In-silico Analysis of Drugs against Non-structural Protein Causing Rift Valley Fever

Swati Srivastava[1], Aman Suri[2], Rishi Sethia[3] and Varun Sharma[4]
[1,2,3,4]*Lovely Professional University, Punjab*
[1] *swati051@gmail.com,* [3] *rishisethia2@gmail.com,* [4] *varunom.12@gmail.com*

ABSTRACT

Rift Valley Fever, also known as viral zoonosis is an acute, fever causing disease which affects domestic animals and livestock. The virus has RNA as genetic material, enveloped by proteins. Its RNA genome is composed of three segments L, M and S of negative polarity. The dual function of Non-Structural protein (NS Protein), one to inhibit the host transcription by interacting with the p44 and affecting the assembly of transcription factor TFII-H in the nucleus and second, inhibition of one interferon (IFN-β) for this it uses transcription factor YY1 to maintain the repressor complex of SAP30 (Sin3A-associated protein 30), YY1 and Sin3A-ssociated protein 30 co repressor factors, which suppresses the transcription of INF- β. NS protein was focused for this study. To get the 3D structure of NS Protein, Homology Modeling was performed by MODELLER 9.11 and the structure obtained was validated through PROCHECK. A set of library was collected by Drugbank. There 2D and 3D conformers were generated by FROG 2.1. Top 11 Molecules were selected and docked with the help of online server PatchDock and ranked based on their Energy and score. To make ligand act as a drug it should follow druglike property which was measured by Molinspiration server calculating Lipinski Rule of Five. A Comparative analysis was done of which Leflunomide showed best result with Molecular Weight = 301.39 Da, Hydrogen Bond Acceptor =6, Hydrogen Bond Donor = 5, Log P= 2.2, Global energy = -58.98 and Docking Score= 5923.

Keywords: Rift Valley Fever, TFII-H, IFN-β, transcription factor YY1, SAP30, Non-Structural protein (NS Protein), Leflunomide

1. INTRODUCTION

Rift Valley Fever is a natural infectious disease which primarily affect livestock and responsible for 50-90% mortality rates [1]. In 1931, it was discovered that this virus was transmitted by mosquitoes [2], and the virus was first time isolated from naturally occurring mosquitoes [3]. RVF is capable of spreading between the different species as a result humans also get infected by this disease as an acute febrile illness [1].

The genome sequencing and serial mutagenesis were used to produce derivative of ZH548 isolate from RVF virus. In this specific derivative mutations were there in all three segments L, M and S segments and it is used to sequence the RVF virus genome in fig:1 [4].

Fig 1. Sequence of L, M, S segments in 3' and 5' direction

L	3'	TGTGTTTCCGCGGGTTAGTACCTAGATA...
M	3'	TGTGTTTCTGGCCACGTTGAATTTCTCA.....
S	3'	TGTGTTTCTGGGGGATCACGAATAGTTC.....
L	5'	ACACAAAGGGCCTTATAAATGGAGCCCG.....
M	5'	ACACAAAGATGGGCATTAAATGTATGTT.....
S	5'	ACACAAAGACCCCCTAGTGATACAAACA.....

Vaccination for the treatment for the RVF was referred as the most effective approach. Variety of vaccines developed for the RVF such as formalin-inactivated RVFV vaccines (NDBR 103 and TSI-GSD 200), live attenuated vaccines (MLVV, MP-12 and GFP-ΔNSm) and other new generation vaccines vaccines(recombinant proteins, virus-like particles and immunization with plasmid encoding a viral protein) [5].

Variability in NSs coding region was analyzed in detail and it was found that Non-stuctural protein NSss (potential target) has less selection pressure considering evolution [6].

A dual function of RVFV NSs protein was observed by various findings**,** one of the functions was to inhibit the host transcription by interacting with the p44 which in turn affects the assembly of transcription factor TFIIH in the nucleus. And the second function was inhibition of one interferon (IFN-β) [7].

