

Identification of Potential Lead Molecules for Zika Envelope Protein from In Silico Perspective

Selvaa Kumar Chellasamy* and Shine Devarajan

Faculty of Biotechnology and Bioinformatics, D.Y. Patil Deemed to be University, CBD Belapur, Navi Mumbai, India

Abstract

Background: Zika virus is the family member of flavivirus with no reported clinically approved drugs or vaccines in the market till date. This virus is spread by *Aedes* mosquitoes, and can also be transmitted through sexual contact or blood transfusions. There are reported medical conditions like microcephaly among new-borns delivered by infected pregnant women. The envelope protein of Zika virus is associated with virulence, tropism, mediation of receptor binding and membrane fusion. ED1-EDII domain (K1 loop pocket) is an integral part of the envelope protein and a potential drug target. In the present study, the purpose was to identify the potential lead molecules to dock against K1 loop which could be later considered as flavivirus entry inhibitors.

Methods: Multiple sequence alignment method was considered for the analysis of indels in envelope protein. Phylogenetic tree was constructed based on the alignment. Aliphatic index, GRAVY scores and hydropathy plot of the envelope proteins were calculated for the flavivirus family members. Zika envelope protein was homology modeled and considered for protein-ligand docking analysis with chemical compounds of known functions.

Results: As per in silico based analysis, the envelope protein of Zika virus is highly hydrophilic with the least number of amino acid deletions compared to rest of the family members. During docking studies, it was observed that compounds like NITD, compound 6, PO2, Doxytetracycline and Rolitetracycline show better binding affinity with Zika envelope protein compared to dengue virus.

Conclusion: These better binding compounds could be the promising lead molecules for Zika envelope protein which could better block the viral entry.

Avicenna J Med Biotech 2019; 11(1): 94-103

Keywords: Aedes, Dengue virus, Envelope protein, Flavivirus, Microcephaly, Zika virus

Introduction

Zika virus (ZIKV) belongs to *flavivirus* family which was first isolated from a sentinel rhesus monkey in the Zika Forest, Uganda in 1947¹. Human population gets infected by the bite of an infected *Aedes* species². In recent years, Brazil has reported Zika viral infection at a larger scale³. Even Iran is highly exposed to the Zika infection due to favorable environment and the presence of three members of *Aedes* genus^{4,5}. Common symptoms associated with the infection are fever, skin rashes, conjunctivitis, joint pain, malaise and headache which are quite similar to that of dengue and chikungunya^{6,7}. Other members of this family include West Nile, dengue, yellow fever, and Japanese encephalitis^{8,9}. A recent study states that this viral infection can be sexually transmitted and can also be passed on from

the pregnant women causing micro cephal among the new borns^{10,11}. Interestingly, *Aedes* mosquitoes remain the common vector for transmitting both dengue and Zika virus². Till date, no drugs or vaccines were reported against this viral infection⁷. The serious threat of viral infection has recently hard-pressed WHO to declare a global public health emergency¹².

The completely sequenced Zika viral genome is available online with 10,272 nucleotides¹²⁻¹⁴. This gets translated into a single polyprotein. Furthermore, they were post and co-translationally cleaved by both host and viral proteases much like rest of their family members¹⁵. This polyprotein comprises three structural (capsid, envelope and pre-membrane) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A,

* Corresponding author:
Selvaa Kumar Chellasamy,
Ph.D., Faculty of Biotechnology
and Bioinformatics, D.Y. Patil
Deemed to be University, CBD
Belapur, Navi Mumbai, India
Tel: +91 22 27563600
E-mail:
selvaakumarc@gmail.com
Received: 23 Jul 2017
Accepted: 3 Nov 2017

NS4B, NS5) ¹⁶⁻¹⁸. These structural proteins were reported to be involved in the formation of the viral particle ¹⁹. The non-structural proteins were involved in flavivirus assembly ²⁰. The present study focused on the envelope protein (E protein) which is considered as the major determinant of virulence, tropism since it plays a critical role in mediation of receptor binding and membrane fusion ²¹⁻²⁴.

The N-terminal region of E protein contains three well characterized domains as determined by crystallographic studies which include ED1, EDII and E-DIII ²⁵⁻²⁸. As such, EDI has no reported functions in ZIKV which is otherwise required for viral entry into the host cell in other flaviviruses. Furthermore, EDII contains a hydrophobic fusion loop at its distal end which is a dimerization domain. It is proposed to bind to the membrane of the endosome to facilitate fusion between virus and endosomal membrane. EDIII with immunoglobulin fold participates in both receptor binding and fusion. In particular, EDI-EDII combo forms a hydrophobic fusion loop (K1 pocket loop) which happens to be a potential drug target. As per the crystal structure report, the hinge angle between ED1 and EDII varies among the family members. This was found to be highly flexible and is required for flexing of the EDI-EDII during the fusion process in order to expose the fusion loop ^{24,29,30}.

In dengue envelope protein (DENV), EDI-EDII combo interacts with β -N-octyl-glucoside (β -OG) which in turn can sterically hinder the conformational change between these domains which is essential for virus-host membrane fusion ²⁴. A single glycosylation site (Asn 154) was observed in Zika envelope protein (ZIKV E) which is two (Asn67 and Asn153) in DENV E. It has also been reported that the amino acids surrounding Asn154 differ in ZIKV E and in other flavivirus, which may provide insight into the pathobiology of Zika virus ³¹. The C-terminal region of E protein consists of two alpha helices (EH1 and EH2) in the stem region and two helices in the transmembrane region (ET1 and ET2). Both ET1 and ET2 are associated with the assembly of E-protein ³²⁻³⁴. The main objective of this study was to identify the potential lead molecules for the ZIKV E protein which shows better interactions with ED1-EDII domain from in silico perspective. This study explored the molecular level interactions of ZIKV E protein with the leads which is not feasible with the conventional method. These compounds could be further used against ZIKV E protein for therapeutics and thus can arrest the virus recognition to the host cell.

