iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 7 **Issue:** V **Month of publication:** May 2019

DOI: https://doi.org/10.22214/ijraset.2019.5276

www.ijraset.com

Call: 08813907089 | E-mail ID: ijraset@gmail.com

International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.177
Volume 7 Issue V, May 2019- Available at www.ijraset.com

In Silico Screening and Identification of Antiviral Inhibitors against Envelope Protein of Yellow Fever Virus

Aman Pratap Singh Chauhan[1], Ajay Kumar[2], Vivek Srivastava[3]
[1, 2, 3] *Department of Biotechnology, Faculty of Engineering & Technology, Rama University, Uttar Pradesh, Kanpur- 209217, India*

Abstract: ***Yellow fever is a mosquito-borne hemorrhagic disease that is possibly related with jaundice and is caused by the infection of yellow fever virus (YFV). Regardless of the accessibility of Vaccine, yellow fever virus affect 2, 00,000 people worldwide every year and causes approximately 30,000 deaths. There are no effective antiviral drugs to cure the dangerous malady and repress YFV replication would meet a squeezing medicinal need. Envelope protein of yellow fever virus has an important role in host cell viral infections. In this study, we performed molecular docking analysis of antiviral drug Ribavirin against envelope protein of yellow fever virus using Autodock 4.2 tool. It has shown binding affinity of -5.85 kcal/mol and it is binding around the active site region of the protein. In docked complex, Ribavirin formed four H-bonds with ASP312, GLU392, TRP389 and HIS390 of Envelope protein. Active site region of envelope protein was predicted by CASTp Server. The docked complex was analyzed through Python Molecular Viewer software for their interaction studies.***
The docked complex structure was optimized by molecular dynamics simulation for 5 ps with the CHARMM-22 force field using NAMD incorporated in VMD 1.9.2 and then evaluating the stability of complex structure by calculating RMSD. Observations made in putative binding site analysis on the protein surface can be very helpful for rational drug design on target Envelope protein of Yellow Fever Virus.
Keywords: Envelope protein; Yellow Fever Virus., CASTp, Autodock, Drug design

I. INTRODUCTION

Flavivirus is a large genus consisting more than 70 pathogenic viruses of different pathologies with significant public health impact in across the tropical world. Yellow fever virus (YFV) and dengue virus (DENV) are some of the most important human pathogenic flaviviruses, sharing the same vectors and causing almost similar symptoms, ranging from low fever and malaise in mild infections, to haemorrhage and rapid terminal events with shock and failure of various organs in haemorrhagic fever syndrome. Yellow fever is endemic in several South American and African countries. It has been estimated that up to 1.7 million cases of yellow fever occur in Africa each year, mostly in West African countries, resulting in 29,000–60,000 deaths. Since late 2016, yellow fever outbreaks have occurred in the Southeast region of Brazil, with 792 confirmed cases, including 274 deaths in about 1 year. The transmission of YFV occurs through the bite of mosquito vectors of different species. Usually human infection occurs through occasional transmission of YFV from nonhuman primates to humans through mosquito vectors belonging to the sylvatic YFV cycle from the Haemagogus and Sabethes genus in South America and the Aedes genus in Africa when humans come into endemic jungle and forest Areas. In the urban cycle, the ubiquitous vector Aedes aegypti is responsible for the transmission. The disease can spread rapidly and affect thousands of individuals. In 25 to 50% of cases, the acute phase can progress to an intoxication stage characterized by high fever, haemorrhagic syndrome, jaundice and kidney disease, which can be fatal. The patients surviving the acute period enter the convalescence phase, characterized by prolonged weakness and fatigue that can last several weeks. Thus, in addition to presenting high lethality rates, yellow fever also causes heavy socio-economic burden in afflicted populations.

In humans, wild-type YFV infection is primarily viscerotropic and affects the liver before damaging other tissues, including kidneys, spleen, lymph nodes and heart. In the case of apparent Yellow fever (YF) infections, after an incubation period of three to six days, the “infection” phase is characterised by a flu-like illness starting with an abrupt onset of fever with headache, nausea and myalgia. As for all flaviviruses, the YFV genome consists of a positive single-stranded RNA molecule comprising about 11,000 nucleotides with a type I cap at the 50 terminus but lacking a polyA tail at the 30 terminus. The cap structure of flaviviral genomes is thought to be important for cap-dependent translation and to protect the genome from degradation by cellular 50-30 exonucleases. This positive single stranded RNA corresponds to one large open reading frame (ORF), flanked at its 50 and 30 termini by untranslated regions (UTRs) that are required forRNA replication and translation. Yellow fewer virus ORF spans 10,233 to 10,236

