No	High	No	No	none	None	none	None	-7.5
10219	Yes	High	No	Yes	none	High	none	High	-7.5
12315516	No	Low	No	No	none	None	none	None	-7.5
5280459	No	Low	No	No	none	None	none	None	-7.5
5281855	No	High	No	No	none	None	none	None	-7.5
932	No	High	No	Yes	none	None	none	None	-7.5
21582894	No	Low	No	No	none	None	none	None	-7.4
5281607	Yes	High	Yes	No	none	None	none	None	-7.4
5281643	No	Low	No	No	none	None	none	None	-7.4
5281647	No	Low	No	No	high	None	none	None	-7.4
5282102	No	Low	No	No	none	None	none	None	-7.4
92765	Yes	High	Yes	Yes	low	High	none	None	-7.4
24360	No	High	No	Yes	none	None	none	None	-7.3
3220	No	High	No	No	high	High	high	High	-7.3
443013	No	High	Yes	No	none	None	high	None	-7.3
13893946	No	Low	No	No	none	None	none	None	-7.2
215159	No	High	Yes	No	none	None	none	None	-7.2
40305	Yes	High	Yes	Yes	none	None	none	None	-7.2
442835	Yes	High	Yes	No	none	None	none	None	-7.2
5280961	No	High	Yes	No	high	High	high	None	-7.2
72276	No	High	No	Yes	none	None	none	None	-7.2
259846	No	Low	No	No	none	None	none	None	-7.1
3884	Yes	High	No	No	none	None	high	None	-7.1
44584517	No	Low	No	No	none	None	high	High	-7.1
11012233	Yes	High	No	Yes	none	None	none	None	-7
5281677	No	High	Yes	No	high	None	none	None	-7
5487772	No	High	Yes	No	none	None	low	None	-7

Molecular dynamic simulation

Docked protein and ligand complex was subjected to molecular dynamics simulation using NAMD software (Phillips et al., 2020). The success of MD simulation depends on the selection of the initial protein and ligand structures. Initially, the structure was checked for inconsistencies. Out of 86 compounds selected from the docking results, we have selected the Taxifolin with PubChem number 439533 as the best ligand. The docked complex was studied for its stability during the simulation. The root means square deviation, root mean square fluctuation, and radius of gyration was studied for protein backbone residue and ligand within the binding site of the simulated system (Bornot et al., 2011, Kufareva & Abagyan, 2012, Lobanov et al., 2008).The stability of the complex was examined by monitoring its root mean square deviation (RMSD) during 50,00,000 steps for a 10 ns simulation. MD simulations were performed using the CHARMM36 force field (Soteras et al., 2016). Visual molecular dynamics (VMD) was used to generate PSF files for the complex

(Sander et al., 2009). The complex was solvated in cubic water boxes containing transferable intermolecular potential with 3 points (TIP3P) water molecules. The box size was chosen to match the molecular dimensions so that there was a distance of 5˚A between the protein surface and the edges of the periodic box. A 5˚A cut off distance was used to calculate short-range non-bonded interactions. The particle mesh Ewald (PME) method was used to calculate long-range electrostatic interactions. The SHAKE method was used to constrain all the bonds involving hydrogen atoms. A conjugated gradient system was used for energy minimization, with all parameters set to default. The system first performed 10000 steps of Conjugated gradient with energy minimization. We used Langevin Dynamics with pressure control so our system was not an NVT ensemble. The Nose–Hoover method was used to maintain a constant temperature. The time step of each simulation was set to 2 fs (Phillips et al., 2020, Phillips et al., 2005, Ribeiro et al., 2016). Visualizations and data analysis were performed with VMD software (Hsin et al., 2008).

Results and discussion

Virtual screening and docking results

Virtual screening helps us to screen the biological molecules with good binding affinity. In this study, we have used PyRx 8.0 tool to screen out the molecules. A total of 162 natural ligands were selected and were docked to the target protein.

The docked compounds were examined in the Auto dock tool and binding free energy was calculated (Cosconati et al., 2010, Morris et al., 2009). We have selected a total of 86 compounds on their binding affinity ranging from -13.2 to -7.0 kcal/mol **(Table 2)**.