Materials and Methods

Sequence analysis

The complete genome polyprotein of Zika virus, West Nile, yellow fever, Japanese encephalitis and dengue was downloaded from Uniprot Database ³⁵, [Q32ZE1: Zika; P27395: Japanese encephalitis; P06935:

West Nile; P17763: Dengue; P03314: Yellow fever] ³⁵. Out of these, only the envelope protein domains were extracted from all five organisms. These envelope proteins were subjected to sequence analysis using ProtParam software ³⁶ wherein, the purpose was to study their overall aliphatic index and GRAVY score (Grand Average of hydropathy). Basically, Aliphatic Index method ^{37,38} predicts regional stability by calculating the relative volume occupied by aliphatic side chains. This is a positive indicator of globular protein thermostability. However, the GRAVY value is calculated by adding the hydropathy value for each residue and dividing them by the length of the sequence by using Kyte-Doolittle method ³⁹. This can be calculated as the sum of the hydropathy values for all the amino acids in a protein divided by the total number of residues in it. Next, all five sequences were considered for hydrophobicity analysis using Kyte-Doolittle hydropathy plot ³⁹. It is a quantitative analysis of the degree of hydrophobicity or hydrophilicity of amino acids of a protein which is used to characterize or identify possible structure or domains of a protein. The graph above zero defines them as hydrophobic whereas below zero is considered as hydrophilic. Finally, all five sequences were considered for multiple sequence alignment using Clustal Omega software ⁴⁰. A phylogenetic rooted tree (Neighbour Joining method) was constructed based on the multiple alignment to identify the close homolog of Zika virus.

Homology modeling

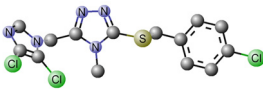
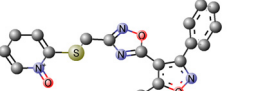
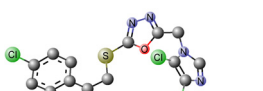


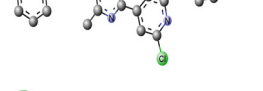
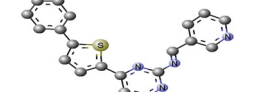
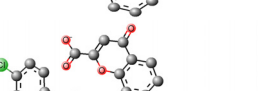
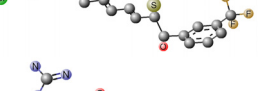
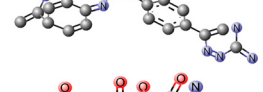
Search for crystal structure of ZIKV E protein has listed nine structures docked with antibody in Protein Data Bank (PDB) (<https://www.rcsb.org/pdb/home/home.do>) (PDB ids: 5JHM, 5JHL, 5KVD, 5KVE, 5KVG, 5KVF, 5VIG, 5GZN and 5GZO) ⁴¹⁻⁴⁴. All these crystal structures were available in post-fusion form with closed ED1-EDII loop. These structures cannot be considered for docking due to their closed hydrophobic pocket. Further search within PDB has listed a pre-fused crystal structure of dengue with open hydrophobic pocket (PDB id: 1OKE). This conformation was due to the local rearrangement of the K1 beta hairpin between residues 268-280. Here, the envelope protein is in complex with n-octyl-beta-D-glucoside within the hydrophobic pocket ²⁴. To generate the similar conformation in ZIKV E, homology modeling was opted using SWISSMODEL server ⁴⁵. Finally, generated model was energy minimized using Swisspdbviewer software ⁴⁵ and validated using Ramachandran plot using RAMPAGE ⁴⁶ and PROSA (Protein Structure Analysis) software ⁴⁷.

Protein-ligand docking

Based on literature review, 10 ligands [A1-A5, NIT-D, compound-6 (with a quinazoline core), P02, Doxytetracycline (with tetracyclic ring structure) and Rolite-tetracycline (with tetracyclic ring structure)] were downloaded from Maybridge chemical database ⁴⁸. These

Lead Identification for Zika Envelope Protein

Table 1. The structure and activity of 10 compounds (μM) against DENV envelope protein

No.	Compound	Structure	Activity against DENV (μM)	Reference
1.	A1		$>100^*$	(46)
2.	A2		$>100^*$	(46)
3.	A3		$>100^*$	(46)
4.	A4		$32 \pm 17^*$	(46)
5.	A5		$1.2 \pm 0.7^*$	(46)
6.	Compound-6		$0.119 / >20^\#$	(47)
7.	NITD-448		$9.8 / 48.7^\#$	(48)
8.	P02		$13 / 371^\#$	(49)
9.	Doxycycline		$55.6 / >500^\#$	(50)
10.	Rolitetracycline		$67.1 / >500^\#$	(50)

* IC_{50} (μM), # (EC_{50}/CC_{50})

compounds have a significant biological affinity (μM) with DENV shown in table 1. In particular, compound A4 and A5 showed good antiviral activity in DENV⁴⁹⁻⁵³. Their physiochemical properties are listed in table 2. Mostly, they are thiazole derivatives critically involved in arresting viral replication in cell-based assays⁵⁴. These chemical compounds are available in 2D form, which were converted into 3D conformers using Che-

mAxon (<http://www.chemaxon.com>) software. The modeled ZIKV E protein was considered as receptor for docking against these ten ligands using AutoDock software (Version 4.2)⁵⁵. In the parameters section, Lamarckian genetic algorithm was selected as a scoring function for identifying the favorable conformation in the binding site. A grid box was constructed at the interface of D1-DII domain of the receptor with a map

Table 2. Physicochemical properties of ten chemical compounds

Compound	Hydrophobicity LogP	Aquous solubility LogS	Blood brain barrier (BBB)	Human Intestinal absorption (HIA)	PgP substrate/Inhibitor or	Acute oral toxicity class	Caco2 permeability (LogPapp,cm/s)	Rat acute toxicity (LD50, mol/kg)
A1	3.67	-3.88	+	+	No	III	1.50	2.37
A2	3.70	-4.01	+	+	No	III	1.42	2.38
A3	4.12	-3.83	+	+	No	III	1.64	2.50
A4	4.39	-4.24	+	+	No	III	0.97	2.50
A5	6.77	-6.12	+	+	No	III	1.32	2.19
Compound-6	5.86	-6.22	+	+	No	III	1.31	2.43
P02	3.57	-4.44	+	+	No	III	0.98	2.50
NITD	6.44	-6.58	+	+	No	III	0.50	3.07
Doxytetracycline	1.14	-2.37	+	+	No	III	1.40	2.51
Rolitetracline	1.48	-2.55	-	+	No	III	0.68	2.78

Table 3. Comparison of GRAVY and Aliphatic Index score of envelope proteins of Zika, dengue, Japanese encephalitis, West Nile and Yellow fever

