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Chikungunya Virus: New Drug Prospects Emerging from Molecular Docking Studies for Medicinal Biotechnology

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ABSTRACT

The Chikungunya virus (CHIKV) cases were ubiquitously reported in several countries of the North American region, but with time this virus has been spread throughout the world. The Indian subcontinent is not an exception. Till date, the absence of any appropriate drugs or vaccines against the CHIKV makes the research scenario more challenging towards the identification and development of novel lead compounds essential for the same. The Cysteine protease (nsp2) has been identified as a key drug target molecule for combating infections induced by alpha-viruses like the CHIKV. CHIKV nsp2 has an extremely compact structure with RNA-binding surface domains, which make nsp2 more efficient for genome replication during pathogenesis. The present study aims to investigate the novel inhibitors for the nsp2 protein domain using in-silico approach. The Tertiary structure of target protein and various antimicrobial drugs were retrieved from protein data bank and drug bank database respectively. The docking studies are performed and it is observed that Telaprevir is having the highest binding affinity followed by Doxycycline, Sennoside A, Acarbose, and Trobicin. Telaprevir is a widely used antiviral drug for the treatment of chronic Hepatitis c virus. Therefore these drugs can be reprofiled as a potential inhibitor of nsp2.

KEY WORDS: ANTIVIRAL DRUGS, CHIKUNGUNYA VIRUS, MOLECULAR DOCKING, NSP2, DRUG REPROFILING.

INTRODUCTION

Chikungunya (CHIKV) is an epidemic arbovirus that is often used to describe both the virus and the disease. The virus is transmitted mainly to humans through the bite of an infected mosquito of the genus Aedes (Pialoux et al., 1953). The disease generally consists of such a severe infection that cause fever, rashes, and musculoskeletal pain (to walk bent over) is the hallmark of chikungunya that characterizes this dengue-like illness (Staples,

Breiman and Powers, 2009; M Dubrulle et al - 2009; Caglioti et al., 2013; Lo Presti et al., 2014).

There have been several CHIKV outbreaks that have been contributed to describing chikungunya fever in detail and identified maculopapular rash predominantly on the thorax, facial edema a bullous rash with pronounced sloughing, and localized petechial rash. It intensely, affects main extremities, large and small joints eg: ankles, wrists, phalanges (Lo Presti et al., 2014). CHIKV is been carried by an infected female mosquito to the host the mosquito inoculates virus-containing saliva into the bloodstream of a new victim (Lo Presti et al., 2014) (Fig. 1).

CHIKV is an enveloped, spherical body of about 70nm in diameter. The virion genome consists of a Monopartite, linear single-stranded (ss), positive-sense RNA molecule

of approximately 11.8 kb long, where the 5' end is capped with a 7-methylguanosine while the 3' end is poly-adenylate. The viral genome contains 2 polyproteins represent four non-structural proteins and five structural proteins (Fig. 2). The replication and propagation of the virus is regulated by nsp2 protein, therefore, it is hypothesized that a compound that inhibits the nsp2 will be a promising and potential drug molecule. In the Era of drug reprofiling efforts can be made to identify a promising inhibitory molecule from the existing antiviral drugs for the treatment of CHIKV the identified potential inhibitors for CHIKV may serve as an inhibitory molecule for other viruses also.which may provide a clear potential path towards the identification of broad-spectrum drugs. (Singh et al., 2011) (Fig. 2).

Figure 1: Transmission cycle of CHIKV
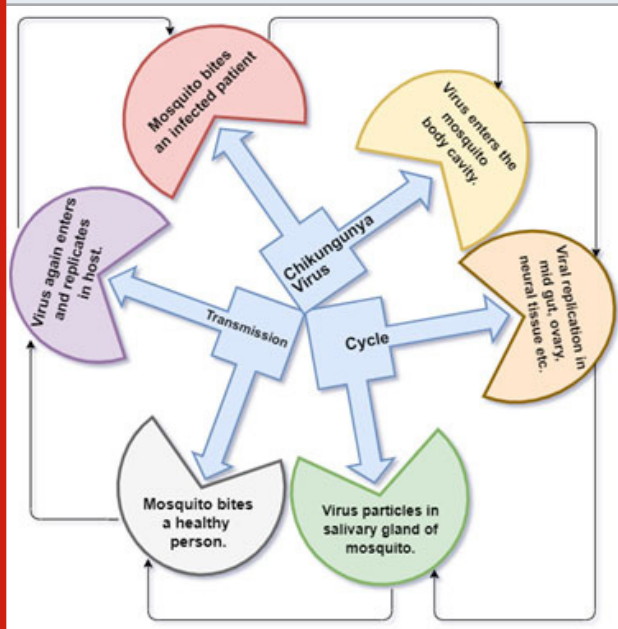
MATERIAL AND METHODS