ADMET analysis

We had selected 86 compounds and the same compounds were studied for their ADMET properties. The properties like human intestinal absorption, irritability, reproductive effect, inhibition to cytochrome p450 enzyme, and several others were predicted. It was clear from the results that compound number 439533, 246330, 275196, 5317435 and 101974031 have a high intestinal absorption value. From the Table 2, it was also observed that we had several compounds like 357293, 222154, 73659, 11385155, and 11729855 have high intestinal absorption values but at the same time they are also showing their inhibitory properties towards the Cytochrome P450 enzymes and PGP substrate binding, so such compounds were removed from the further selections. Similarly, from the selected compounds with high intestinal absorption values and negative inhibitory actions to cytochrome P450 enzymes, compounds were also studied for their mutagenic, tumorigenic, irritability, and reproductive effect. So the compounds having either of the side effects were also removed from the study. Finally, we selected four ligands namely Taxifolin_CID_439533, Dihydrofisetin_CID_246330, Narcotine_CID_275196 and Fustin_CID_5317435 which complied all the parameters of ADMET. Among these we selected Taxifolin_CID_439533 for further analysis considering its higher binding affinity compared to other three compounds. The ligand selected for further study was having hydrogen bonds and also presents a hydrophobic

interaction with the protein **Table 3** &**Table 4**.

Protein-ligand interaction

The hydrogen bond and hydrophobic interactions of protein-ligand complexes were analyzed by LigPlot+ (v 1.4.5)(38) and Protein-ligand interaction profiler. "LigPlot+" is a graphical system that generates multiple three-dimensional (3D) diagram of ligand-protein interactions from docked complexes. PLIP is complementary to another state of the art tools like a Swiss dock, galaxy site, or ProBis and thus it can be used to study the protein-ligand complex. The server allows comprehensive detection and visualization of protein and ligand complexes along with interaction patterns.

Table 3. Hydrophobic Interaction between Protein and Ligand complex.

Ligand	Residue	AA	Distance	Ligand Atom	Protein Atom
Taxifolin	1191A	PRO	3.46	3216	1873
	1191A	PRO	3.84	3201	1874
	1191A	PRO	3.58	3215	1874
	1203A	LEU	3.58	3199	1988
	1203A	LEU	3.62	3212	1988
	1221A	ILE	3.54	3209	2154
	1239A	LYS	3.43	3219	2344
	1243A	LEU	3.67	3219	2388

Table 4. Hydrogen bond interaction between Ligand and Protein complex.

Ligand	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor	Side chain	Donor Atom	Acceptor Atom
Taxifolin	1203A	LEU	2.38	2.93	115.54	×	×	3193[O3]	1987[O2]

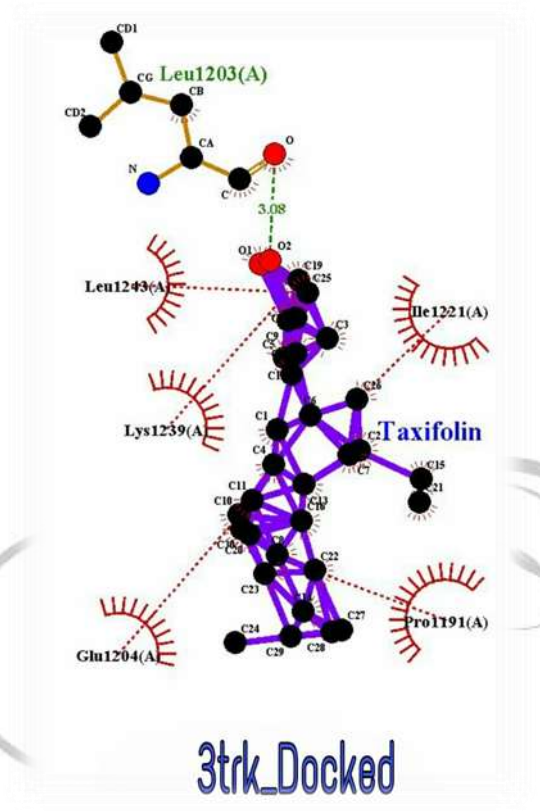

Figure 1. Protein and Ligand interaction Diagram.

Molecular Dynamic Simulation studies

We assessed the residue RMSD to study the residue behaviour of the protein during the simulations. In general, a residue's RMSD value was considered to represent the local flexibility of a protein and ligand complex. It reflected the mobility of an atom during the MD simulation trajectory. Therefore, a higher residue RMSD value indicated higher mobility; conversely, a lower residue RMSD value indicates lower mobility. To investigate the fluctuations in the ligand-binding energy as well as the motions of the amino acid residues within the complex during the simulation, the root

means square fluctuation (RMSF) of the complex was also monitored. Besides, the compactness of the complex was determined by carefully examining how folded or unfolded the protein-ligand complex was by calculating the radius of gyration (Lobanov et al., 2008). Based on the docking analysis 86 compounds were selected for further ADMET investigation and it leads us to select the final compound (Taxifolin_CID_439533) to consider the structural stability of the protein-ligand complex by molecular dynamic simulation. The stability of the complex (3TRK_Taxifolin) was monitored using root mean square deviation (RMSD) during 10 ns simulation studies.