Due to high rejection rates at clinical stage a decline was observed in the field of innovative drug discovery, fewer computational studies are there but due to increasing combinatorial science and enrichment of data of proteins, genomes and compounds in the field of drug discovery is becoming fast effective and less hectic [8]. Five million compounds were virtually screened against a protease (nsP2) of chikunguniya virus (CHIKV). Ultimately 26 compounds were selected which were capable of producing good therapeutic effects [9]. Therefore comparative analysis of existing drugs was done so as to come through and novel drug.

2. MATERIALS AND METHODS

Target identification

Non-structural protein (NSs) of RVF virus was selected as a target on the basis of its critical role in virulence of RVF virus. Protein BLAST (Basic Local Alignment Search tool) was used for the identification of homologous sequences of NSs protein. With the help of homology modeling structure was obtained and the structure for the NSs protein was predicted by MODELLER, the obtained model was validated by two tools; first, to check stereo chemical properties via residue-by-residue geometry through PROCHECK, another was Ramachandran Plot.

Ligand binding site prediction

After the structure is predicted it is necessary to predict ligand binding as it helps in docking compounds to specific active site, this helps in increasing the efficiency of molecular docking. LIGSITE a web server is used for this prediction; it is based on the surface-solvent-surface events and the degree of conservation of residues. The amino acid residues present in the binding site were analyzed.

Ligand Identification by Virtual Screening

An online server ATOME 2 was used for identification of natural ligands of homologous proteins. Several different alignments were performed by this server and then the ligands associated with the homologous proteins were retrieved. In accordance with the chemical nature of amino acid residues which were present in the protein ligand binding site, intense database searching was conducted by using DrugBank. Different 3D conformations for all possible ligands (Leads) were generated by using an online server Frog 2.1

Molecular docking

For molecular docking of the leads with the best model generated for NSs protein an online server Patch Dock was used. As it generates a number of different conformations in which the protein and the ligand can interact. The output is a list of different possible poses along with the score and the binding energy.

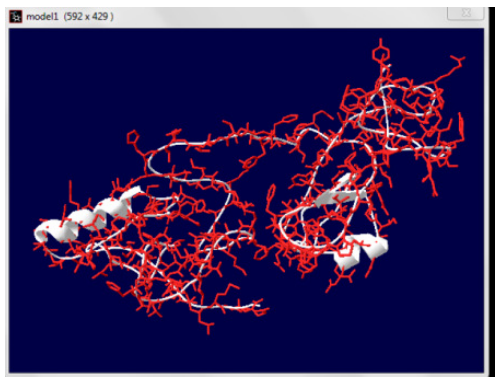
Comparative analysis of Ligand through Molinspiration

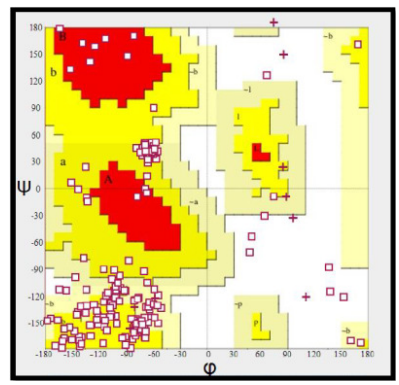
An online server molinspiration was used for the comparative analysis of the newly designed ligands. It calculates some important chemical properties like, *logP* (partition coefficient) etc used in QSAR studies and rational drug design as a measure of molecular hydrophobicity, polar surface area, number of non-hydrogen atom, number of hydrogen bond accepter, number of hydrogen bond donors and molecular weight.

3. RESULT AND DISSCUSSION

Protein database in NCBI was searched for "NSs Rift Valley Fever", blastp was performed of NSs against Protein Data Bank.

A crystal structure of ß-Ketosynthase (1MZJ-A) from the R1128 was identified with 38% similarity. Three model were generated and compared out of these 1st model was showing best result and has many allowed regions in Ramachandran Plot.





(a). Model 1 generated by MODELLER **(b). Ramachandran plot result**

Fig: 2 (a) Model 1 generated by MODELLER 9.11 (b) Ramachandran Plot, of 3-D models generated by MODELLER 9.11.