Virus	Swissprot accession number	Envelope protein	GRAVY score	Aliphatic index score
Zika	Q32ZE1	291-790	-0.078	82.22
Dengue	P17763	281-775	-0.052	85.23
Japanese encephalitis	P27395	295-794	-0.008	80.56
West Nile	P06935	291-787	0.025	82.19
Yellow Fever	P03314	286-778	-0.020	85.98

dimension of 30×30×30 and kept 1 Å grid spacing for accommodating all the amino acids in the binding site. The grid center of the x y z box coordinates were set as -8.141, 80.423 and 45.672, respectively. Based on the above settings, AutoGrid parameters for each ligand within the binding sites were calculated. After successful generation of each grid box, Lamarckian genetic algorithm based docking parameters were prepared to generate the conformations of the ligands. A population size of 150 was used for generating 50 conformations for each ligand with a maximum number of 2500000 evaluations per cycle. The rate of gene mutation and crossover parameters in the algorithm were set as 0.02 and 0.8, respectively. Among 50 conformations, the most favorable compound was selected based on their binding affinity. Similar steps were followed for docking dengue envelope protein with the ten ligands as a reference.

Results

Sequence analysis

Multiple sequence alignment of all five envelope proteins reveals that minimum number of deletions were observed in Zika and maximum in yellow fever (Figure 1). Based on this alignment, a phylogenetic rooted tree (Neighbour Joining) was generated wherein Zika and dengue envelope proteins share a common internal node. Thus, these two OTUs (Operational Taxonomic Unit) are close homologs. Other OTUs, like Japanese encephalitis and West Nile share a common ancestral internal node. However, yellow fever is

the outlier (Figure 2). A separate alignment of the K1 loop (268-280 of 13 residues length) of Zika and dengue confirms six identical residues followed by two conservative and three non-conservative substitutions (Figure 3). Next, the GRAVY score (Grand Average of hydropathy) was calculated for all five E proteins. The ZIKV E protein is highly negative followed by dengue virus. However, West Nile has a positive GRAVY score. Conversely, aliphatic index value was calculated. Yellow fever has the maximum index value and Japanese encephalitis has the minimum (Table 3). Finally, Kyte-Doolittle hydropathy plots were generated for all five envelope proteins. In these plots, Zika and dengue virus falls below the zero which confirms them as hydrophilic in nature. However, most of the segments of West Nile, Japanese encephalitis and yellow fever moves above zero marking them as hydrophobic (Figures 4A-E).

Homology modeling

Availability of post-fused crystal structures of ZIKV E protein has compelled homology modeling to generate an opening conformation of the K1 loop. Thus, the 3D structure of DENV E protein (PDB id: 1OKE) with a bound n-octyl-beta-D-glucose was considered as the template for modeling ZIKV E protein. From sequence perspective, both proteins share 55.87% identity making them a perfect template for modeling. Modeled structure was energy minimized and considered for model validation. As per the Ramachandran plot analysis, only six residues were observed in the disallowed region (Figure 5A). Mostly, residues were within the

Lead Identification for Zika Envelope Protein

Yellow Japan Westnile Zika Dengue	AHCIGITDRDFIEGVHGGTWSATLEQDKCVTVMAPDKPSLDISLETVAIDRPAEVRKVC FNCLGMGNRDFIEGASGATWVDLVEGDSCVTIMANDKPTLDVRMINIEASQLAEVRSYC FNCLGMSNRDFIEGVSGATWVDLVEGDSCVTIMSKDKPTIDVMMNMEANLADVRSYC IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPAVDIELVTTTNSMAEVRSYC MRCVIGIGNRDFVEGLSGATWVDVLEHGGCVTTMAKDKPTLDIELLTEVTNPVLRKLC *.*.: :***:* *.*. .** .*: * :***:.*: : . * : * *
Yellow Japan Westnile Zika Dengue	YNAVLTHVKINDKCPSTGEAHLAEENEGDNACKRTYSDRGWNGCGLFGKGSIVACAKFT YHASVTDISTVARCPTTGEAHNEKRADSSVYCKQGFDRGWGNGCGLFGKGSIDTCAKFS YLASVSDLSTRAACPTMGEAHNEKRADPAFVCKQGVVDRGWGNGCGLFGKGSIDTCAKFA YEASISDMASDRCPQTQGEAYLDKQSDTQYVCKRTLVDGWGNGCGLFGKGSIVTCAKFA IEAKISNTTDSRCPQTQGEATLVEEQDTNFVCRRTFVDRGWGNGCGLFGKGSITCAKFK * : : . * : * * : : . : . * : : *
Yellow Japan Westnile Zika Dengue	CAKMSLFEVDQTKIQYVIRAQLHVGAKQENWNTDI-----KTLKFDALSGSQVEVF CTSKAIGRTIQPENIKYEVGIFVHGTTTTSENHGNYSQVQASQAQAFVTPNPASITLKL CTTKATGWI IQKENIKYEVAFVHGPTTVE SHGK----IGATQAGRFSITPSPASYTLKL CSKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHE TDENRAKVEITPNSPRAEATL CVTKLEGKIVQYENLKYSVIVTVHTGDQH-----QVGNETTEHGTTATITPQAPTSEIQL * . . : : * : : * : : *
Yellow Japan Westnile Zika Dengue	IGYGKATLECVQQTAVDFGNSYIAEMETE SWIVDRQWAQDLTLPWQSGS---GGVWREMH GDYGEVTLDCPEPRSLNTEAFYVMTVGSKSFLVHREWFHDLALPWTSPS---STAWRNRE GEYGEVTVDCPEPRSGIDTSAYYVMSVGEKSFLVHREWFMDLNL PWSSAG---STTWRNRE GGFGSLGLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNKQD TDYGALTLDCSPRTGLDFNEMVLLTMEKKSFLVHKQWFLDLPLPWTSGASTSQETWNRQD :* : * . : : : : : : : : * : * * * : * * * * * * * * * * * * * * * *
Yellow Japan Westnile Zika Dengue	HLVEFEPHAATIRVLALGNQEGSLKTALTGAMRVTKDNDNNLYKLHGGHVSCRVKLSA LLMEFE GAHATKQSVVALGSQEGGLHQLAGAI VVEYSS---SVKLTSGHLKCRLLKMDK TLMEFE PHATKQSVVALGSQEGALHQLAGAI PVEFSSN---TVKLTSGHLKCRVKMEK ALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGA---KGRLLSSGHLKCRLLKMDK LLVTFKTAHAKKQEVVVLGSQEGAMHTALTGATEIQTSGT---T-TFAGHLKCRLLKMDK * : * : * * * : * * * * * : * : * * * . : * * * * * * * * * * * * * * * *
Yellow Japan Westnile Zika Dengue	LTLKGTSYKICTDKMFFVKNPTDGTGHGTVMQVKVS-KGAPCRIPVIVADDL TAAINKGI LALKGTTYGMCTEKFSFAKNPVDTGHTVVIELSYSGSDGPKIPIVSVASLNDMTPVGR LQLKGTTYGVC SKAFKFARTPADTGHGTVLELQYTGTDGPKVPISSVASLNDLTPVGR LRLKGVSYSLCTAAF TFKIPAE TLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGR LTLKGMSYVMCTGSFKLEKEVAETQHGTVLVQKYEGTDAPCKIPFSSQ-DEKGVQTQNGR * * * * * : * : * : : : : . * * * * * : : . . . * * * * * * * * * * * *

