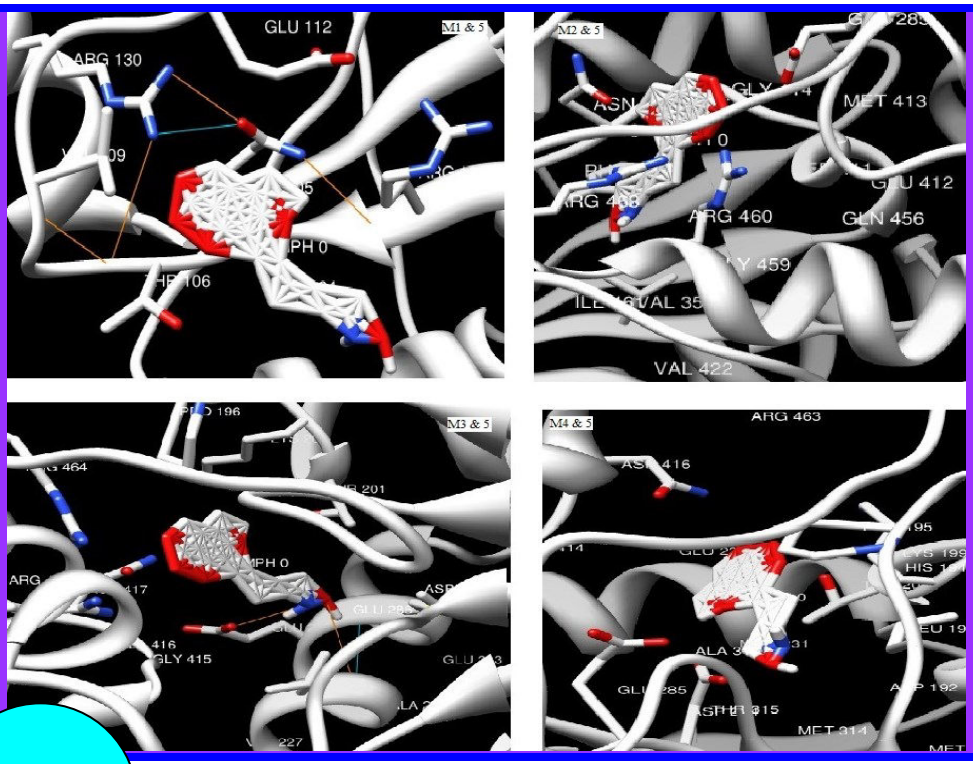

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In silico antigenic site evaluation and antiviral therapy against dengue serotypes

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Abstract

Nonstructural protein 3 (NS3) constitute protease, helicase and polymerase that are essential for dengue virus replication. The aim of the present study is to block the replication of the virus by targeting the NS3 Protein. The retrieved sequences of NS3 protein from National Centre for Biotechnology information shows that the antigenic sites of the protein are highly variable in all the four serotypes of dengue virus (DENV) i.e. DENV I, DENV II, DENV III and DENV IV. DENV III found to be most distantly related serotype among all the serotypes studied using UPGMA method. The 3D structure of NS3 protein was modeled using homology modeling by MODELLER 9v8. Evaluation of the constructed NS3 protein models were done by PROCHECK, WhatIf using Exome Horizon. The derived compounds of mycophenolic acid and ribavirin were docked as ligands to the constructed models of NS3 protein using AutoDock 4.2 for Protein-ligand interaction study.

Introduction

Dengue disease, caused by dengue virus infection which is found to be an endemic in over 100 countries (Brinkworth et al., 1999). It was found that 100 million cases of dengue fever occur annually. Of which, 500,000 cases require hospitalization, and 25,000 are fatal (Gubler et al., 1998; Ligon et al., 2005; Gratz et al., 1999; Halstead, 2007), due to limited healthcare facilities in developing and underdeveloped countries. DENV (Dengue virus) is an arthropod-borne flavivirus that comprises four distinct serotypes (DENV I, DENV II, DENV III and DENV IV) that constitute an antigenic complex of the genus flavivirus, family *Flaviviridae* (Mason et al., 1990; Henchal et al., 1982; Gentry et al., 1982; Monath et al., 1986; Russell et al., 1967). Every step in the life cycle of the dengue virus is a potential target for inhibiting viral replication (Qi et al., 2008). NS3 protein constitutes protease, helicase and polymerase that are essential for dengue virus replication (Bera et al., 2008). NS3 is responsible for proteolysis of den [gue viral RNA polyprotein as well as carrying out various enzymatic reactions that are mandatory for replication of dengue virus (Luo et al., 2008). Currently there is no antiviral therapy available for Dengue (Muhamad et al., 2010). We assessed the ability of myco -phenolic acid (MPA) and ribavirin (RBV), drug currently used as an immunosuppressive agent, to inhibit dengue virus antigen expression, RNA replication, and virus production (Diamond et al., 2012; Allison et al., 1993; Koff et al., 1983; Conner et al., 1984). The aim of this study was to examine the mutation in antigenic site of dengue virus and the antiviral action of mycophenolic acid (MPA) and ribavirin (RBV) on NS3 proteins DENV I-IV determining the best drug that can be most active against the virus from the binding energy and the pocket which fits the drug.