nts and encodes a polypeptide of 3411 to 3412 amino acids. The amino-terminal residues correspond to the three structural proteins while the remainder of the ORF encodes the seven NS proteins with the following organisation: 50cap-C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-2k-NS4B-NS5-30. The 50UTR of YFV (~110 nts) is much shorter than the 30UTR (~400–650 nts), the size of which varies among YF `strains. The largest of the structural proteins, the envelope (E) protein, is the major component of the virion surface. It is the primary immunogen and plays a central role in receptor binding and membrane fusion. The structure of the ectodomain (the soluble N-terminal portion, consisting of 395 residues) of the E protein of TBEV was determined by x-ray crystallography. Based on this structure, three distinct structural domains, domains I, II and III, have been identified in the ectodomain. Because no treatment exists for yellow fever, there is great interest in developing strategies to control the disease. Unlike other mosquito-borne flaviviruses, YFV has a tropism for the liver and causes a viscerotropic disease whereas many other mosquito-borne flaviviruses have a tropism for the brain, or in the case of the DEN viruses, they target cells of reticuloendothelial origin. Drug discovery process is a very serious issue in the pharmaceutical industry since it is a very costly and time taking process to produce new drug potentials and enlarge the scope of diseases incurred. The process of drug development targets towards the identification of compounds with pharmacological interest to assist in the treatment of diseases. Drug development for YFV has been in advance for quite a few years. In this study, we docked Ribavirin with envelope protein using AutoDock4.2. The docked complex structure was optimized by using molecular dynamics simulation and evaluated the stability of complex structure.

II. METHODOLOGY

A. Protein Target Structure

Crystal structure of the precursor membrane protein-envelope protein heterodimer of the yellow fever virus was retrieved from PDB. PDB is the repository for 3-D structure data of proteins and nucleic acid.

B. Binding Site Analysis

CastP server uses the weighted Delaunay triangulation and the alpha complex for the measurement of shape. This software allows the identification and measurements of surface accessible pockets as well as interior inaccessible cavities of protein and other molecules.

C. Molecular Docking

Docking is an automated computer algorithm that determines how a compound will bind to the binding site of a protein. The approach includes verifying the orientation of the compound, its conformational geometry, and the docking scores. Docking of Ribavirin against envelope protein structure was done using molecular docking program AutoDock 4.2 and ArgusLab 4.0.1. During the docking procedure a Lamarckian Genetic Algorithm (LGA) was used for flexible ligand rigid protein docking calculation.

D. Molecular Dynamics Simulations

Molecular dynamics simulations were done by using the NAMD (NAnoscale Molecular Dynamics program; v2.7) graphical interface module incorporated visual molecular dynamics (VMD 1.9.2). After the simulations, the results were analyzed in VMD by calculating the Root mean square deviation (RMSD) of the complex and was accessed in Microsoft excel.

III. RESULTS AND DISCUSSION

A. Binding Site Analysis

CASTp Server predicted binding site residues of envelope protein consist of residues ASP312, GLY314, GLY316, THR317, VAL318, PRO369, PHE371, TRP389, HIS390, LYS391, GLU392, ASP393. Molecular surface area and volume are 80.975 and 49.023, respectively.

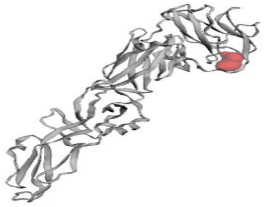


Fig 1: The predicted binding site residues were shown with red color in space fill model and backbone of protein is represented by gray color in ribbon model.

B. Molecular Docking By Autodock

Docking studies predicted the interaction of ligand with protein and residues involved in binding. After that, the ligand was allowed to run using GA algorithm and a Score scoring function complex. For such interaction 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. The docking results of Ribavirin with matrix protein VP40 was shown in table 1.

Table 1: Docking result of Rivavarin with envelope protein structure.

Sl. No.	Inhibitor	BE	IME	IE	TorE	VdwE	EE
1	Ribavirin	-5.85	-5.87	-1.62	1.65	-5.4	-0.47

BE: Binding Energy; IME: Intermolecular Energy;IE: Internal Energy; TorE: Torsional Energy; VdwE:Vdw-lbDesolv Energy; EE: Electrostatic Energy.