Figure 1. Multiple sequence alignment of yellow fever, Japanese encephalitis, West Nile, Zika and dengue envelope proteins. The identical, conservative and non-conservative substitutions are shown as asterisk (*), colon (:), and dot (.), respectively. Deletions were denoted as hyphen (-).

This is a Neighbour-joining tree without distance corrections.

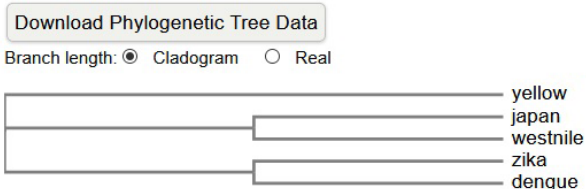


Figure 2. The pairwise alignment of the K1 loop of dengue and Zika envelope proteins (268-280) highlighting the region of identical and semi-conservative residues which are critically involved in drug interaction.

allowed region. As per PROSA report, most of the regions were lying below zero confirming their overall structural stability (Figure 5B).

Protein-ligand docking

Modeled ZIKV E protein was docked with ten chemical compounds. The K1 loop of Zika envelope protein exhibits better protein-ligand interaction with compound-6, Doxycycline, NITD, P02 and Rolitetracycline. However, compounds like A1-A5 show lesser binding affinity (Figures 6A-J). In contrast, the K1 loop of DENV E protein shows better interaction with A1-A5 compounds as reported earlier in the literature⁴⁹. Furthermore, compound-6, Doxycycline, NITD, P02

and Rolitetracycline display lesser binding affinity (Figures 7A-J) (Table 4). The RMSD scores for A1 to A5 between DENV and ZIKV are 2.386Å, 1.532Å, 2.961Å, 1.838Å and 2.338Å, respectively. However, compound-6, Doxycycline, NITD, P02 and Rolitetracycline show large deviation in their values due to the distinct binding behavior between the two viral proteins.

Conclusion

Outbreak of Zika viral infection and non-availability of drug therapeutics against this disease have compelled identification of better lead molecules. In this study, envelope protein of ZIKV was the main focus with reference to rest of their family members. ZIKV E protein is quite distinct from rest of them with the least number of amino acid deletions. Furthermore, from phylogenetic perspective, they are closer to DENV E protein. Based on this report, the chemical compounds better binding with DENV E proteins were docked with ZIKV E protein. Here, it was observed that compounds like NITD, compound-6, P02, Doxytetracycline and Rolitetracycline showed better binding affinity with ZIKV. Irrespective of being structurally similar, lead interactions differ between DENV and ZIKV proteins which was evident through their binding affinity and RMSD score. Thus, it was concluded that these



Figure 3. The rooted phylogenetic tree generated using Clustal Omega software for Japanese encephalitis (P27395), dengue (P17763), West Nile (P06935), yellow fever (P03314) and Zika (Q3ZE1) envelope proteins.