Materials and Methods

Sequence retrieval

The nonstructural protein 3 sequence of DENV I, DENV II, DENV III and DENV IV were obtained from the

National Centre for Studies in Biotechnology (http://www.ncbi.nlm.nih.gov). The numbers of sequences were found to be 25, 17, 103 and 25 for DENV I, DENV II, DENV III and DENV IV respectively.

Antigenic site finding

The Exome Horizon antigenic site finder tool was used to find the antigenic sites of protein. The accession No., sequence length, hits, positions, antigenic sites, antigenic site length and the highest score were given in the Table I-IV.

Phylogenetics analysis

The phylogenetic trees were also built for analyzing the highly variable sequences and to find the conserved domains of the non structural protein sequences by using UPGMA method using Exome Horizon.

Sequence alignment and homology modeling

The amino acid sequences of NS3 of DENV were taken from the National Center for Biotechnology Information (NCBI) Database (Wheeler et al., 2008). A modified Needleman and Wunsch (1970) method implemented in Exome Horizon and the BLOSUM62 substitution matrix (Henikoff et al., 1992) was applied for aligning the sequences.

The homology models of DENV NS3 were constructed applying the MODELER 9v8 program using same template structures for each domain. All the models (M1, M2, M3, and M4 for DENV I, DENV II, DENV III and DENV IV respectively) were generated using the Crystal Structure of the NS3 Protease-Helicase from Dengue virus as template structures (2VBC) (Bhattacharya et al., 2008).

Ligand preparation

The Molecules (In this study referred as ligands) were prepared using moldraw tool of Exome Horizon. The different parameters of the ligands were studied given in the Table V and VI.

Molecular docking study

The docking of ligands to the catalytic triad of NS3-NS2B protease was performed using AutoDock 4.0 software (http://autodock. scripps.edu/). AutoDock is reported to be a very common docking program (Sousa et al., 2006) and is reliable (Hetényi et al., 2002). Using the software, polar hydrogen atoms were added to the enzyme and its nonpolar hydrogen atoms were merged, whereas for the ligand, nonpolar hydrogen atoms were merged and Gasteiger charges were added. All rotatable bonds of ligands were set to be rotatable. All calculation for protein-fixed ligand-flexible docking was done using the Lamarckian Genetic Algorithm (LGA) method. A population size of 150 and 10 millions energy evaluations were used for 100 search runs. The grid box with a dimension of 60 × 60 × 60 points and 0.375 Å grid spacing was used around the catalytic triad to cover the entire enzyme binding site and allow accommodated ligands to move freely. After the docking searches were completed, clustering histogram analysis was performed based on an RMSD (root mean square deviation) of not more than 1.5 Å. The best conformation was chosen from the most populated cluster with the lowest docked energy. The interactions of complex enzyme-ligand conformations, including hydrogen bond and other interactions, were analyzed using Viewerlite (www.accelrys.com) and UCSF Chimera software (www.cgl.ucsf.edu/chimera/) (Figure 1-4).

Results

The antigenic sites analyzed from all the serotypes were found to be highly variable. In DENV-I the sites were found to be IVGLYGNGVVTTSGTYVSPIAQAK, MRLLSPVRVP, EVQVIAVE, MAVGIVSILLSSLLKND VPLAGPLIAGGMLIACYVISG, FTVVVGDVVGILAQ and HGTVLVQVKY. It is observed that the amino acids present at the site producing highest scores were found to be A, P, V, C, G and Q for DENV I. For DENV-II, the antigenic sites were found to be EVQVLALE, MRLLSPVRVPNYNLI, QLGQVMLLVLCVTQVLM, RYLPAIVREA, TSLSVSLVLVGIVTLYLGVMVQAD, and QLGQVMLLVLCVTQVLM. The amino acids producing highest scores for DENV-II were found to be V, P and L. However DENV-III showed much conserved antigenic sequences and was found to be KYLPAIVREA, DRVIDPRRCLKPVILT, and ADRVI DPRRCLKPVILTD. Proline was found to be the most conserved antigenic site for DENV-III. The DENV-IV found to be having the most similar antigenic sites as of DENV-III. The sites were found to be IVDLMCHAT, GRVIDPRRCLKPVILT, MADLSLEKAANVQ, QLGQVMLLVLCAGQLLLM, KHMILVVVITLCAIIL GG, and GRVIDPRRCLKPVILT. The high scoring regions were found to be L, P, E and I. Hence, the antigenic site P is found to be the most conserved region among all the four serotypes, suggesting as the potent inhibition point of the ligands. The geometry and stereochemistry of the models were evaluated by using the program PROCHECK (Sousa et al., 2006). The stereochemical quality of the generated homology models as well as the crystal structure was evaluated using Ramachandran plots. Results revealed that 79.7 and 2.4% of the residues of the models are located in the most favored regions and the additional allowed regions for Model1. Similarly the residues present in favored region and disallowed regions were found to be 88.6 and 0.2, 87.1 and 11.2, 89.3 and 0.4% for Model2, Model3 and Model4 respectively. The obtained results indicate that all models possess sufficient stereo-