C. Docking Studies By Arguslab

The YFV envelope protein was downloaded into ArgusLab program and binding site was made by choosing "Make binding site for this protein" option. The inhibitor was chosen, centered and added hydrogens. In next step, the ligands was allowed to run using Genetic algorithm and Alignment Score scoring functions. ArgusLab 4.0.1 program has two options for docking algorithm which are GA (Genetic Algorithm) dock and Argusdock (shape-based search algorithm). We chose GA dock only to compare with AutoDock 4.2. For GA parameters of ArgusLab, population size 50, grid resolution 0.35 Å, maximum generation 1,000, crossover rate 0.8, mutation rate 0.2 and dock engine used Lamarckian Genetic Algorithm. In Argus lab software, docking calculation type was set to "Dock" and "Flexible" ligand docking mode and used for each docking run.

Table 2: Binding energy of Ribavirin with envelope protein inhibitors from AutoDock 4.2 and ArgusLab 4.0.1.

Compound Name/ Pubchem CID	Binding Energy (Kcal/mol) from AutoDock	Binding Energy (Kcal/mol) from ArgusLab
Ribavirin	-5.85	-5.94

From the study, the docking results with AutoDock 4.2 and ArgusLab 4.0.1 were compared in table 2. Both programs show almost similar results.

Docking poses of the best conformation of Ribavirin with YFV envelope protein was analysed by Python molecular viewer were shown in figure 2.

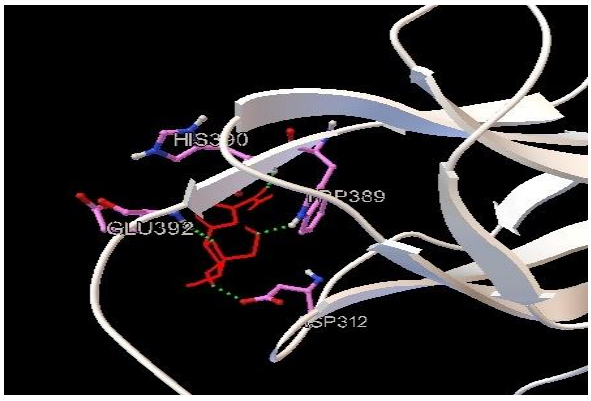


Fig 2: Docking orientation of Ribavirin with YFV envelope protein. Complex depicting compound formed four H-bond with ASP312, GLU392, TRP389 and HIS390 of protein. Ribavirin is represented as lines and colored as red.

The docking activity gives proteins the ability to boost or inhibit chemical reactions and to accelerate or prevent the processes that keep cells alive and maintain a balanced micro environment. More over the specific effects of a drug could depend on the structure of the molecular aggregates formed. Ribavirin was screened from pubchem compound database was further dock and verified by ArgusLab with envelope protein.

D. Molecular Dynamics Simulations

RSMD, a crucial parameter to analyze the equilibration of MD trajectories, is estimated for backbone atoms of the compound Ribavirin with envelope protein complex. Measurements of the backbone RMSD for the complex provided insights into the conformational stability.

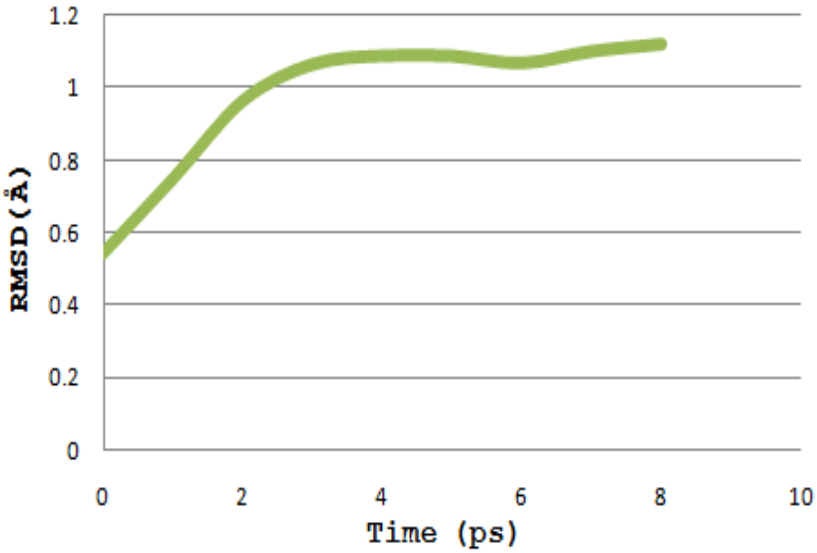


Fig 3: Graph displaying root mean square deviation (RMSD) of the backbone atoms of docked complex (Ribavirin - envelope protein) versus time at 310 K, resulted in highest peak at 1.12 Å.