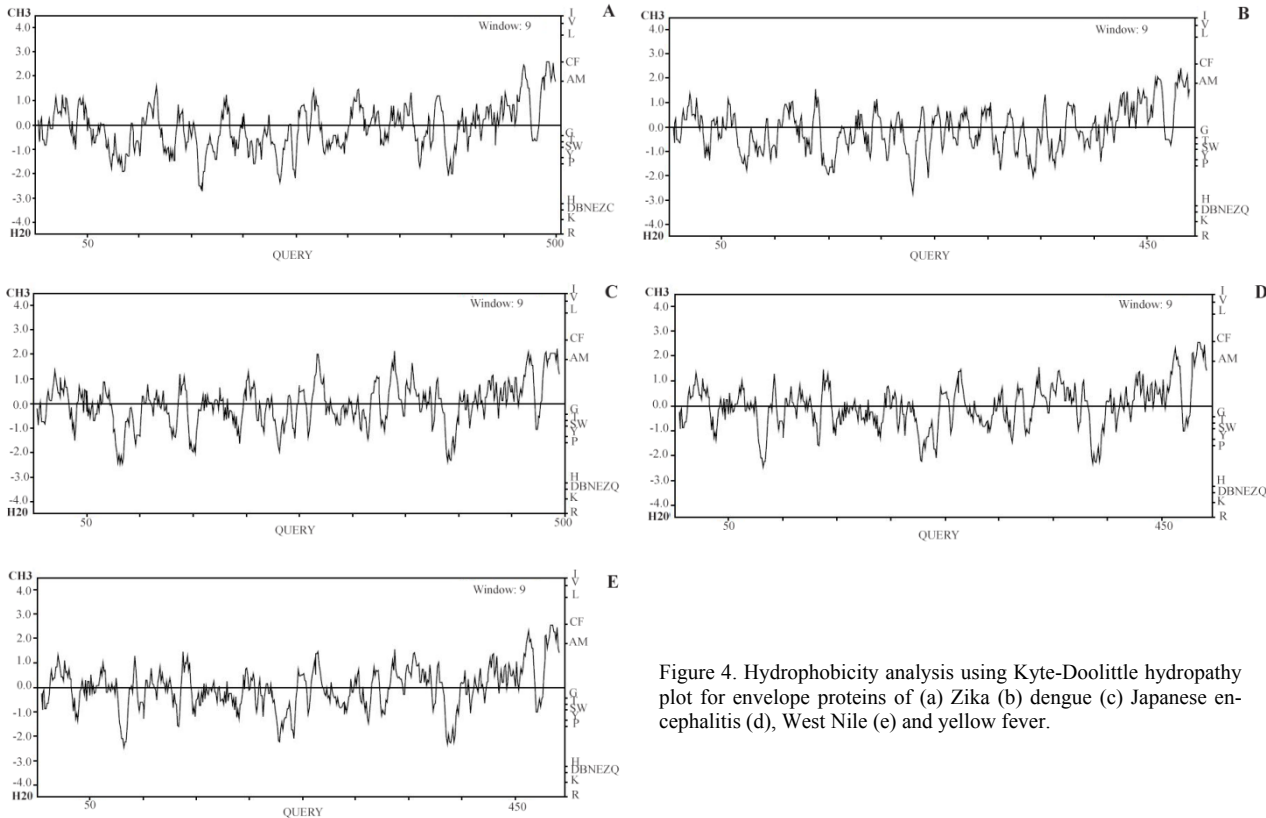


Figure 4. Hydrophobicity analysis using Kyte-Doolittle hydropathy plot for envelope proteins of (a) Zika (b) dengue (c) Japanese encephalitis (d), West Nile (e) and yellow fever.

Lead Identification for Zika Envelope Protein

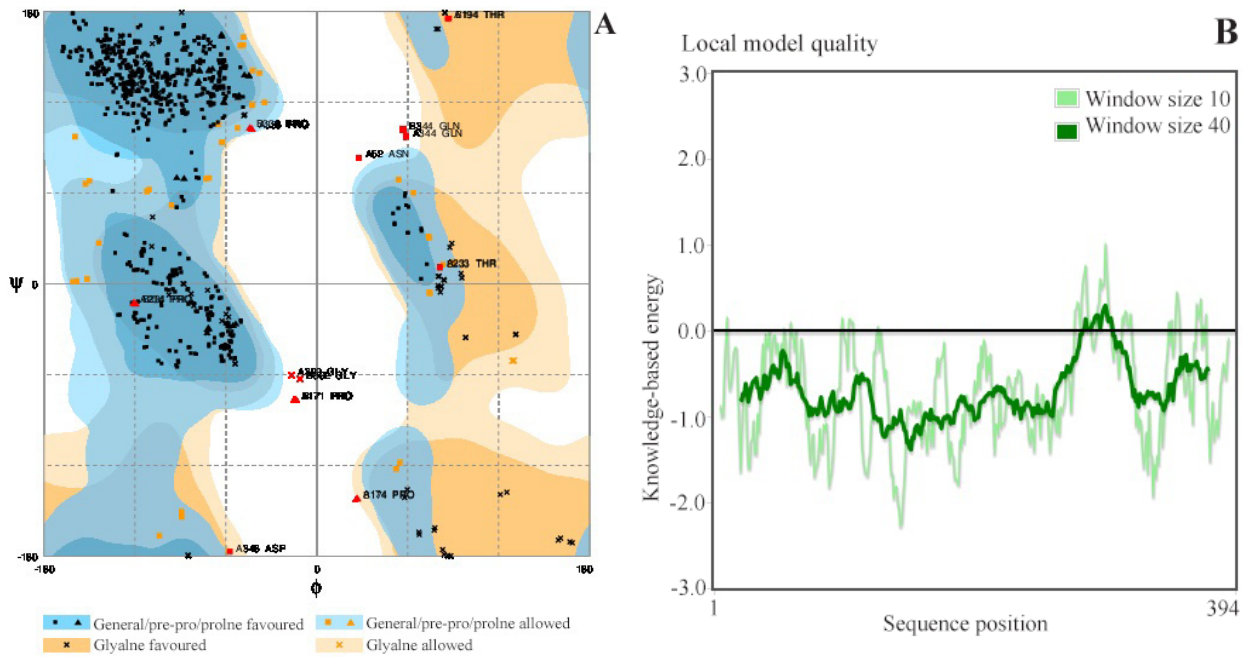


Figure 5. A) Ramachandran plot of modeled Zika envelope protein. B) PROSA showing the local model quality of the modeled Zika protein. The plot is generated with a window size of 40.