Table I

Antigenic site of nonstructural proteins (DENV I)

Accession No.	Seqeunce length	Hits	Positions	Antigenic sites	Antigenic site length	Maximum score pose at	Score
ACJ05959.1	58	1	14->37	IVGLYGNGVVTTSGTYVSPI-AQAK	24	A	1.122
ACJ05958.1	55	1	14->37	IVGLYGNGVVTTSGTYVSAI-AQAK	24	A	1.122
ACJ05957.1	55	1	14->37	IVGLYGNGVVTTSGTYVSAI-AQAK	24	A	1.122
ACJ05956.1	56	1	14->37	IVGLYGNGVVTTSGTYVSAI-AQAK	24	A	1.122
ACJ05955.1	55	1	14->37	IVGLYGNGVVTTSGTYVSAI-AQAK	24	A	1.122
ACJ05954.1	56	1	14->37	IVGLYGNGVVTTSGTYVSAI-AQAK	24	A	1.122
ACJ05953.1	57	1	14->37	IVGLYGNGVVTTSGTYVSPI-AQAK	24	P	1.122
ACJ05952.1	56	1	14->37	IVGLYGKGVVTTSGTYVSAI-AQAK	24	K	1.122
ACJ05951.1	57	1	14->37	IVGLYGNGVVTTSGTYVSPI-AQAK	24	P	1.122
ACJ05950.1	57	1	14->37	IVGLYGNGVVTTSGTYVSPI-AQAK	24	P	1.122
ACJ05949.1	56	1	14->37	IVGLYGKGVVTTSGTYVSAI-AQAK	24	K	1.122
ACJ05948.1	56	1	14->37	IVGLYGNGVVTTSGTYVSAI-AQAK	24	A	1.122
ACJ05947.1	55	1	14->37	IVGLYGNGVVTTSGTYVSAI-AQAK	24	A	1.122
ACJ05946.1	56	1	14->37	IVGLYGKGVVTTSGTYVSAI-AQAK	24	K	1.122
AAA18245.1	142	6	129->138	MRLLSPVRVP	10	P	1.174
AAB03618.1	143	6	129->138	MRLLSPVRVP	10	P	1.174
AAB03617.1	143	6	129->138	MRLLSPVRVP	10	P	1.174
AAB03616.1	143	6	129->138	MRLLSPVRVP	10	P	1.174
3LKWA	236	9	144->151	EVQVIAVE	8	V	1.176
3L6PA	236	9	144->151	EVQVIAVE	8	V	1.176
POLG_DENV IW	3392	134	1354->1391	MAVGIVSILLSSLLK-NDVPLAGPLIAGGMLIACYVISG	38	C	1.225
POLG_DENV IS	3396	137	860->873	FTVVVGDVVGILAQ	14	G	1.225
POLG_DENV IC	791	39	597->606	HGTVLVQVKY	10	Q	1.236
POLG_DENV IA	792	38	597->606	HGTVLVQVKY	10	Q	1.215

Table II

Antigenic site of nonstructural proteins (DENV II)

Accession No.	Seqeunce length	Hit	Position	Antigenic sites	Antigenic site length	Score
CAA40704.1	618	26	94->101	EVQVLALE	8	1.171
NP_739587.2	618	25	267->281	MRLLSPVRVPNYNLI	15	1.174
AAA73185.1	3391	135	2410->2426	QLGQVMLLVLCVTQVLM	17	1.262
AAA73186.1	3391	134	2410->2426	QLGQVMLLVLCVTQVLM	17	1.262
AAB03619.1	108	5	64->73	RYLPAIVREA	10	1.135
AAA66406.1	886	38	331->348	TGPLVAGGLLTVCYVLTG	18	1.25
POLG_DENV IIT	1683	69	473->503	TSLSVSLVLVGIVTLYL-GVMVQADSGCVVSW	31	1.239
POLG_DENV IIJ	3391	135	753->783	TSLSVSLVLVGVVTLYL-GAMVQADSGCVVSW	31	1.272
POLG_DENV IID	1127	49	753->783	TSLSVSLVLVGVITLYL-GAMVQADSGCVVSW	31	1.239
POLG_DENV IIU	679	34	653->676	TSLSVSLVLVGIVTLYLGVMVQAD	24	1.239
POLG_DENV II6	3391	135	2410->2426	QLGQVMLLVLCVTQVLM	17	1.262
POLG_DENV IIN	3391	131	753->783	TSLSVSLVLVGVVTLYL-GVMVQADSGCVVSW	31	1.272
POLG_DENV II8	3391	133	2410->2426	QLGQVMLLVLCVTQVLM	17	1.262
POLG_DENV IIQ	3391	134	2410->2426	QLGQVMLLVLCVTQVLM	17	1.262
POLG_DENV II7	3391	134	2410->2426	QLGQVMLLVLCVTQVLM	17	1.262
POLG_DENV IIP	3388	132	2407->2423	QLGQVMLLVLCVTQVLM	17	1.262