Table 5. RMSD values for the simulated complexes.

Protein-ligand complex	Mean RMSD(Å)	Min RMSD(Å)	Max RMSD(Å)
3TRK_Taxifolin	2.018	0.057	3.074

The values presented in (Table 5) for the protein-ligand complex studied for its stability during 10 ns simulation. From the values, it is clear that the range of RMSD obtained for the complex complies with the acceptance range of 1 to 3.5 (Å). It is also observed from the graphs that the complex was also equilibrated as the average RMSD values are stabilized at the end of the 10 ns simulation. This fixed range of RMSD was indicating the interaction between bound ligand and flexible loop region, as it reduces the flexibility of the protein-ligand complex.

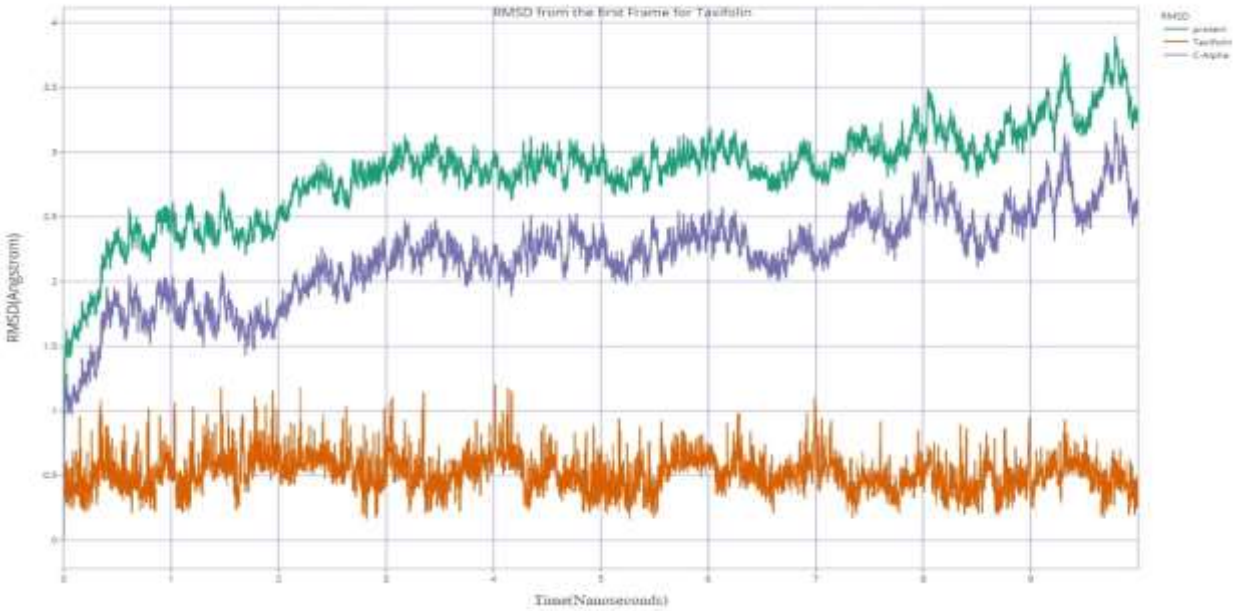

Figure 2. RMSD results for Taxifolin with 3TRK protein, based on 10 ns simulation.

The root means square fluctuations (RMSF) were assessed and plotted to equate the flexibility of the residue in the ligand-protein complexes. The RMSF of the protein-ligand complex denoted the minimized fluctuation for the complex. The RMSF did not deviate much during the simulation period of 10 ns and the average RMSF values were kept constant for the complex.

The radius of gyration was also monitored during the 10-nsMD simulation for the protein-ligand complex to ascertain whether the complex was stably folded or unfolded. If the radius of gyration remained relatively constant, the complex was considered to be stably folded, otherwise, it was considered to be unfolded.