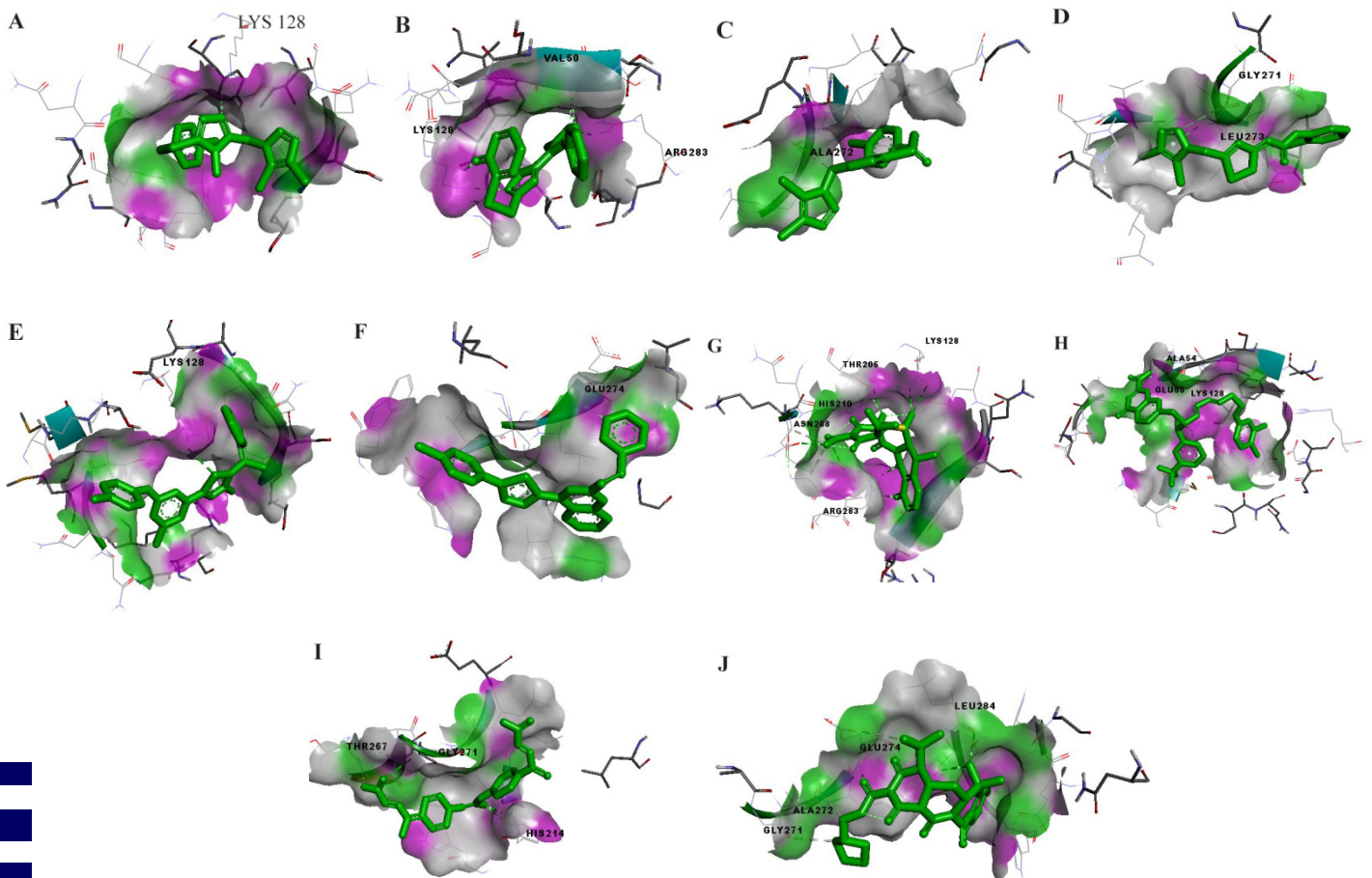


Figure 6. The docked poses of ligands A1-A5 with the envelope protein of dengue (a-e). The interactions between compound 6, Doxycycline, NITD, PO2 and Rolitetracycline (f-j). The purple color denotes hydrogen bond donor and the green color denotes hydrogen bond acceptor.

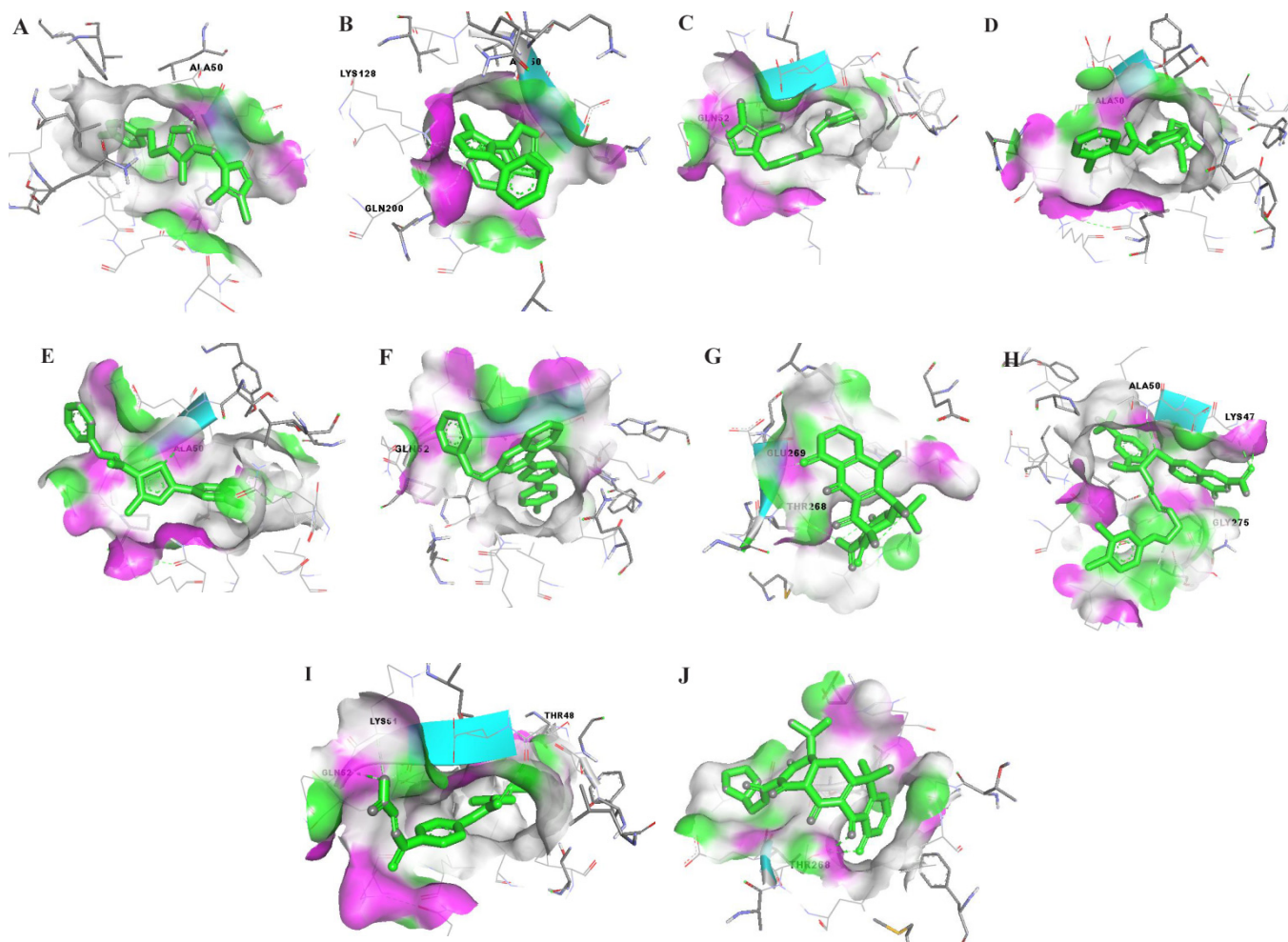


Figure 7. The docked poses of ligands A1-A5 with the envelope protein of Zika (a-e). The interactions between compound 6, Doxycycline, NITD, PO2 and Rolitetracycline (f-j). The purple color denotes hydrogen bond donor and the green color denotes hydrogen bond acceptor

Table 4. The docking score of ten compounds docked against dengue and zika envelope protein

S.No	Chemical Compounds	Dengue		Zika	
		Amino acids	Binding energy (kcal/mol)	Amino acids	Binding energy (kcal/mol)
1	A1	Ala50	-4.25	Lys128	-1.27
2	A2	Ala50, Lys128, Gln200	-2.8	Lys128, Val50, Arg283	-2.23
3	A3	Gln52	-3.32	Ala272	-1.16
4	A4	Ala50	-3.68	Gly271, Leu273	-2.54
5	A5	Ala50	-4.48	Lys128	-1.14
6	Compound6	Gln62	-1.4	Glu274	-2.17
7	Doxycycline	Gln269, Thr268	-4.14	Thr205, Lys128, His210, Asn208, Arg283	-5.93
8	NITD	Ala50, Lys47, Gly275	-3.92	Ala54, Glu55, Lys128	-6.25
9	P02	Gln62, Lys51, Thr48	-3.32	Thr267, Gly271, His214	-5.20
10	Rolitetracycline	Thr268	-3.34	Gly271, Ala272, Glu274, Leu284	-5.21

five small molecules which were exhibiting better interaction with ZIKV E protein could be promising lead molecules. A wet lab based study could assist in understanding the role of these molecules in blocking the function of viral envelope proteins to prevent viral entry.