Table III

Antigenic site of nonstructural proteins (DENV III)

Accession No.	Sequence length	Hits	Positions	Antigenic sites	Antigenic site length	Score
ABU88348.1	102	3	P	KYLPAIVREA	10	1.143
ABU88347.1	102	3	P	KYLPAIVREA	10	1.143
YP_001531172.2	619	24	P	DRVIDPRRCLKPVILT	16	1.206
ACJ06087.1	86	3	P	KYLPAIVREA	10	1.143
ACJ06086.1	88	3	P	KYLPAIVREA	10	1.143
ACJ06085.1	18	1	P	PAIVREA	7	1.143
ACJ06081.1	87	2	P	KYLPAIVREA	10	1.143
ACJ06082.1	88	2	P	KYLPAIVREA	10	1.143
ACJ06083.1	88	2	P	KYLPAIVREA	10	1.143
ACJ06080.1	87	2	P	KYLPAIVREA	10	1.143
ACJ06079.1	89	4	P	KYLPAIVREA	10	1.143
ACJ06078.1	89	2	P	KYLPAIVREA	10	1.143
ACJ06077.1	88	2	P	KYLPAIVREA	10	1.143
ACJ06076.1	88	2	P	KYLPAIVREA	10	1.143
ACJ06074.1	88	2	P	KYLPAIVREA	10	1.143
ACJ06075.1	88	2	P	KYLPAIVREA	10	1.143

Table IV

Antigenic site of nonstructural proteins (DENV IV)

Accession No.	Sequence length	Hits	Positions	Antigenic sites	Antigenic site length	Score
AAA18247.1	140	7	115->123	IVDLMCHAT	9	1.142
2VBCA	618	28	420->435	GRVIDPRRCLKPVILT	16	1.206
2VBCB	31	1	4->16	MADLSLEKAANVQ	13	1.06
2WHXA	618	28	420->435	GRVIDPRRCLKPVILT	16	1.206
POLG_DENV IVT	3387	150	2406->2423	QLGQVMLLVLCAGQLLLM	18	1.251
POLG_DENV IVH	3387	151	2406->2423	QLGQVMLLVLCAGQLLLM	18	1.251
POLG_DENV IVS	3387	147	1156->1173	KHMILVVVITLCAIILGG	18	1.267
POLG_DENV IVP	3387	151	2406->2423	QLGQVMLLVLCAGQLLLM	18	1.251
POLG_DENV IVD	3387	148	1156->1173	KHMILVVVITLCAIILGG	18	1.267
2JLXB	451	20	253->268	GRVIDPRRCLKPVILT	16	1.206
2JLXA	451	20	253->268	GRVIDPRRCLKPVILT	16	1.206
2JLZB	451	20	253->268	GRVIDPRRCLKPVILT	16	1.206
2JLZA	451	20	253->268	GRVIDPRRCLKPVILT	16	1.206
2JLYB	451	19	253->268	GRVIDPRRCLKPVILT	16	1.206
2LYBA	451	19	253->268	GRVIDPRRCLKPVILT	16	1.206
2JLWB	451	20	253->268	GRVIDPRRCLKPVILT	16	1.206
2JLWA	451	20	253->268	GRVIDPRRCLKPVILT	16	1.206
2JLVB	451	20	253->268	GRVIDPRRCLKPVILT	16	1.206
2JLVA	451	20	253->268	GRVIDPRRCLKPVILT	16	1.206
2JLUB	451	20	253->268	GRVIDPRRCLKPVILT	16	1.206
2JLUA	451	20	253->268	GRVIDPRRCLKPVILT	16	1.206
2JLSA	451	20	253->268	GRVIDPRRCLKPVILT	16	1.206
2JLRA	451	20	253->268	GRVIDPRRCLKPVILT	16	1.206
2JLQA	451	19	253->268	GRVIDPRRCLKPVILT	16	1.206
NP_740321.1	618	28	420->436	GRVIDPRRCLKPVILPD	17	1.206