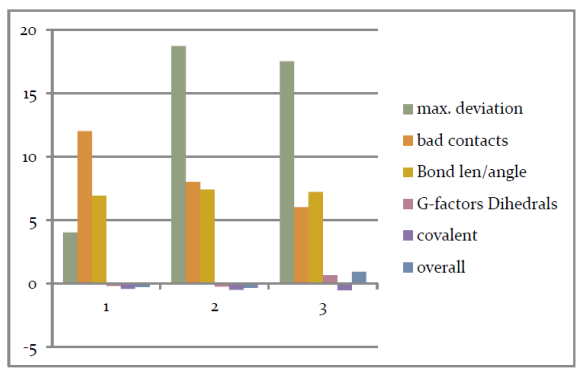
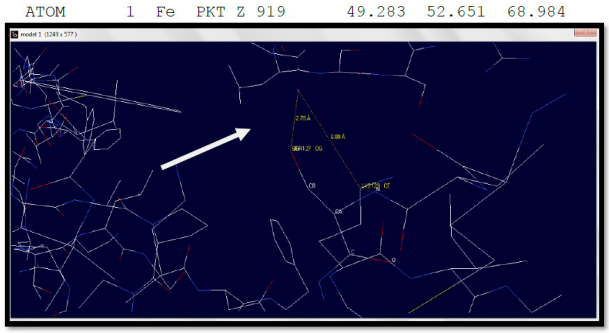


Fig 3 Graph comparing different properties of protein structure generated by MODELLER 9.11

Model 1 generated by the MODELLER has lesser deviations and optimal characteristics among three models (as shown in Fig 3).



ATOM	977	O	ALA	121	hydrophobic
ATOM	1001	CG	MET	125	hydrophobic
ATOM	1017	N	SER	127	polar
ATOM	1018	CA	SER	127	polar
ATOM	1019	CB	SER	127	polar
ATOM	1020	OG	SER	127	polar
ATOM	1093	CA	SER	137	polar
ATOM	1095	OG	SER	137	polar
ATOM	1185	OE2	GLU	148	negative charged
ATOM	1200	O	ILE	150	hydrophobic
ATOM	1206	CD2	LEU	151	hydrophobic
ATOM	1343	O	VAL	168	hydrophobic
ATOM	1345	CA	ALA	169	hydrophobic
ATOM	1346	CB	ALA	169	hydrophobic
ATOM	1558	CB	MET	198	hydrophobic
ATOM	1564	N	GLU	199	negatively charged

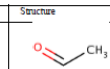
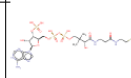
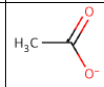
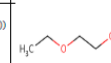
Fig 4. Binding site found by LIGSITE **Fig 5.Amino acid binding site on NSs protein**

Figure 4 and 5, shows coordinates for the binding site of model 1, using LIGSITE and Amino acid residues present binding site of NSs protein with their chemical nature respectively.

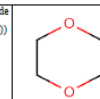
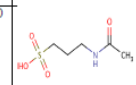
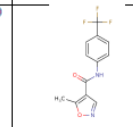
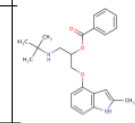
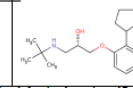
Virtual screening and Database Search results:

Ligands chosen from Drug Bank also are of mixed chemical nature because of the mix nature of ligand binding site of the NSs protein. After this these compounds were processed in a online server Frog 2 and 3D new conformations were generated respectively for each of the compound.

Table: 1 List of all possible drugs which were selected after Virtual screening and Database searching.

Serial no.	Lead molecule	Structure
01	Acetyl group (ACE)	
02	Coenzyme A (COA)	
03	Sulphate ion (SO4)	
04	Acetate ion (ACT)	
05	1-ethoxy-2-(2-ethoxyethoxy)ethane (DB08357)	
06	2-ethoxyethanol (DB02249 (EXPT01374))	

(a)

07	1-4-diethylene dioxide (DB03316 (EXPT01208))	
08	Acamprosate (DB00659)	
09	Leflunomide (DB01079)	
10	Bopindolol (DB08807)	
11	Penbutolol (DB01359)	

(b)

All the 11 Lead molecules screened were docked to the predicted protein structure target. Out of these Lead 9 has minimum energy of -58.91 and Binding score of 5923.