Acknowledgement

The authors would like to acknowledge the Faculty of Biotechnology and Bioinformatics, D.Y Patil Deemed to be University, Navi Mumbai for carrying out this project. This project was not funded by any external funding agencies.

Conflict of Interest

The authors declare they have no conflicts of interest.

References

- Dick GW, Kitchen SF, Haddow AJ Zika virus. I. Isolations and serological specificity. *Trans R Soc Trop Med Hyg* 1952;46(5):509-520.
- Dick GW. Zika virus. II. Pathogenicity and physical properties. *Trans R Soc Trop Med Hyg* 1952;46(5):521-534.
- de Oliveira WK, Carmo EH, Henriques CM, Coelho G, Vazquez E, Cortez-Escalante J, et al. Zika virus infection and associated neurologic disorders in Brazil. *N Engl J Med* 2017;376(16):1591-1593.
- Azari-Hamidian S. Checklist of Iranian mosquitoes (Diptera: Culicidae). *J Vector Ecol* 2007;32(2):235-242.
- Mardani M. Update on Zika virus infections. *Arch Clin Infect Dis* 2016;11(2):69-71.
- Payne S. Family Flaviviridae. *Viruses from understanding to investigation*. Academic Press; 2017,129-139.
- Beltrán-Silva SL, Chacón-Hernández SS, Moreno-Palacios E, Pereyra-Molina JA. Clinical and differential diagnosis: Dengue, chikungunya and Zika. *Rev Med Hosp Gen Méx* 2018;81(3):146-153.
- Malone RW, Homan J, Callahan MV, Glasspool-Malone J, Damodaran L, Schneider Ade B, et al. Zika virus: medical countermeasure development challenges. *PLoS Negl Trop Dis* 2016;10(3):e0004530.
- Sikka V, Chattu VK, Popli RK, Galwankar SC, Kelkar D, Sawicki S G, et al. The emergence of Zika virus as a global health security threat: a review and a consensus statement of the INDUSEM joint working group. *J Glob Infect Dis* 2016;8(1):3-15.
- Oster AM, Brooks JT, Stryker JE, Kachur RE, Mead P, Pesik NT, et al. Interim guidelines for prevention of sexual transmission of Zika virus-United States, 2016. *MMWR Morb Mortal Wkly Rep* 2016;65(5):120-121.
- Schuler-Faccini L, Ribeiro EM, Feitosa IM, Horovitz D, Cavalcanti DP, Pessoa A, et al. Possible association between Zika virus infection and microcephaly-Brazil. *MMWR Morb Mortal Wkly Rep* 2015;65(3):59-62.
- Logan IS. ZIKA-How fast does this virus mutate? *Don-gwuxue Yanjiu* 2016;37(2):110-115.
- Kuno G, Chang GJ. Full-length sequencing and genomic characterization of Bagaza, Kedougou, and Zika viruses. *Arch Virol* 2007;152(4):687-696.
- Enfissi A, Codrington J, Roosblad J, Kazanji M, Rousset D. Zika virus genome from the Americas. *Lancet* 2016; 387(10015):227-228.
- Baronti C, Piorkowski G, Charrel RN, Boubis L, Leparco-Goffart I, de Lamballerie X. Complete coding sequence of Zika virus from a French polynesia outbreak in 2013. *Genome Announc* 2014;2(3):pii: e00500-514.
- Bollati M, Alvarez K, Assenberg R, Baronti C, Canard B, Cook S, et al. Structure and functionality in flavivirus NS-proteins: perspectives for drug design. *Antiviral Res* 2010;87(2):125-148.
- Lindenbach BD, Thiel HJ, Rice CM. *Flaviviridae: The viruses and their replication*. 5th rev.ed. Knipe DM, Howley PM. Fields virology. Philadelphia: Lippincott Williams & Wilkins; 2007.1101p.
- Stadler K, Allison SL, Schalich J, Heinz FX. Proteolytic activation of tick-borne encephalitis virus by furin. *J Virol* 1997;71(11):8475-8481.
- Speight G, Coia G, Parker MD, Westaway EG. Gene mapping and positive identification of the non-structural proteins NS2A, NS2B, NS3, NS4B and NS5 of the flavivirus Kunjin and their cleavage sites. *J Gen Virol* 1998; 69(Pt 1):23-34.
- Zhang X, Ge P, Yu X, Brannan JM, Bi G, Zhang Q, et al. EM structure of the mature dengue virus at 3.5-Å resolution. *Nat Struct Mol Biol* 2012;20(1):105-110.
- Kudelko M, Braut JB, Kwok K, Li MY, Pardigon N, Peiris JS, et al. Class II ADP-ribosylation factors are required for efficient secretion of dengue viruses. *J Biol Chem* 2012;287(1):767-777.
- McMinn PC. The molecular basis of virulence of the encephalogenic flaviviruses. *J Gen Virol* 1997;78(Pt 11): 2711-2722.
- Rey FA, Heinz FX, Mandl C, Kunz C, Harrison SC. The envelope glycoprotein from tick-borne encephalitis virus at 2 Å resolution. *Nature* 1995;375(6529):291-298.
- Modis Y, Ogata S, Clements D, Harrison SC. A ligand binding pocket in the dengue virus envelope glycoprotein. *Proc Natl Acad Sci USA* 2003;100(12):6986-6991.
- Zhang Y, Zhang W, Ogata S, Clements D, Strauss JH, Baker TS, et al. Conformational changes of the flavivirus E glycoprotein. *Structure* 2004;12(9):1607-1618.
- Lee E, Lobigs M. Substitutions at the putative receptor-binding site of an encephalitic flavivirus alter virulence and host cell tropism and reveal a role for glycosaminoglycans in entry. *J Virol* 2000;74(19):8867-8875.
- Seligman SJ, Bucher DJ. The importance of being outer: consequences of the distinction between the outer and inner surfaces of flavivirus glycoprotein. *Trends Microbiol* 2003;11(3):108-110.
- Modis Y, Ogata S, Clements D, Harrison SC. Variable surface epitopes in the crystal structure of dengue virus type 3 envelope glycoprotein. *J Virol* 2005;79(2):1223-1231.
- Modis Y, Ogata S, Clements D, Harrison SC. Structure of the dengue virus envelope protein after membrane fusion. *Nature* 2004;427(6972):313-319.
- Bressanelli S, Stiasny K, Allison SL, Stura EA, Duquerois S, Lescar J, et al. Structure of a flavivirus envelope glycoprotein in its low-pH induced membrane fusion conformation. *EMBO J* 2004;23(4):728-738.
- Sirohi D, Chen Z, Sun L, Klose T, Pierson TC, Rossmann MG, et al. The 3.8 Å resolution cryo-EM structure of Zika virus. *Science* 2016;352(6284):467-470.
- Stiasny K, Allison SL, Marchler-Bauer A, Kunz C, Heinz FX. Structural requirements for low-pH-induced rearrangements in the envelope glycoprotein of tick-borne encephalitis virus. *J Virol* 1996;70(11):8142-8147.