Table V

Molecular properties of mycophenolic acid derivatives

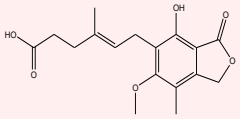
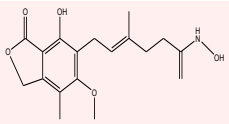
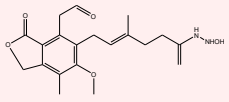
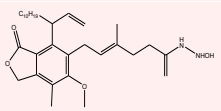
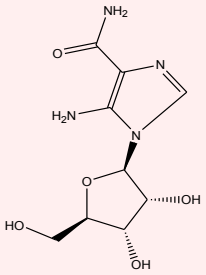
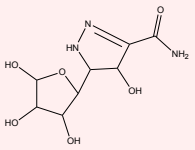
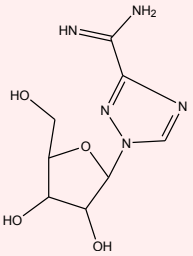
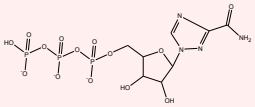
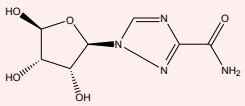
Sl. No	IUPAC Name	Chemical formula	Molecular weight	Log P	Structure
1	6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydro-isobenzofuran-5-yl)-4-methyl-hex-4-enoic acicd	$C_{17}H_{20}O_6$	320.34	2.55	
2	7-hydroxy-6(6-hydroxyamino-3-methyl-hepta-2,6-dienyl)-5-methoxy-4-methyl-3H-isobenzofuran-1one	$C_{18}H_{23}NO_5$	333.38	2.63	
3	ACMPHA	$C_{20}H_{26}N_2O_5$	374.43	1.91	
4	LMPHA	$C_{31}H_{46}N_2O_4$	510.71		

Table VI

Molecular properties of ribavirin derivatives

Sl. No.	IUPAC	Chemical formula	Mol. Wt.	Log P	Structure
5	5-amino-1-(3,4-dihydroxy-5-hydroxymethyl-tetrahydro-furan-2-yl)-1H-imidazole-4-carboxylic acid amide	$C_9H_{14}N_4O_5$	258.23	-3.28	
6	4-hydroxy-5-(3,4,5-trihydroxy -tetrahydro-furan-2-yl)-4,5-dihydro-1H-pyrazole-3-carboxylic acid amide	$C_8H_{13}N_3O^6$	247.21	-3.73	
7	1-(3,4-dihyroxy-5-hydroxymethyl-tetrahydro-furan-2-yl)-1H-[1,2,4]triazole-3-carbozamidine	$C_8H_{13}N_5O_4$	243.22	-1.61	
8	Ribavirin 5 triphosphate	$C_8H_{12}N_4O_{14}P_3^{3-}$	481.12		
9	1-(3,,4,5-trihydroxy-tetrahydro-furan-2-yl)-1H-[1,2,4]triazole-3carboxylic acid amide	$C_7H_{10}N_4O_5$	230.18	-1.38	

chemical quality. The Phylogenetic analysis showed the high intraspecies variation in NS3 protein of all the serotypes (Figure 5). The distances within group was found to be 0.05, 1.27, 0.70 and 0.20 for DENV-I, DENV-II, DENV-III and DENV-IV respec-tively. The mean distances within groups showed that DENV-I has high distance related from DENV-II, DENV-III and DENV-IV. The homology models of the NS3 protein of four serotypes also showed very good variation in Rama-chandran plot. The docking study of the derived compound of mycophenolic acid (4 nos.) and ribavirin (5 nos.) was done with AutoDock4.2. The binding energy obtained in the range of -0.97 to +190.03. The ligand 3 of mycophenolic acid was found to be the best drug in Model1 having the minimum binding energy -9.2 kcal/mol. The ligand 4 of the ribavirin revealed as the best inhibitor for model2 and showed the minimum binding energy of -16.5 kcal/mol and was found to be the best ligand among all the ligands studied. The number 4 ligand of the mycophenolic acid found to be second best inhibitor for model3 among all the selected ligands after the ligand4 of mycophenolic acid with binding energy -15.9 kcal/mol. The ligand1 of ribavirin was found to be the best inhibitor for model4 having minimum binding energy -10.7 kcal/mol (Figure 6).

Discussion

Dengue fever epidemics has increased numerously over the last few decades (Ligon et al., 2005) therefore Developing antiviral drug and vaccine is becoming very important due to the global threat of viral disease pandemics (Noble et al., 2010; Wang et al., 2009). The functional similarity between the NS2B/NS3 proteases from the four genetically and antigenically distinct serotypes was identified by the differences in their substrate specificity using tetrapeptide and oc-