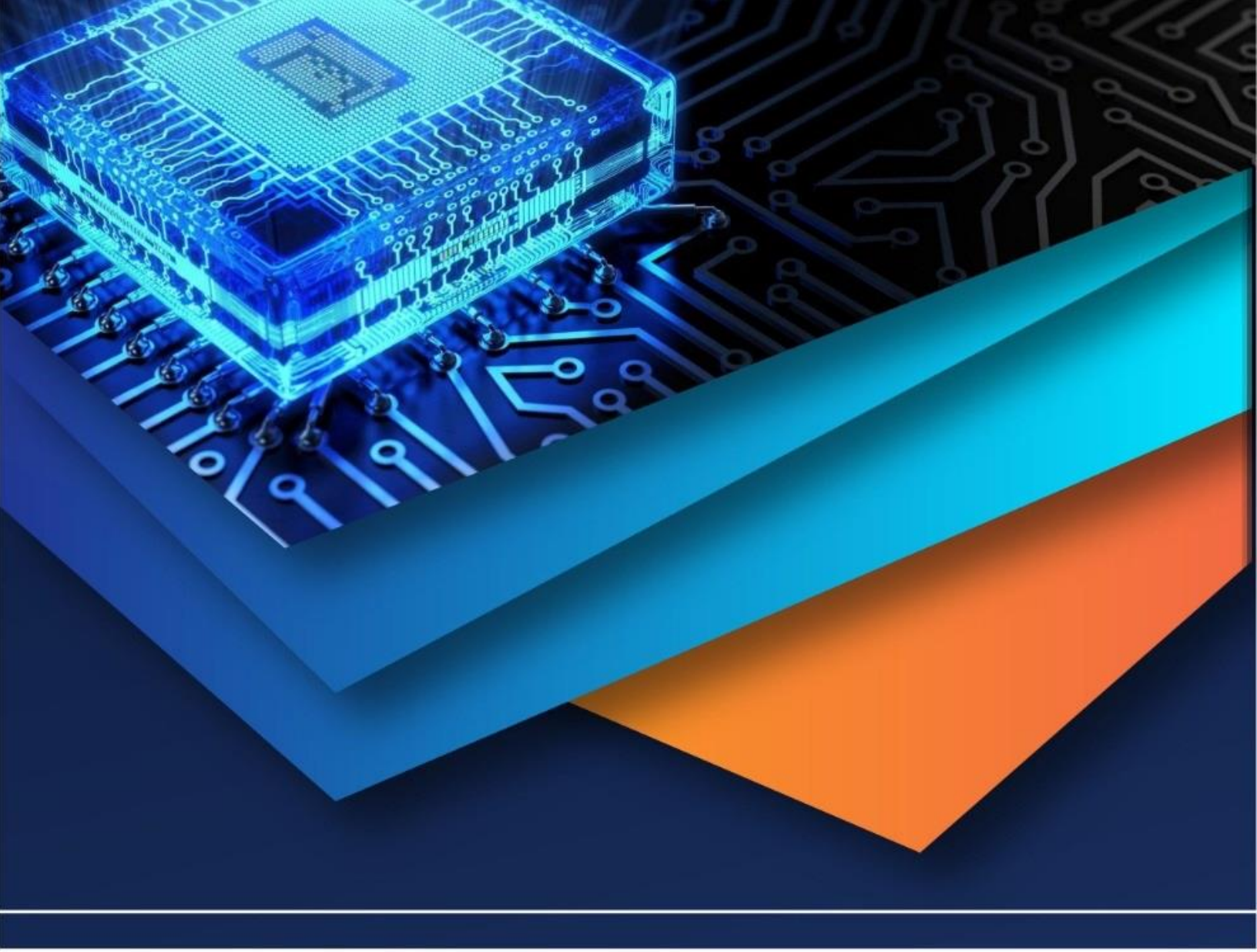
IV. CONCLUSION

Since chemical synthesis of natural products was problematic and expensive due to their structural complexity, we have used Ribavirin for docking studies against Yellow Fever Virus envelope protein. We used AutoDock and ArgusLab for docking studies in this work because they are convenient for users who are familiar with the Windows operating system. Both the tools were used Lamarckian Genetic Algorithm for docking calculation. Thus from the docking of Ribavirin, we got optimal binding energy -5.85 kcal/mol with AutoDock and -5.94 kcal/mol with ArgusLab. Both the docking tool predicts almost same binding energy. Anaysis of docked complex using Python Molecular Viewer shows Ribavirin formed four H-bond with ASP312, GLU392, TRP389 and HIS390 of protein. Optimizatin of docked protein - inhibitor complexe shows its stability.