33. Allison SL, Stiasny K, Stadler K, Mandl CW, Heinz FX. Mapping of functional elements in the stem-anchor region of tick-borne encephalitis virus envelope protein E. *J Virol* 1999;73(7):5605-5612.
34. Orlinger KK, Hoenninger VM, Kofler RM, Mandl CW. Constructon and mutagenesis of an artificial bicistronic tick-borne encephalitis virus genome reveals an essential function of the second transmembrane region of protein E in flavivirus assembly. *J Virol* 2006;80(24):12197-12208.
35. Boutet E, Lieberherr D, Tognolli M, Schneider M, Bansal P, Bridge AJ, et al. UniProtKB/Swiss-Prot, the manually annotated section of the UniProt Knowledge Base: how to use the entry view. *Methods Mol Biol* 2016; 1374:23-54.
36. Gasteiger E, Hoogland C, Gattiker A, Hoogland C, Gattiker A, Duvaud S, et al. Protein identification and analysis tools on the ExPASy server. In: Walker JM, editor. *The proteomics protocols handbook*. UK: Humana Press; 2005. p. 571-607.
37. Argos P, Rossman MG, Grau UM, Zuber H, Frank G, Tratschin JD. Thermal stability and protein structure. *Biochemistry* 1979;18(25):5698-5703.
38. Ikai A. Thermostability and aliphatic index of globular proteins. *J Biochem* 1980;88(6):1895-1898.
39. Kyte J, Doolittle RF. A simple method for displaying the hydropathic character of a protein. *J Mol Biol* 1982;157(1):105-132.
40. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol* 2011;7:539.
41. Dai L, Song J, Lu X, Deng YQ, Musyoki AM, Cheng H, et al. Structures of the Zika virus envelope protein and its complex with a Flavivirus broadly protective antibody. *Cell Host Microbe* 2016;19(5):696-704.
42. Zhao H, Fernandez E, Dowd KA, Speer SD, Platt DJ, Gorman MJ, et al. Structural basis of Zika virus-specific antibody protection. *Cell* 2016;166(4):1016-1027.
43. Robbiani DF, Bozzacco L, Keffe JR, Khouri R, Olsen PC, Gazumyan A, et al. Recurrent potent human neutralizing antibodies to Zika virus in Brazil and Mexico. *Cell* 2017;169(4):597-609.e11.
44. Wang Q, Yang H, Liu X, Dai L, Ma T, Qi J, et al. Molecular determinants of human neutralizing antibodies isolated from a patient infected with Zika virus. *Sci Transl Med* 2016;8(369):369ra179.
45. Guex N, Peitsch MC, Schwede T. Automated comparative protein structure modeling with SWISS-MODEL and Swiss-PdbViewer: a historical perspective. *Electrophoresis* 2009;30 Suppl 1:S162-173.
46. Lovell SC, Davis IW, Arendall WB 3rd, de Bakker PI, Word JM, Prisant MG, et al. Structure validation by C α geometry: phi,psi and C β deviation. *Proteins* 2003;50(3):437-450.
47. Wiederstein M, Sippl MJ. ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. *Nucleic Acids Res* 2007;35(web server issue):W407-410.
48. Maybridge Chemical Company Ltd. Available online: <http://www.maybridge.com> (accessed on 16 February 2011).
49. Kampmann T, Yennamalli R, Campbell P, Stoermer MJ, Fairlie DP, Kobe B, et al. In silico screening of small molecule libraries using the dengue virus envelope E protein has identified compounds with antiviral activity against multiple flaviviruses. *Antiviral Res* 2009;84(3): 234-241.
50. Poh MK, Yip A, Zhang S, Smit JM, Wilschut J, Priestle JP, et al. A small molecule fusion inhibitor of dengue virus. *Antiviral Res* 2009;84(3):260-266.
51. Wang QY, Patel SJ, Vangrevelinghe E, Xu HY, Rao R, Jaber D, et al. A small-molecule dengue virus entry inhibitor. *Antimicrob Agents Chemother* 2009;53(5): 1823-1831.
52. Zhou Z, Khaliq M, Suk JE, Patkar C, Li L, Kuhn RJ, et al. Antiviral compounds discovered by virtual screening of small-molecule libraries against dengue virus E protein. *ACS Chem. Biol* 2008;3(12):765-775.
53. Yang JM, Chen YF, Tu YY, Yen KR, Yang YL. Combinatorial computational approaches to identify tetracycline derivatives as flavivirus inhibitors. *PLoS One* 2007;2(5): e428.
54. Li Z, Khaliq M, Zhou Z, Post CB, Kuhn RJ, Cushman M. Design, synthesis, and biological 284 evaluation of antiviral agents targeting flavivirus envelope proteins. *J Med Chem* 2008;51(15):4660-4671.
55. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, et al. AutoDock4 and AutoDock Tools4: automated docking with selective receptor flexibility. *J Comput Chem* 2009;30(16):2785-2891.