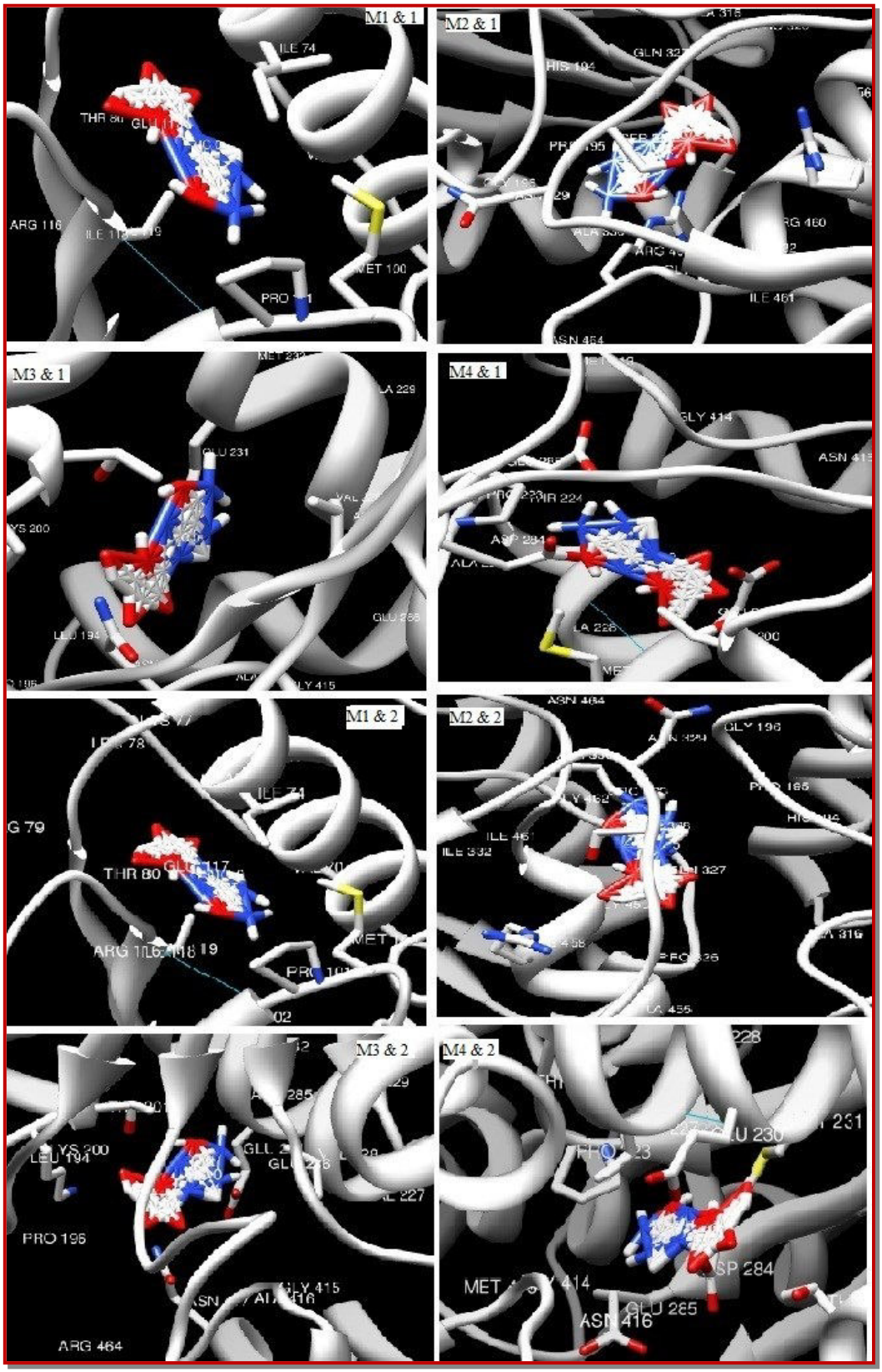


Figure 1: Docking Interaction of Models with ligand1 and 2. The interaction energy was calculated and the hydrogen bonds were observed

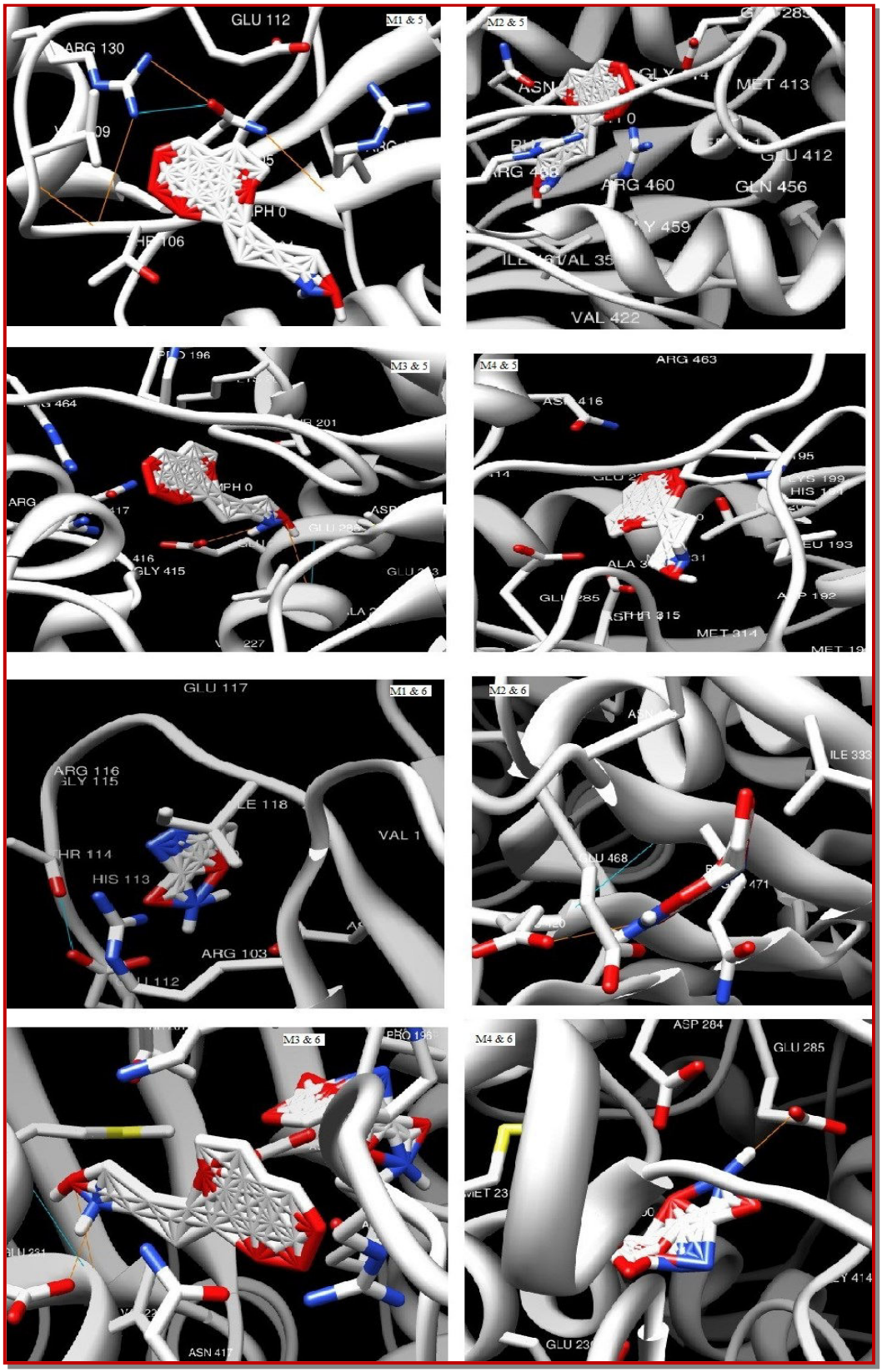


Figure 2: Docking interaction of Models with ligand 5 and 6. The interaction energy was calculated and the hydrogen bonds were observed