Retrieval of Target and Lead molecule: The nsp2 crystal structure was retrieved from the protein data bank (PDB) (www.rcsb.org). The retrieval of protein was followed by energy minimization using PYMOL (a user-sponsored molecular visualization system, version 2). The minimization process includes the removal of water molecules, sodium ion, l-peptide linking, and the gaps between amino acids. The lead compound for nsp2 protease was retrieved from PubChem and Drug bank database (Table1). The small molecules were optimized with AVOGADRO: open-source molecular builder and visualization tool(version 1). The optimization process was done with the false parameters that is the force field is off, steps per update is 4, the algorithm is the steepest descent.

Molecular docking studies: Before performing molecular docking studies, we need to identify the binding pockets of the protein molecule. The Automated active site

Figure 2: Schematic description of both structural and nonstructural proteins within the polyprotein CHIKV. CHIKV RNA 11811 bases (top bar, purple color), translates into non-structural and structural precursor polyproteins of 2474 and 1244 residues, respectively, after maturation by protease cleavage, it gives 4 non-structural proteins (left bar, green color) and 5 structural proteins (right bar, red color).
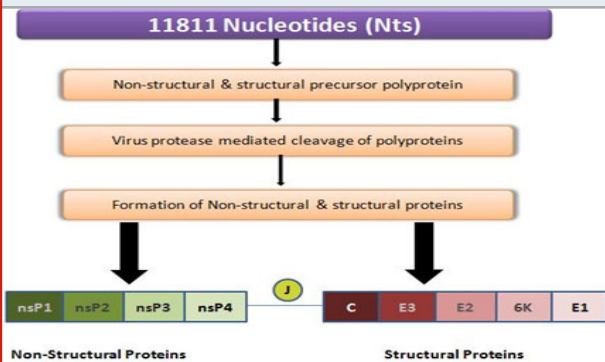
docking and scoring (AADS) is used in this analysis to identify binding pockets. The AADS (http://www.scfbio-iitd.res.in/dock/ActiveSite_new.jsp) utilizes the 3D structure of target molecules and identify top 10 possible binding sites with 100% precision in identifying the real (active) binding sites (Table 4). Once the protein binding pockets are identified, the Small Molecules Library (Table 1) is screened against these sites to identify the hit molecules using the software. For this study, PyRx and AutoDockVina software were used to analyze the ligand-protein binding properties to the protein.

The blind dockings were performed in which the grid boxes' size was adjusted to cover the binding site. Once the docking is complete the resulting PDBQT output file was opened in the PyMOL software for converting all protein conformations into one file analysis on further studies. Afterward, each conformation was examined using Discovery Studio 2.5 software, using information like binding affinities, interaction energies, van der Waals energies, electrostatic energies, hydrogen bonding,pi-pi interactions, pi-cation interactions and close contacting residues were obtained and recorded. The compounds were screened against nsp2 using the PyRx tool to identify the ligands with the best conformers to the target protein.

RESULTS AND DISCUSSION

During Retrieval of the target molecule, the nsP2 with the PDB ID – 3TRK was retrieved and 52 lead compounds were listed (Table1) these lead compounds were screened for potential inhibitory activities against the top 10 binding sites (Table 4) CHIKV's non-structural protein nsp2. The docking studies for the top 10 binding sites of nsp2 (Table3) the docking studies of all 52 lead compounds with 10 binding sites.

Table 1. List of ligands involved in protein–ligand interaction.