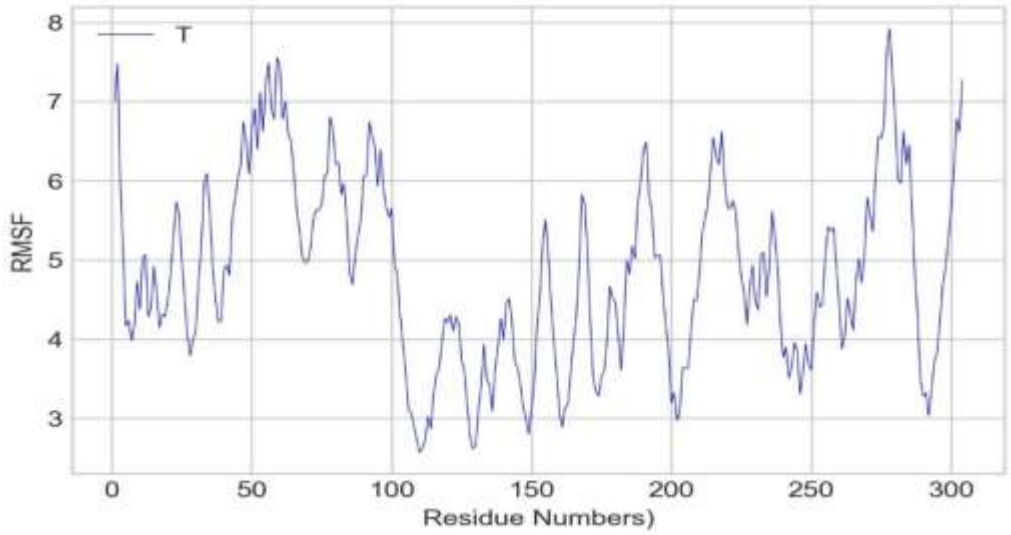

Figure 3. RMSF results for Taxifolin with 3TRK protein, based on the data from 10 ns simulation.

Table 6. The radius of Gyration for Protein-Ligand complexes.

Protein-Ligand complex	Mean	Min	Max
3TRK_Taxifolin	21.257	20.343	21.963

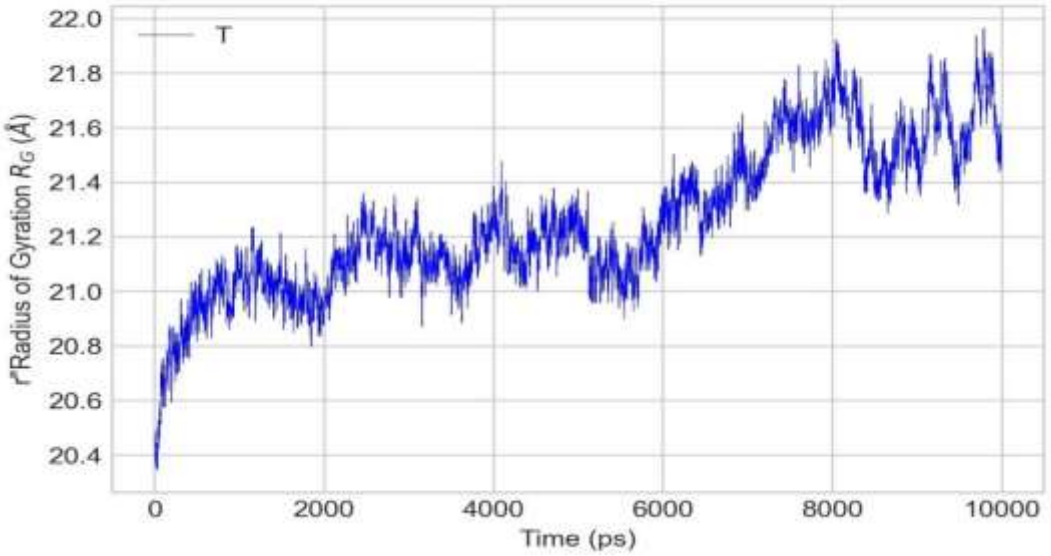

Figure 4. The radius of Gyration results for Taxifolin with 3TRK protein, based on the data from the 10 ns simulation.

In this study, the radius of gyration values obtained is listed in Table 6. All the values obtained for the test ligand Taxifolin showed a relatively constant radius gyration during the simulation. So, we can conclude that all of the complexes formed relatively stable folded

polypeptide structures during the 10-ns MD simulation.

CONCLUSION

In our entire study, we tried to come up with an active natural compound for the treatment of Chikunguniya infection. We investigated 162 natural compounds for their activity against NsP2 Protease of the Chikunguniya Virus through computational methods including molecular docking and ADMET profiling using available software, and found four compounds (Taxifolin_CID_439533, Dihydrofisetin_CID_246330, Narcotine_CID_275196 and Fustin_CID_5317435) that complied with all the parameters. Among these we selected Taxifolin_CID_439533 for further analysis considering its higher binding affinity compared to Dihydrofisetin_CID_246330, Narcotine_CID_275196 and fustin_CID_531735. Further the molecular dynamic simulation of 3TRK_Taxifolin complex was carried out to study its structural stability by investigating its RMSD, RMSF and radius of gyration values. By these investigations we conclude that Taxifolin can be a potential drug against Chikunguniya Virus (CHIKV).

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