REFERENCES

[1] Heinz FX, Stiasny K. Flaviviruses and their antigenic structure. J Clin Virol. 2012;55: 289-295.
[2] Mukhopadhyay S, Kuhn RJ, Rossmann MG. A structural perspective of the flavivirus life cycle. Nat Rev Microbiol. 2005;3: 13-22.
[3] Beasley DWC, McAuley AJ, Bente DA. Yellow fever virus: Genetic and phenotypic diversity and implications for detection, prevention and therapy. Antiviral Res. 2015;115: 48-70.
[4] Monath TP. Yellow fever : an update. Lancet Infect Dis. 2001;1:11-20.
[5] PAHO/WHO, Pan American Health Organization/World Health Organization. Epidemiological Update: Yellow Fever.; 2017.
[6] Couto-Lima D, Madec Y, Bersot MI, et al. Potential risk of re-emergence of urban transmission of Yellow Fever virus in Brazil facilitated by competent Aedes populations. Sci Rep. 2017;7(4848):1-12.
[7] Vasconcelos PFC. Yellow Fever. In: Arthropod Borne Diseases. Springer International Publishing; 2017:101-113.
[8] Mutebi J-P, Barrett ADT. The epidemiology of yellow fever in Africa. Microbes Infect. 2002;4:1459-1468.
[9] Paules CI, Fauci AS. Yellow Fever — Once Again on the Radar Screen in the Americas. N Engl J Med. 2017;376 (15):1397-1399.
[10] Barrett, M.G.S. Yellow fever vaccine. In Vaccines, 6th ed.; Elsevier: New York, NY, USA, 2012; pp. 870–968.
[11] Monath, T.P. Yellow fever: A medically neglected disease. Report on a seminar. Rev. Infect. Dis. 1987, 9, 165–175.
[12] Zhou, Y.; Ray, D.; Zhao, Y.; Dong, H.; Ren, S.; Li, Z.; Guo, Y.; Bernard, K.A.; Shi, P.Y.; Li, H. Structure and function of flavivirus ns5 methyltransferase. J. Virol. 2007, 81, 3891–3903
[13] Cleaves, G.R.; Dubin, D.T. Methylation status of intracellular dengue type 2 40 S RNA. Virology 1979, 96, 159–165.
[14] Wengler, G.;Wengler, G.; Gross, H.J. Studies on virus-specific nucleic acids synthesized in vertebrate and mosquito cells infected with flaviviruses. Virology 1978, 89, 423–437.
[15] Corver, J.; Lenches, E.; Smith, K.; Robison, R.A.; Sando, T.; Strauss, E.G.; Strauss, J.H. Fine mapping of a cis-acting sequence element in yellow fever virus RNA that is required for RNA replication and cyclization. J. Virol. 2003, 77, 2265–2270.

[16] Ng, W.C.; Soto-Acosta, R.; Bradrick, S.S.; Garcia-Blanco, M.A.; Ooi, E.E. The 5' and 3' untranslated regions of the flaviviral genome. Viruses 2017, 9, 137.
[17] Goodsell DS, Olson AJ.Automated docking of substrates to proteins by simulated annealing. Proteins. 1990;8(3):195-202.
[18] Dundas J, Ouyang Z, Tseng J, Binkowski A, Turpaz Y, Liang J. CASTp: computed atlas of surface topography of proteins with structural and topographical mapping of functionally annotated residues. Nucleic Acids Res. 2006 Jul 1;34(Web Server issue):W116-8.
[19] Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem. 2004 Oct;25(13):1605-12.
[20] V. Srinivasa Rao and K. Srinivas. Modern drug discovery process: An in silico approach. Journal of Bioinformatics and Sequence Analysis Vol. 2(5), pp. 89-94, June 2011
[21] Humphrey W, Dalke A, Schulten K. VMD: visual molecular dynamics. J Mol Graph. 1996; 14(1):33-8.
[22] Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. Autodock4 and AutoDockTools4: automated docking with selective receptor flexiblity. J. Computational Chemistry 2009; 16: 2785-91.
[23] Phillips JC, Braun R, Wang W, Gumbart J, Tajkhorshid E, Villa E, Chipot C, Skeel RD, Kalé L, Schulten K. Scalable molecular dynamics with NAMD. J Comput Chem. 2005; 26(16):1781-802.
[24] ArgusLab 4.0.1 Mark A. Thompson Planaria Software LLC, Seattle, WA
[25] HyperChem (TM) Release 7.5, Hypercube, Inc., 1115 NW 4th Street, Gainesville, Florida 32601, USA.



10.22214/IJRASET

45.98

IMPACT FACTOR: 7.129

IMPACT FACTOR: 7.429

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY