International Journal of Scientific and Innovative Research 2017; 5(2) : 47 - 54
P-ISSN 2347-2189, E- ISSN 2347-4971

MOLECULAR DOCKING AND ADME/TOX STUDY OF NIC LOSAMIDE DERIVATIVES AGAINST NS3 HELICASE OF JEV

Anjali Singh, Ajay Kumar, Vivek Srivastava*

Department of Biotechnology, Rama University, Kanpur, Uttar Pradesh, India

* Address for Correspondence: Vivek Srivastava, Department of Biotechnology, Rama University, Kanpur, Uttar Pradesh, India
Email ID : viveksrivastavabio@gmail.com

ABSTRACT

Japanese encephalitis virus (JEV) can cause severe central nervous disease with a high mortality rate. It is dire to recognize successful and cheap antiviral medications for JEV treatment. The protease activity of NS3 is necessary for viral replication and its prohibition could be considered as a strategy for treatment of JEV infection. We reported previously that Niclosamide had lowest binding affinity at catalytic site region of the NS3 helicase in our previous work. In this work, Niclosamide similar compounds were retrieved from PubChem database and a structure-based virtual screening against NS3 helicase was carried out by AutoDock4.2. We found three potent drug-like compounds CID44240996, CID44565834 and CID88834438 among them having binding energy of -9.22, -9.27 and -9.05 kcal/mol with NS3 helicase, respectively. The docked complex structures were optimized by molecular dynamics simulation for in ps with the CHARMM-22 force field using NAMD incorporated in VMD and then evaluating the stability of complex structures by calculating RMSD. Further, in silico ADME/Tox of best predicted derivatives of NS3 helicase were evaluated. Compounds showed satisfactory results for oral administration and had HIA in the range of well absorbed compounds. P_{CaCO2} of these compounds were in moderate range. The cell permeability in MDCK of compounds was low varying from -4.47326nm/s to -2.80936 nm/s. Skin permeability showed negative values. Derivatives bind strongly to plasma proteins and blood-brain barrier value had less than 1. The Ames test showed compounds were mutagen and carcinogenicity in mouse and rat showed negative value except compound CID88834438.

Keywords: Flavivirus; NS3 helicase; Japanese encephalitis virus; ADME/Tox study; Niclosamide derivatives.

INTRODUCTION

Japanese encephalitis virus JEV is the most important cause of viral encephalitis in Asia. It is a mosquito-borne flavivirus, and belongs to the same genus as dengue, yellow fever and West Nile viruses. JEV was first reported in Japan in the 1871. It primarily affects children, a literature review estimates nearly 68,000 clinical cases of JE occur globally each year, with approximately 13,600 to 20,400 deaths (WHO, 2015). JEV has spread to most countries in south, east and south-east Asia (Mackenzie et al., 2007). Several flavivirus non-structural (NS) proteins, such as NS2B-NS3, NS3 helicase, NS4B, NS5 methyl-transferase, and NS5 RNA-dependent RNA polymerase (RdRp), have been identified as potential anti-viral drug targets (Luo et al., 2015; Xie et al., 2015; Lim et al., 2015). Many compounds have an inhibitory effect on the virus, raising hopes that new treatments are on the horizon for prevention and treatment of flavivirus-associated diseases (Lim et al., 2013). Vaccination can prevent the disease, but no specific antiviral drug is yet available for clinical therapy, and the death rate caused by JEV can reach as high as 60%. The NS3 protein of JEV is a large multifunctional protein possessing protease, helicase, and nucleoside 5'-triphosphatase (NTPase) activities, and plays important roles in the processing of a viral polyprotein and replication. JEV helicase is composed of three domains containg a tunnel large enough to accommodate single-stranded RNA. Each of the motifs I, II and VI was composed of an NTP-binding pocket. Mutation analyses revealed that all of the residues in I (GLY199, LYS200 and THR201), in addition to the polar residues within

the NTP-binding pocket (GLN457, ARG461 and ARG464), and also ARG458 in the outside of the pocket in the motif IV were crucial for ATPase and helicase activities and virus replication (Yamashita et al., 2008). The C-terminus of NS3 helicase was identified as a potential drug target.