S.No.	Drugs	REFERENCE
1.	(R)-Chloroquine	Andersag H et al., 1941
2.	Acarbose	S. P. Clissold et al 1988
3.	Acetaminophen	Kis B et al., 2005
4.	Amikacin Sulfate	Overington JP et al., 2006.
5.	Aspirin	Sneader W ., 2000
6.	Arbidol	Hui Peng et al., 2020
7.	Baicalein (Natural Compound)	Oliveira et al., 2017
8.	Bisdesethylchloroquine	Ajayi FO et al., 1989
9.	Boceprevir	Jennifer J Kiser et al, 2013
10.	Boswellic acid	Arne Henkel et al, 2012
11.	Cefadroxil (Sumacef)	Leonardo Marsili., 1978
12.	Celecoxib	Yi Yu Ke et al., 2020
13.	Chloroquine	Vincent MJ et al., 2005
14.	Cletoquine	Dongre VG et al., 2009
15.	Curcumin	Fatemeh Zahedipour et al, 2020
16.	Desethylchloroquine	Frisk-Holmberg M et al., 1984
17.	Didesethylchloroquine Hydroxyacetamide	Abraham MJ et al., 2015
18.	Dihydrostreptomycin Sulfate	CURCI G ., 1951
19.	Diminazene Aceturate	R. Ghildiyal et al., 2019
20.	Docosanol	Hardman et al 2001
21.	Doxycycline	Dahl EL et al 2006
22.	E-64 (Zinc13493525)	Zheming Wang et al. 2008
23.	Etidronate (Etidronic Acid)	Rogovin et al 1968
24.	Fisetin (Natural Compound)	Liu L et al 2019.
25.	Glucosamine Sulphate	Arvind Chopra et al, 2013
26.	Hesperetin	Samie A et al., 2018
27.	Hydroxychloroquine	Lim HS et al. 2009
28.	Ibandronate Sodium	Epstein S et al. 2005
29.	Ibuprofen	Casper D et al., 2000
30.	Imatinib	Deininger MW et al2003
31.	Iron Sucrose	Hörl WH 2007.
32.	Kanamycin Sulfate	Vetting MW et al. 2002.
33.	Ketotifen	Roy W. Bryant et al. 2011
34.	Leupeptin Hemisulfate	Pérez-Pérez et al 2019
35.	Mitoxantrone Hydrochloride (Novantrone)	Fox EJ 2006.
36.	N-Acetyl (Mono) Desthylchloroquine	E. E. Essien et al1989
37.	Naproxen	Wongrakpanich S et al., 2018
38.	Nelfinavir	Kaldor SW et al 1997
39.	Niacin	Briggs gg, et al.,1998
40.	Officinalis acid	Mohammed Bourhia et al., 2019
41.	Pemetrexed Disodium Hemipentahydrate	Prateek Kumar et al.
42.	Pirodavir	Jef Peeters et al. 2007
43.	Pleconaril	Florea NR et al 2003
44.	Prednisolone	Maryam Daneshpazhooh ., 2020
45.	Quercetagetin (Natural Compound)	Weiyou Wang et al 2016,
46.	Ribavirin	Sidwell RW et al. 2005
47.	Ribostamycin Sulfate	Zhou et al.1992
48.	Sennoside A	Esposito F et al 2016
49.	Sofosbuvir	Asselah T 2013
50.	Spectinomycin Hydrochloride Hydrate (Trobicin)	David R. White 1966
51.	Telaprevir	Kim JJ et al. 2012
52.	Zinc Acetate	Berni Canani R et al 2011

The study suggests out of 52 lead compounds the four compound Telaprevir, Doxycycline, Acarbose, Sennoside A showed significant binding affinity whereas spectinomycin hydrochloride (trobicin), Baicalin, Ibandronate sodium, Quercetagetin, Mitoxantrone hydrochloride, and Fisetin showed promising binding affinity (Table.4 and5.). Telaprevir showed the strongest binding affinity (-12.3kcal/mol), is a member of protease blockers (a group of antiviral medicine). These affinities and energies are due to interaction and bond formation between lead molecules and binding site amino acid of nsp2.

Table 2. Parameters used for molecular docking of top ten ligands with the protein of interest. All grid boxes with a spacing size of 1.000 A˚ have sufficient sizes to cover the entire protein structures during molecular docking.

S.No.	Ligands with Protein	Center-X	Center-Y	Center-Z	Size-X	Size-Y	Size-Z
1.	3trk_Acarbose	12.815566 6274	26.263485 9746	21.59923 82951	82.01098 38983	84.3446 57921	61.15847 77994
2.	3trk_Baicalin	11.6975 98268	23.474 79489	28.50747 38773	67.57002 08963	85.802520 3587	98.212213 4629
3.	3trk_Doxycycline	11.3741 9874	23.059632 7058	21.68560 01381	71.67382 45391	86.78730 81266	53.17848 38552
4.	3trk_Fisetin	28.92522 80574	24.68480 42594	19.22511 98554	115.8926 12756	88.46105 80017	86.8902 924293
5.	3trk_Ibandronate sodium	28.9252 280574	24.6848 042594	19.2251 198554	115.8926 12756	88.4610 580017	86.8902 924293
6.	3trk_Mitoxantrone hydrochloride	28.6614 606151	20.2939 940445	19.3423 23373	104.00 1803984	95.79584 89908	74.5569 102783
7.	3trk_Quercetagetin	12.2522 153681	25.5314 252099	22.5563 495924	70.093 582201	93.8091 943285	81.4775 121373
8.	3trk_Sennoside A	12.2522 153681	25.5314 252099	22.5563 495924	70.093 582201	93.80919 43285	81.47751 21373
9.	3trk_spectinomycinhydrochloride	16.17368 49469	22.7882 300823	17.3275 975767	88.09715 54836	88.323 2612193	73.2683 286745
10	3trk_Telaprevir	12.25221 53681	25.53142 52099	22.5563 495924	70.0935 82201	93.8091 943285	81.47751 21373

Table 3. Cavity details of Nsp2 Protein

S.No.	Cavity Points			V	A	D	R	h	
1.	124.023	55.309	63.320	0.94	0.39	0.42	1.00	0.68	0.6857
2.	100.718	64.861	70.832	0.94	0.56	0.50	0.75	0.63	0.6755
3.	109.046	35.144	85.035	1.00	0.17	0.77	0.50	0.80	0.6465
4.	86.588	66.635	79.871	0.71	0.39	0.46	0.62	1.00	0.6371
5.	92.209	49.198	90.901	0.78	1.00	0.62	0.25	0.41	0.6107
6.	-16.257	-22.551	-4.530	0.98	1.00	1.00	0.83	0.35	0.8326
7.	-23.802	-30.007	2.100	1.00	0.22	0.57	1.00	0.61	0.6804
8.	-7.096	-43.641	-3.465	0.98	0.72	0.53	0.67	0.40	0.6595
9.	-5.740	-45.699	-23.319	0.62	0.72	0.77	0.50	0.48	0.6173
10.	-24.954	-46.789	4.600	0.30	0.67	0.47	1.00	0.53	0.5934

The result shows the amino acid residue found in the binding pocket between Telaprevir and nsP2, are SER1048, GLN1241, TRP1084, TYR1047, ASN1082, TYR1079, ALA1046, CYS1013, LYS1091, GLU1048, VAL1051, ARG1271, THR1268, ARG1267, TRP1014, HIS1083, LEU1205, and GLU1204 a Fig 3. The hydrogen bonds between Telaprevir, Doxycycline, Acarbose, Sennoside A, spectinomycin hydrochloride (trobicin), Baicalin, Ibandronate sodium, Quercetagetin, Mitoxantrone hydrochloride, Fisetin, and 3TRK are also as shown in (Table 4). between Telaprevir and nsP2, are SER1048, GLN1241, TRP1084, TYR1047, ASN1082, TYR1079, ALA1046, CYS1013, LYS1091, GLU1048, VAL1051, ARG1271, THR1268, ARG1267,

TRP1014, HIS1083, LEU1205, and GLU1204 a Fig. 3. The hydrogen bonds between Telaprevir, Doxycycline, Acarbose, Sennoside A, spectinomycin hydrochloride

(trobicin), Baicalin, Ibandronate sodium, Quercetagetin, Mitoxantrone hydrochloride, Fisetin, and 3TRK are also as shown in (Table 4).

Table 4. Hydrogen Bonding Between the top hit compounds from the blind docking and CHIKV Nsp2. This table documents the Residues involved in the Discovery Studio 2.5. The binding affinities as ranked by the PyRx 8.0 and Auto Dockvinal 1.5.6 are recorded in the final column of the table.

Ligands with Protein	Hydrogen bonds	AngleDHA(')	Distance(A°)	Binding affinity (kcal/mol)
3trk andTelaprevir	:UNL1:HN – A:ASN1082:O	119.232	2.43416	-12.3
	:UNL1:HN – :UNL1:O	133.404	2.79452	
	:UNL1: HN – A: TYR1047:O	151.652	2.0751	
3trk and doxycycline	A: TYR1047: HN – :UNK0: O	146.817	2.86321	-11.8
	A: TRP1084: HE1 – :UNK0: O	155.005	1.69013	
3trk andAcarbose	A: TYR1047: HN – :UNK0: O	148.924	2.3149	-10.9
	A: SER1048: HG – :UNK0: O	107.507	2.42619	
	A: TRP1084: HE1 – :UNK0: O	147.087	2.46832	
3trk andSennoside A	A: TYR1079: HH – :UNK0: O	152.837	1.82834	-10.9
	A: TRP1084:HE1 – :UNK0: O	135.362	2.37549	
	A: GLN1241: HE22 – :UNK0: O	108.266	2.53045	
3trk and spectinomycin hydrochloride(trobicin)	A: TRP1084: HE1 – :UNK0: O	160.856	2.14139	-8.9
	A: TRP1084: HE1 – :UNK0: O	142.698	2.27101	
	:UNK0: H – A:TYR1079: OH	94.399	2.72027	
3trk and baicalin	A: TRP1084: HE1 – :UNK0: O	150.648	2.03652	-8.1
	A: GLN1241: HE22 – :UNK0: O	99.059	2.87101	
	:UNK0: H – A:TYR1079: OH	138.871	2.70173	
	:UNK0: H – A:ASN1082: OD1	150.896	2.76228	
3trk and Ibandronate sodium	A: TYR1047: HN – :UNK0: O	162.318	2.22615	-8
	A: TRP1084: HE1 – :UNK0: O	132.257	2.66686	
	:UNK0: H – A:TYR1079: OH	102.006	2.77491	
	: UNK0: H – A: TYR1047: O	137.74	2.21746	
	: UNK0: H – A: TYR1047: O	147.153	2.12432	
3trk andQuercetagetin	A: TYR1047: HN – :UNK0: O	149.998	2.84355	-7.9
	A: TYR1047: HN – :UNK0: O	165.015	2.2247	
	A: TRP1084: HE1 – :UNK0: O	135.434	2.27803	
3trk and Mitoxantrone hydrochloride	A: TYR1047: HN – :UNK0: O	157.204	2.30123	-7.8
	A: TRP1084: HE1 – :UNK0: O	173.755	1.82032	
3trk andFisetin	A: SER1048: HG – :UNK0: O	154.341	2.30796	-7.7
	: UNK0: H – A: TYR1047: O	140.152	2.06479	
	:UNK0: H – A:ASP1246: OD2	150.743	2.84791	

Figure 3: 2D diagram of the interaction between telaprevir and nsP2. The diagram shows the ligand– receptor interactions and close amino acid residues found in the binding pocket.
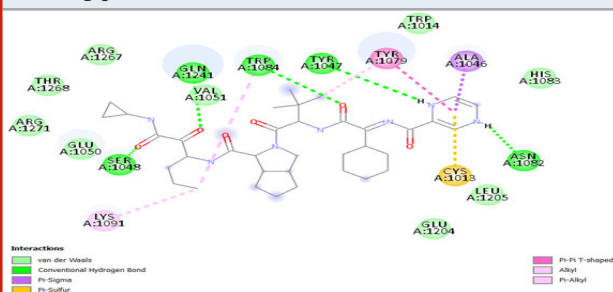

The result of computational studies recommends that Telaprevir, Doxycycline, Acarbose, Sennoside A can be used as nsp2 inhibitors for chikungunya. These lead compounds already exist and were listed in antiviral medicines especially protease blocker so no harm in exploring these drugs for CHIKV inhibition. This significant outcome is for country path in drug reprofiling studies and here we are proposing molecular docking as a tool for exploring new drug prospects from old drugs.

S.No.	Ligand	Binding Affinity	Rmsd/Ub	Rmsd/Lb
	Table 5. Analysis of ligand–receptor interactions			
1.	3trk_Telaprevir	-12.3	2.456	1.087
2.	3trk_Doxycycline	-11.8	5.49	1.561
3.	3trk_Acarbose	-10.9	5.22	2.49
4.	3trk_Sennoside A	-10.9	8.368	0.016
5.	3trk_Spectinomycin hydrochloride (Trobicin)	-8.9	4.485	1.768
6.	3trk_Baicalin	-8.1	8.143	5.091
7.	3trk_Ibandronate sodium	-8	10.415	9.23
8.	3trk_Quercetagetin	-7.9	31.104	30.446
9.	3trk_Mitoxantrone hydrochloride	-7.8	5.817	0.058
10.	3trk_Fisetin	-7.7	6.404	2.937
11.	3trk_Imatinib	-7.7	21.294	19.022
12.	3trk_Proteinase inhibitor E64	-7.7	12.243	10.903
13.	3trk_N acetyl Desethylchloroquine	-7.6	13.291	11.618
14.	3trk_Nelfinavir	-7.5	28.101	24.91
15.	3trk_Beta-Boswellic acid	-7.5	26.375	23.199
16.	3trk_Etidronic acid	-7.4	2.29	0.784
17.	3trk_Celecoxib	-7.4	5.297	3.179
18.	3trk_Officinalic acid	-7.4	13.228	9.67
19.	3trk_Pleconaril	-7.2	19.127	14.524
20.	3trk_Hesperetin	-7.1	8.237	2.364

Figure 4: The receptor-ligand interactions, and bonds between them with the highest binding affinities of Acarbose, Baicalin. Doxycycline, Fisetin, Ibandronate sodium, Mitoxantrone hydrochloride, Quercetagetin, Sennoside A, spectinomycin hydrochloride, and Telaprevir (Grey, Red, and Blue stick structure) when docked against Nsp2 protein (dark green colored ball and stick structure).
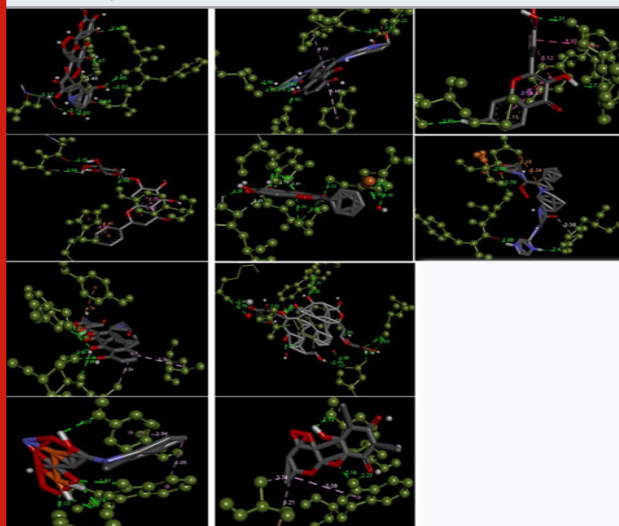
- 3trk_Telaprevir with the binding affinity of -12.3 kcal/mol
- 3trk_Doxycycline with the binding affinity of -11.8 kcal/mol
- 3trk_Acarbose with the binding affinity of -10.9 kcal/mol
- 3trk_Sennoside A with the binding affinity of -10.9 kcal/mol
- 3trk_Spectinomycin hydrochloride with the binding affinity of -8.9 kcal/mol
- 3trk_Baicalin with the binding affinity of -8.1 kcal/mol
- 3trk_Ibandronate sodium with the binding affinity of -8 kcal/mol
- 3trk_Quercetagetin with the binding affinity of -7.9 kcal/mol
- 3trk_Mitoxantrone hydrochloride with the binding affinity of -7.8 kcal/mol
- 3trk_Fisetin with the binding affinity of -7.7 kcal/mol.

CONCLUSION

In our current study, we conclude briefly that Telaprevir, Doxycycline, Acarbose, Sennoside A possesses interactions with CHIKV non-structural protein to (NSP2) which plays a role in the virus replication cycle. These findings enhance our understandings of the possibility of an existing antimicrobial drug molecule to be used for treatment against chikungunya fever. The repurposing of these old drugs to treat chikungunya will become an attractive proposition because it involves the use of no risk compounds with considerably lower development cost and minimal discovery timeline hence further

studies on this target protein and ligands will enhance the development of a novel anti-CHIKV drug.

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