Table-2 PatchDock results of 11 leads showing energy (KJ/mol) and binding score with the best model (model 1) for NSs protein.

S.no.	Ligand name	Binding Score	Global Energy
1	lead1	6375	45.64
2	lead2	7751	17.74
3	lead3	8367	-47.77
4	lead4	7369	-25.65
5	lead5	7073	72.52
6	lead6	8463	-2.58
7	lead7	7345	18.63
8	lead8	6894	73.49
9	lead9	5923	-58.91
10	lead10	8134	31.84
11	lead11	8875	65.45

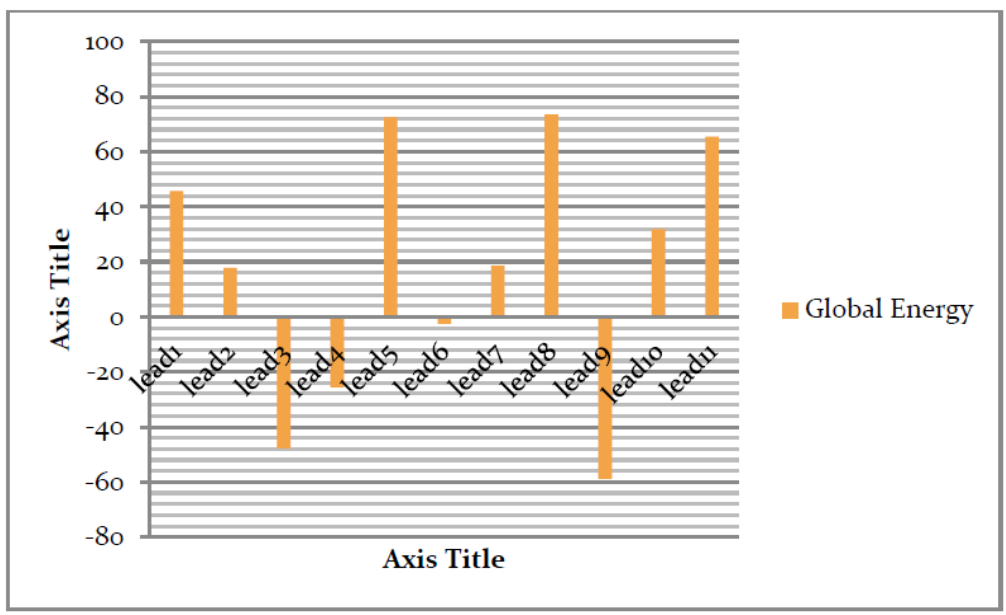


Figure 6: Global energy associated with binding of different ligands.

4. COMPARATIVE STUDY RESULTS

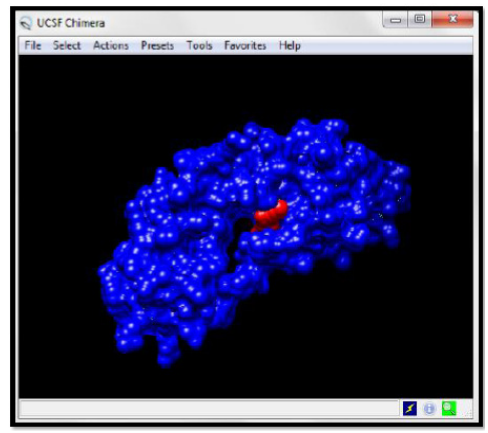


Figure: 7 Visualisation of the best pose of the lead 9 using USCF-chimera.

chemical properties	LogP	polar surface area	no. of non hydrogen atoms	molecular weight	no. of H-bond acceptors	no. of H-bond donors	molecular volume
lead 1	0.509	17.071	3	44.053	1	0	48.179
lead2	-4.44	346.58	48	767.541	23	10	594.458
lead3	-4.155	80.256	5	96.063	4	0	54.617
lead4	-2.78	40.128	4	59.044	2	0	53.455
lead5	0.641	27.702	11	162.229	3	0	173.552
lead6	-0.053	29.462	6	90.122	2	1	96.604
lead7	-0.234	18.468	6	88.106	2	0	86.97
lead8	-3.187	83.468	11	181.213	5	2	150.436
lead9	2.2	95.561	22	301.394	6	5	291.821
lead10	5.144	63.357	28	380.488	5	2	366.964
lead11	3.654	41.489	21	291.435	3	2	303.259