In this study, we have screened Niclosamide derivatives against NS3 helicase. Niclosamide had lowest binding affinity at catalytic site region of the NS3 helicase, which is reported in our previous work (Singh et al., 2017). Further, best predicted Niclosamide derivatives of NS3 helicase of JEV were investigated for physicochemical properties, pharmacokinetic properties: human intestinal absorption (HIA), cellular permeability (Caco2), cell permeability Maden Darby Canine Kidney (MDCK), skin Permeability, plasma protein binding (PPB) and penetration of the blood-brain barrier (BBB), and toxicological: mutagenicity and carcinogenicity.

MATERIALS AND METHODS

Protein preparation

The 3D coordinates of the Crystal Structure of Catalytic Domain of Japanese Encephalitis Virus NS3 Helicase/Nucleoside Triphosphatase (PDB Id: 2Z83) was retrieved from Protein Databank (http://www.rcsb.org/).

Inhibitors dataset

We have screened Niclosamide derivatives from pubchem compound database (Wang et al., 2010) using similar compounds, score>=95. The 3D structures of screen 37 derivatives were downloaded in .sdf format from pubchem compound database. They were later converted in .pdb format with the help of open babel tool (O'Boyle et al., 2011).

Molecular docking

Docking of thirty seven Niclosamide derivatives screened from PubChem comound database were done using molecular docking program AutoDock4.2 (Morris et al., 2009). Gasteiger charges are added to the ligand and maximum 6 numbers of active torsions are given to the lead compounds using AutoDock tool. Kollman charges and the solvation term were added to the protein structure. The Lamarckian genetic algorithm implemented in Autodock was used for docking.

Molecular dynamics simulations

Molecular dynamics simulations were done using the NAMD (Phillips et al., 2005) graphical interface module incorporated in VMD (Humphrey et al., 1996). The protein-ligand complex was immersed in the center of a 50 Å box of water molecules where all water molecule atoms were closer than 1.5 Å and a CHARMM22 parameter file for proteins and lipids was used in the force field for complexes. The psf was created from the initial pdb and topology files using psfgen package of VMD. After running psfgen, two new files were generated protein.pdb and protein.psf and by accessing PSF and PDB files; NAMD generated the trajectory DCD file. After the simulations, the results were analyzed in VMD by calculating the Root mean square deviation of the complex.

ADME and Toxicological properties of Niclosamide derivatives

Absorption, distribution, metabolism, and excretion (ADME) and toxicological properties were essential for pharmacological/clinical applications of identified inhibitors. Therefore, The predicted inhibitors were evaluated for key physicochemical properties like molecular weight, Hydrogen Bond Donor Count, Hydrogen Bond Acceptor Count, XLogP and ADME properties like percentage of human intestinal absorption (HIA), cell permeability (P_{Caco-2}), cell permeability Maden Darby Canine Kidney (MDCK), skin permeability, Plasma protein binding (PPB), blood brain barrier (BBB) using PreADMET tool (https://preadmet.bmdrc.kr/adme/). Toxicological properties of mutagenicity and carcinogenicity were also evaluated using PreADMET tool.

RESULTS AND DISCUSSION

Molecular Docking

In docking studies the most important requirement was the proper orientation and conformation of ligand which fitted to the enzyme binding site appropriately and formed protein-ligand complex. Therefore, optimal interactions and the best AutoDock score were used as criteria to interpret the best conformation among the 10 conformations, generated by AutoDock program. The docking results of 37 compounds with Mca model was shown in Table 1. Among the above

docked compounds CID44240996, CID-44565834 and CID88834438 had the lower binding energy -9.22 kcal/mol, -9.27 kcal/mol and -9.05 kcal/mol, even lower than Niclosamide (-8.10 kcal/mol) with NS3 helicase. Docking poses of the best conformation of CID44240996, CID44565834 and CID88834438 with NS3 helicase protein were shown in figure 1, 2 & 3.

Molecular dynamics simulation

After Molecular dynamics simulation, the results were analyzed in VMD by calculating the Root mean square deviation (RMSD) of the complex using rmsd.tcl source file from the Tk console and finally rmsd.dat was saved and accessed in Microsoft office excel. RSMD, a crucial parameter to analyze the equilibration of MD trajectories, is estimated for backbone atoms of the compounds CID44240996, CID44565834 and CID88834438 with NS3 helicase complex (shown in figure 4, 5 & 6). Measurements of the backbone RMSD for the complex provided insights into the conformational stability.

ADME and Toxicological properties of best predicted Niclosamide derivatives

In analyzing the parameters of the best predicted compounds was observed that all had values within Lipinski parameters, except XlogP to evaluate oral absorption (table2). It was observed that the compounds CID44565834, CID44240996, CID88834438 had human intestinal absorption (HIA) values in the range 90.820693 to 95.072513 (table3). These compounds are categories as in the range of well absorbed compounds (HIA: 70 ~ 100 %) (Yee, 1997). The cell permeability in vitro Caco-2 is an important test to assess intestinal absorption of drugs. It was found that the P_{CaCO2} (nm/s) value were 11.1689 nm/s to 17.4262 nm/s for compounds (table 3). P_{CaCO2} value of compounds was in low range (Yazdanian et al., 1998). The cell permeability in vitro in MDCK system is used as a tool for the rapid analysis of permeability. These derivatives had 0.0714057 nm/s to 5.05717 nm/s as low MDCK (Irvine et al., 1999) (table 3). Skin permeability parameter is used in the pharma-ceutical industry to assess the risk chemical products in case there is accidental contact with skin (Singh and Singh, 1993). Predicted derivatives showed negative perme-ability values (table3). The binding of drug to blood and plasma proteins can alter the half-life of the drug in the body of the individual (Godin, 1995; Pratt and Taylor, 1990). It is verified that derivatives bind strongly to plasma proteins, being 58.605579 to 100.00000%. (table 4).

The blood-brain barrier (BBB) has an importance in the pharmacology of drugs, because the compounds are classified as inactive and active compounds. Derivatives bind strongly to plasma proteins. In relation to the penetration of the blood-brain barrier the inhibitors analyzed showed penetration values less than 1, and ranged from 0.0731198 to 0.411246. The Ames test (Ames et al., 1972) showed compounds were mutagen and carcinogenicity in mouse and rat showed negative value. Except compound CID88834438 shows positive value in rat.

CONCLUSION

We got three best predicted compounds CID44240996, CID44565834 and CID-88834438 having lower binding energy even lower than Niclosa-mide. Molecular dynamics simulations showed that predicted compounds were stable. In Silico ADME and Toxicological properties of predicted compounds showed satisfactory results. Therefore it is predicted that compounds CID44240996, CID44565834 and CID88834438 could be promising inhibitor for NS3 helicase as drug target yet experimental studies have to confirm it.

ACKNOWLEDGEMENT

The Authors gratefully acknowledge the necessary computational facilities and constant supervision provided by Department of Biotechnology, Rama University of Technology and Science, Kanpur, U.P., India for their generous support during the research work.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

Table 1: Docking of Niclosamide derivatives with NS3 helicase protein.

Sl. No.	PubChem CID	BE	IME	IE	TorE	VdwE	EE
1	189036	-7.14	-9.82	-2.7	2.68	-9.73	-0.09
2	487721	-8.39	-9.58	-1.32	1.19	-7.67	-1.43
3	828117	-8.28	-9.47	-1.28	1.19	-8.28	-1.18
4	2781466	-8.46	-9.66	-0.68	1.19	-7.04	-2.61
5	2844786	-5.92	-8.31	-1.62	2.39	-6.9	-1.41
6	2946176	-6.54	-8.93	-1.63	2.39	-7.12	-1.81
7	3353792	-6.81	-9.19	-1.52	2.39	-8.59	-0.6
8	3491605	-6.55	-9.23	-2.76	2.68	-8.8	-0.43
9	3895545	-8.42	-9.61	-1.32	1.19	-8.82	-0.79
10	10093194	-7.66	-10.34	-2.81	2.68	-8.71	-1.63
11	11200749	-7.12	-9.5	-1.7	2.39	-8.63	-0.87
12	11440508	-6.67	-9.35	-3.21	2.68	-8.0	-1.36
13	12220828	-7.4	-10.08	-1.69	2.68	-8.38	-1.7
14	12363049	-7.92	-9.11	-1.31	1.19	-7.63	-1.48
15	18452502	-6.96	-8.45	-1.41	1.49	-6.87	-1.58
16	19027407	-7.37	-8.87	-1.42	1.49	-7.38	-1.48
17	19027409	-7.63	-9.13	-1.31	1.49	-8.29	-0.83
18	19027410	-7.99	-9.48	-1.31	1.49	-8.1	-1.38
19	25217041	-7.56	-8.75	-1.05	1.19	-7.7	-1.05
20	25217042	-8.34	-9.53	-0.97	1.19	-7.99	-1.54
21	25217043	-6.06	-7.25	-0.88	1.19	-6.4	-0.85
22	25217045	-7.28	-8.47	-1.03	1.19	-6.61	-1.86
23	25217155	-7.26	-8.45	-1.01	1.19	-7.5	-0.96
24	25217156	-7.38	-8.57	-1.0	1.19	-7.91	-0.66
25	25217270	-7.22	-8.41	-1.02	1.19	-6.7	-1.72
26	25217492	-8.32	-9.52	-1.11	1.19	-8.63	-0.89
27	25217494	-7.62	-8.81	-1.12	1.19	-7.76	-1.05
28	29557196	-8.34	-9.53	-1.11	1.19	-8.19	-1.34
29	44240996	-9.22	-10.42	-0.73	1.19	-8.28	-2.14
30	44565834	-9.27	-10.46	-0.91	1.19	-8.25	-2.21
31	68168508	-7.54	-8.73	-0.87	1.19	-7.76	-0.97
32	68169781	-8.11	-9.3	-1.04	1.19	-7.59	-1.71
33	71459109	-8.0	-9.19	-0.74	1.19	-7.15	-2.05
34	71720820	-8.63	-10.13	-1.43	1.49	-9.49	-0.64
35	86251702	-8.03	-9.22	-1.08	1.19	-7.51	-1.71
36	88834438	-9.05	-10.24	-1.14	1.19	-9.03	-1.21
37	25217274	-7.69	-8.88	-0.7	1.19	-6.76	-2.12

BE = Binding Energy; IME: Intermolecular Energy; IE = Internal Energy; TorE= Torsional; Energy; VdwE = vdW + Hbond + desolv Energy; EE= Electrostatic energy.

Table 2: Physicochemical properties of best predicted Niclosamide derivatives.

Sl.N	PubChem CID	Molecular Weight	Donor	Acceptor	logP
Φ.	44240996	306.702	2	4	3.8
2	44565834	327.117	2	4	4
3	88834438	396.001	2	4	5.3

Table 3: Absorption properties of best predicted compounds.

Derivative	PubChem CID	Absorption			
		HIA (%)	P_{Caco-2} (nm/sec)	MDCK(nm/sec)	Skin Permeability
1	44565834	90.820693	17.4262	5.05717	-4.47326
2	44240996	93.324583	11.1689	3.24464	-3.12468
3	88834438	95.072513	14.8798	0.0714057	-2.80936

Table 4: Distribution properties in percentages of PPB and penetration of the blood brain barrier of best predicted compounds.

Derivative	PubChem CID	Distribution	
		PPB (%)	BBB
1	44565834	58.605579	0.171371
2	44240996	96.033057	0.0731198
3	88834438	100.000000	0.411246

Table 5: Toxicological properties of mutagenicity (Ames test) and carcinogenicity (mouse and rat).

Derivative	CID	Ames Test	Carcinogenicity	
			Mouse	Rat
1	44565834	Mutagen	Negative	Negative
2	44240996	Mutagen	Negative	Negative
3	88834438	Mutagen	Negative	Positive

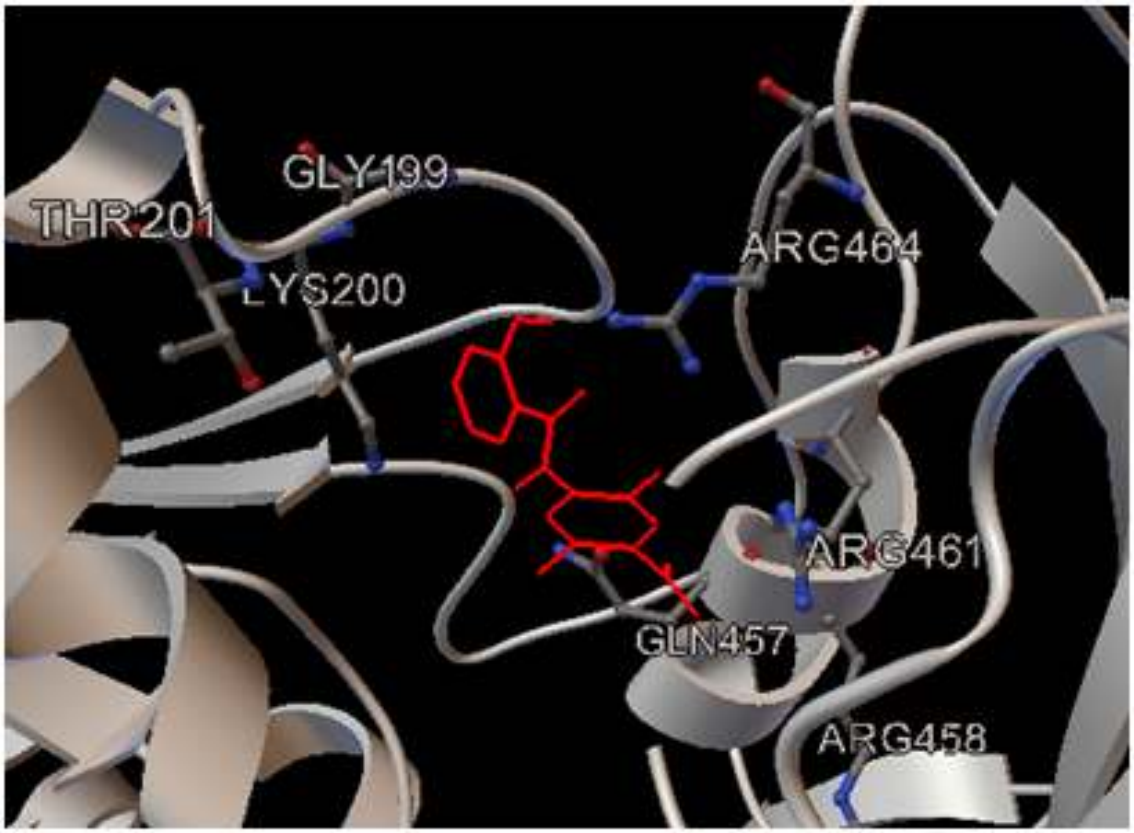

Figure 1: Docking orientation of compound CID44565834 with NS3 protein.

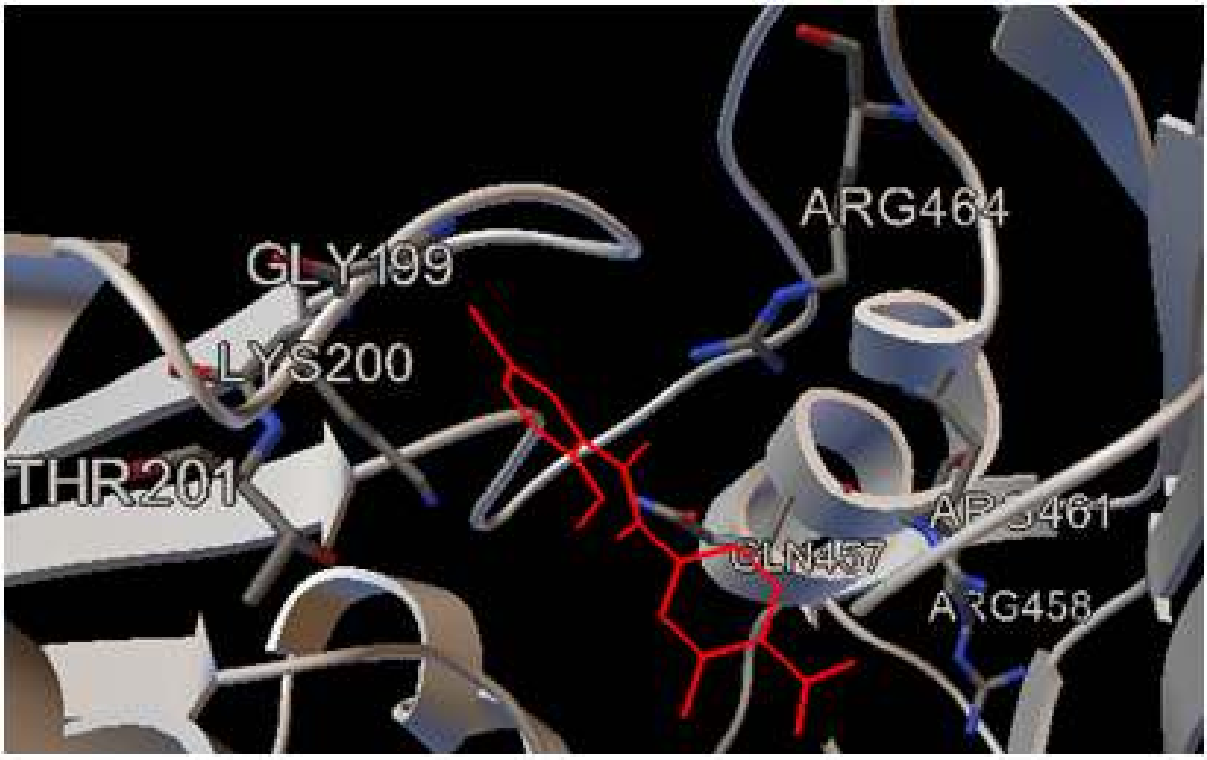

Figure 2: Docking orientation of compound CID44240996 with NS3 protein.

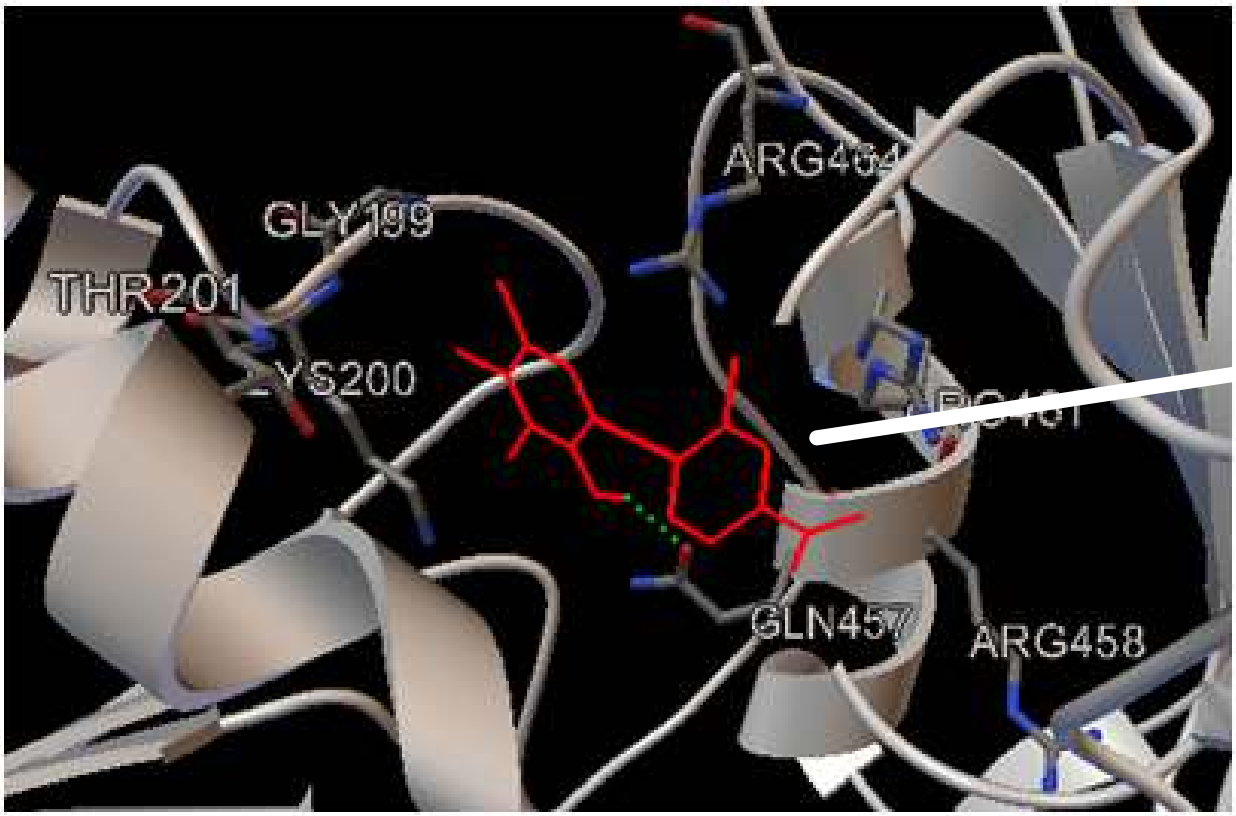

Figure 3: Docking orientation of compound CID88834438 with NS3 protein.

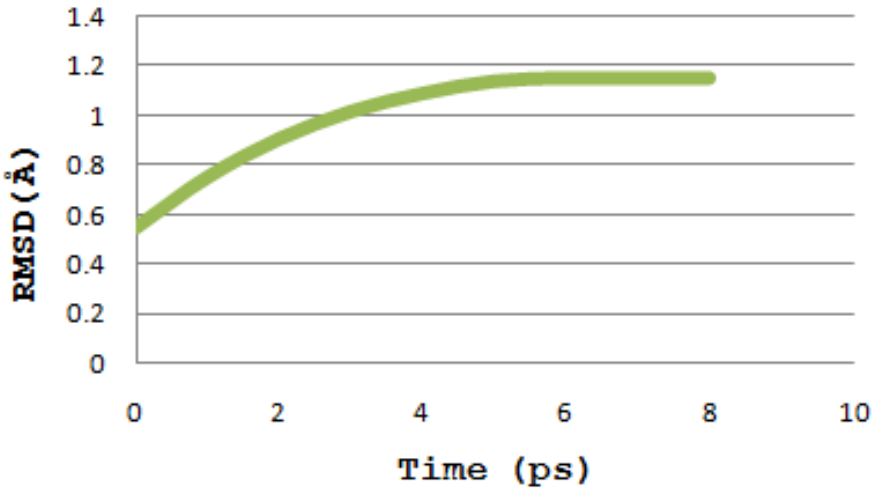

Figure 4: Graph displaying root mean square deviation (RMSD) of CID44240996 - NS3 helicase complex versus time (ps) at 310 K, resulted in highest peak at 1.149 Å.

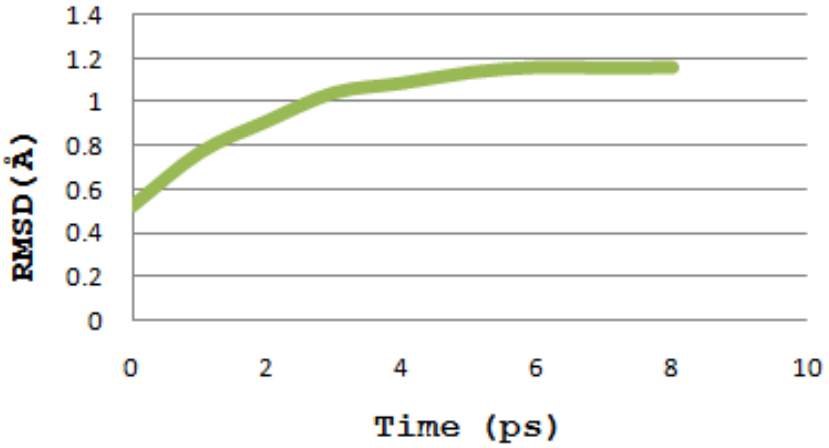

Figure 5: Graph displaying root mean square deviation (RMSD) of CID44565834 – NS3 helicase complex versus time (ps) at 310 K, resulted in highest peak at 1.160 Å.

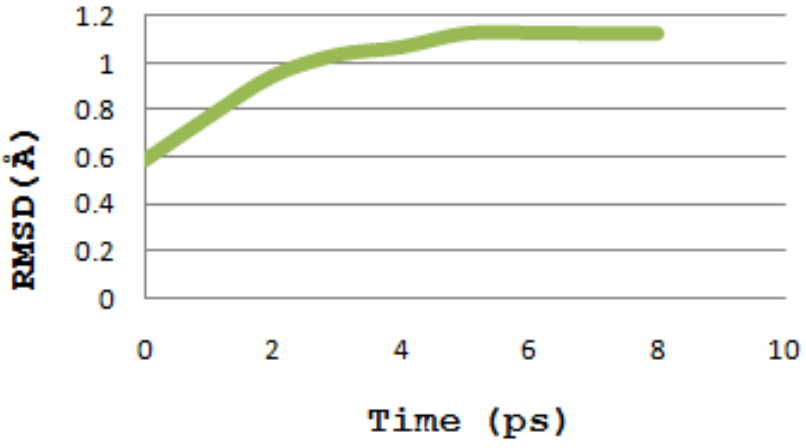

Figure 6: Graph displaying root mean square deviation (RMSD) of CID 88834438 – NS3 helicase complex versus time (ps) at 310 K, resulted in highest peak at 1.128 Å.