Figure: 8 Results obtained by molinspiration server

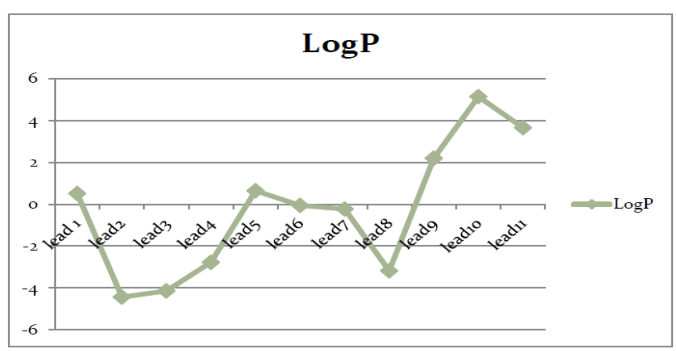


Figure 10, Graph showing logP values of individual leads.

5. CONCLUSION

Rift valley fever has potential to affect human race in many aspects of our life. Many work has been done to design vaccines and drugs against it, but still there is no licensed drug present for this disease. The purpose of the current study was to design novel possible drugs for rift valley fever disease using a computational approach because it is time effective and number of chemical designed is high so the possibility of getting the right drug is also high. Out of all ligands designed in this work one of the ligand (lead 9) showed significantly better results (logP=2.2, Global energy= -58.98). Comparative analysis of all the ligands was also performed. Lead 9 showed significant results and can be used for further research for finding an effective drug for rift valley fever.

REFERENCES

[1] Francis F. Schwentker, Thomas M. River. Report of fatal laboratory infection complicated by thrombophlebitis. J Exp Med 1934; 59(3): 305–313.

[2] Sall A.A., de A Zanotto P.M., Zeller H.G., Digoutte J.P., Thiongane Y. and Bouoy M. Variability of the NSs protein among rift valley fever virus isolates. J Gen Virol 1997 78(11): 2853-2858.

[3] Sotriffer C.A. and Klebe G. Identification and mapping of small molecule binding sites in proteins: computational tools for structure based drug design. Farmaco 2002; 57: 243-251.

[4] Muller R., Poch O., Delarue M., Bishop D.H. and Bouloy M. Rift Valley fever virus L segment: correction of the sequence and possible functional role of newly identified regions conserved in RNA-dependent polymerases. J Gen Virol. 1994; 75 (6): 1345-1352.

[5] Kakach L.T., Suzich J.A., Collett M.S. Rift Valley fever virus M segment: phlebovirus expression strategy and protein glycosylation. Virology 1989; 170(2): 505-510

[6] Smithburn K.C., Haddow A.J. and Gillett J.D. Rift Valley fever; isolation of the virus from wild mosquitoes. Br J Exp Pathol 1948; 29(2): 107-121.

[7] Tetsuro Ikegami, Krishna Narayanan, Sungyong Won, Wataru Kamitani, C. J. Peters and Shinji Makino. Dual functions of ift valley fever virus NSs protein: Inhibition of host mRNA transcription and post-translational downregulation of protein kinase PKR. Ann N Y Acad Sci 2009; 1171(1): E75-85

[8] Talevi A, Bellera C.L., Ianno M., Duchowicz P.R., Bruno-Blanch L.E., and Castro E.A. An integrated drug development approach applying topological descriptors. Curr Comput Aided Drug Des 2012; 8(3): 171-181

[9] Basseto M., De Burghraeve T., Delang L., Massarotti A., Coluccia A., Zonta N., Gatti V., Colombano G., Sorba G., Silvestri R., Tron G.C., Leyssen P. And Brancale A. Computer aided identification, design and synthesis of a novel series of compounds with selective antiviral activity against chikungunya virus. Antiviral Res 2013. 98(1): 12-18