REFERENCES

1. Ames B.N., Gurney E.G., Miller J.A., Bartsch H. Carcinogens as Frame shift Mutagens: Metabolites and Derivatives of 2Acetylaminofluorene and Other Aromatic Amine Carcinogens. Proceedings of the National Academy of Sciences of the United States of America 1972; 69:3128-3132.
2. Godin D.V. Pharmacokinetics: Disposition and Metabolism of Drugs. In: Munson, P.L., R. A. Mueller, G.R. Breese, Eds., Principles of Pharmacology: Basic Concepts and Clinical Applications, Chapman and Hall, New York 1995; 39-84.
3. Humphrey W., Dalke A., Schulten K. VMD: visual molecular dynamics. J Mol Graph. 1996; 14(1):33-8.
4. Irvine J.D., Takahashi L., Lockhart K., Cheong J., Tolan J.W., Selick H.E., Grove J.R. MDCK (Madin-Darby Canine Kidney) Cells: A Tool for Membrane Permeability Screening. Journal of Pharmaceutical Sciences 1999; 88:28-33.
5. Lim S. P. et al. Ten years of dengue drug discovery: Progress and prospects. Antivir Res. 2013; 100:500–519.
6. Lim S.P., Noble C.G., Shi P.Y. The dengue virus NS5 protein as a target for drug discovery. Antivir Res. 2015; 119:57–67.
7. Luo D.H., Vasudevan S.G., Lescar, J. The flavivirus NS2B-NS3 protease-helicase as a target for antiviral drug development. Antivir Res. 2015; 118:148–158.
8. Mackenzie J.S., Williams D.T., Smith D.W. Japanese Encephalitis Virus: The Geographic Distribution, Incidence, and Spread of a Virus with a Propensity to Emerge in New Areas. Persp Med V 2007; 16:201–268.
9. Morris G.M., Huey R., Lindstrom W., Sanner M.F., Belew R.K., Goodsell D.S., Olson A.J. Autodock4 and AutoDockTools4: automated docking with selective receptor flexiblity. J. Computational Chemistry 2009; 16:2785-91.
10. O'Boyle N.M., Banck M., James C.A., Morley C., Vandermeersch T., Hutchison G.R. Open Babel: An open chemical toolbox. J Cheminform. 2011; 3:33.
11. Phillips J.C., Braun R., Wang W., Gumbart J., Tajkhorshid E., Villa E., Chipot C., Skeel R.D., Kalé L., Schulten K. Scalable molecular dynamics with NAMD. J Comput Chem. 2005; 16:1781-802.
12. Pratt W.B., Taylor P. Principles of Drug Action: The Basis of Pharmacology. 3th Edition, Churchill Livingstone, New York, 1990.
13. Singh A., Kumar A., Srivastava V. Molecular docking studies of antiviral drugs against NS3 helicase of japanese encephalitis virus. International Journal of Scientific and Innovative Research 2017; 5(1): 33-36.
14. Singh S., Singh J. Transdermal Drug Delivery by Passive Diffusion and Iontophoresis: A Review. Medicinal Research Reviews 1993; 13:569-621.
15. Wang Y., Bolton E., Dracheva S., Karapetyan K., Shoemaker B.A., Suzek T.O. et al. An overview of the PubChem BioAssay resource. Nucleic Acids Res. 2010; 38:D255-D266.
16. WHO. http://www.who.int/mediacentre/factsheets/fs386/en/. Accessed on December 2015.
17. Xie X.P., Zou J., Wang Q.Y., Shi P.Y. Targeting dengue virus NS4B protein for drug discovery. Antivir Res. 2015; 118:39–45.
18. Yamashita T., Unno H., Mori Y., Tani H., Moriishi K., Takamizawa A., Agoh M., Tsukihara T., Matsuura Y. Crystal structure of the catalytic domain of Japanese encephalitis virus NS3 helicase/nucleoside triphosphatase at a resolution of 1.8 A. Virology 2008; 373(2):426-36.
19. Yazdanian M., Glynn S.L., Wright J.L., Hawi A. Correlating partitioning and caco-2 cell permeability of structurally diverse small molecular weight compounds. Pharm Res. 1998; 15(9):1490-4.
20. Yee S. In vitro permeability across Caco-2 cells (colonic) can predict in vivo (small intestinal) absorption in man--fact or myth. Pharm Res.1997; 14(6):763-6.