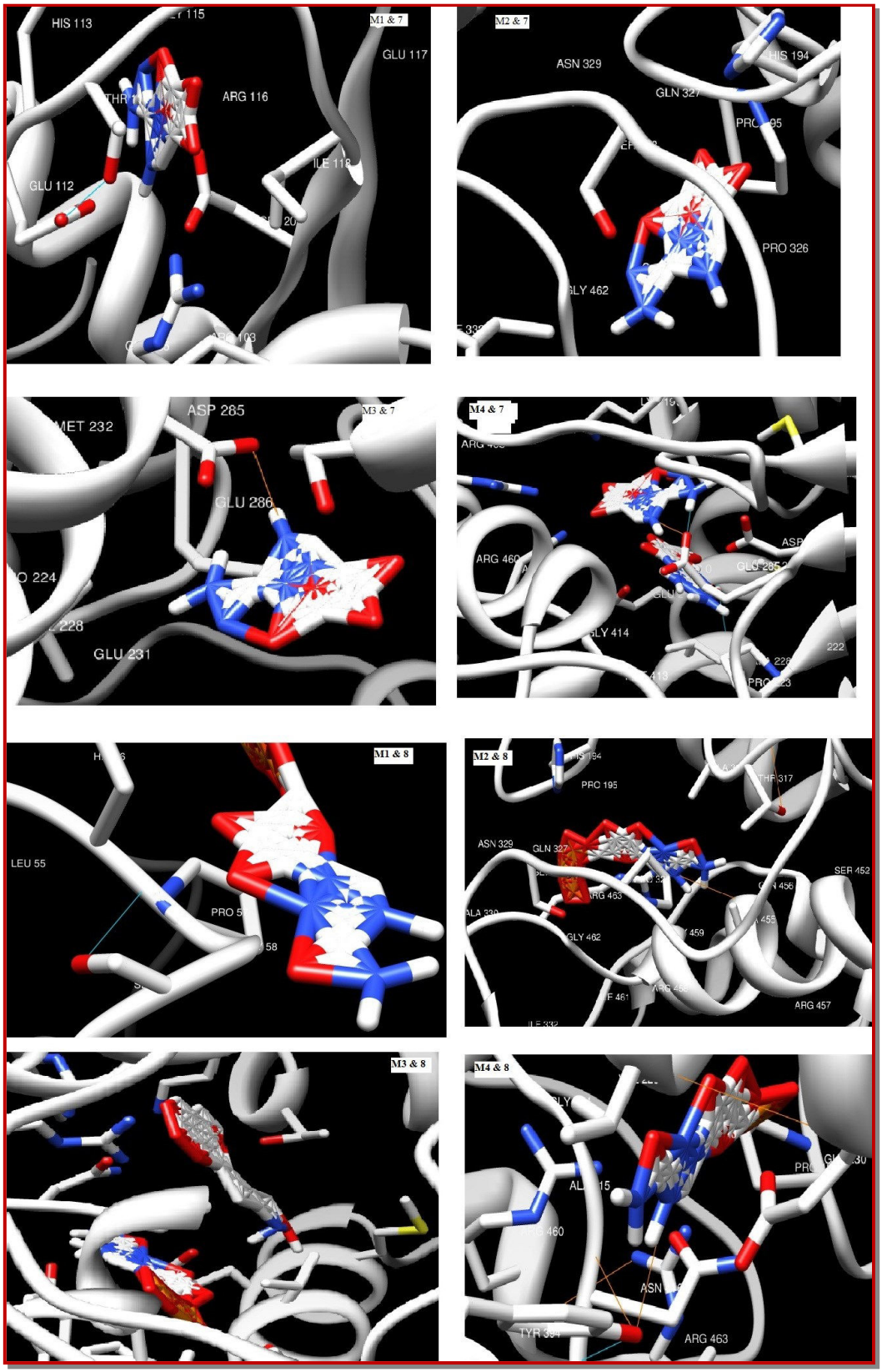


Figure 3: Docking interaction of Models with ligand 7 and 8. The interaction energy was calculated and the hydrogen bonds were observed

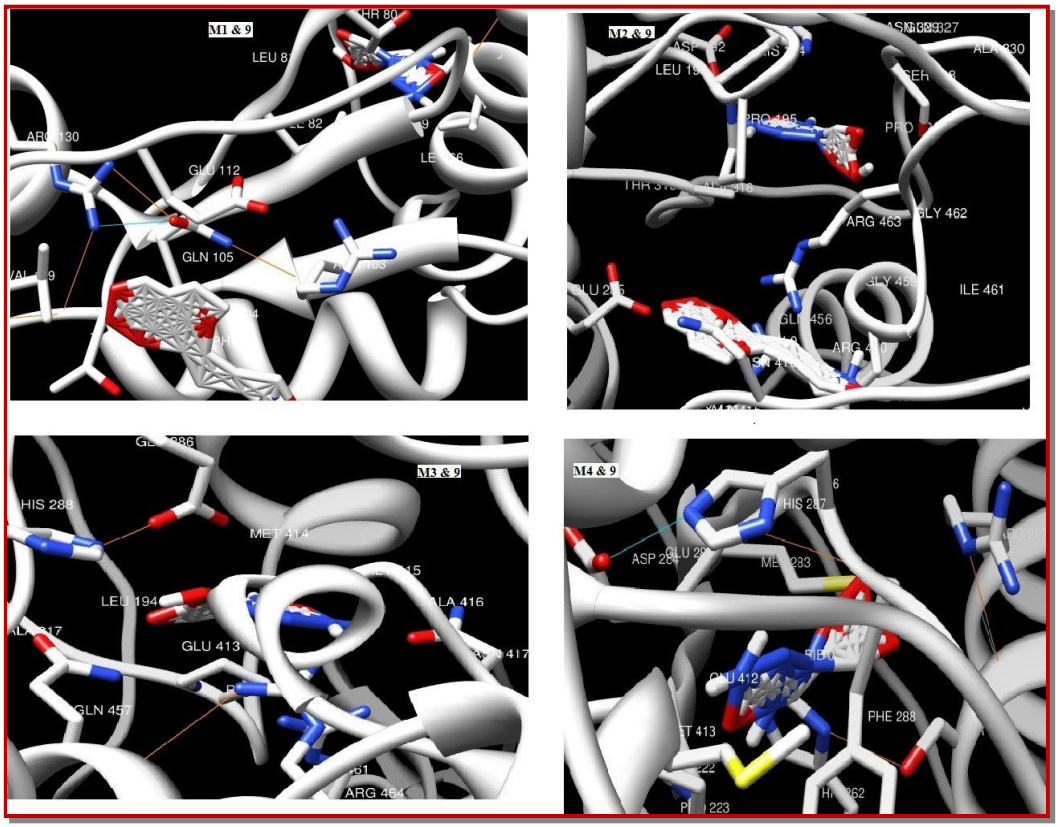


Figure 4: Docking interaction of Models with ligand 9. The interaction energy was calculated and the hydrogen bonds were observed

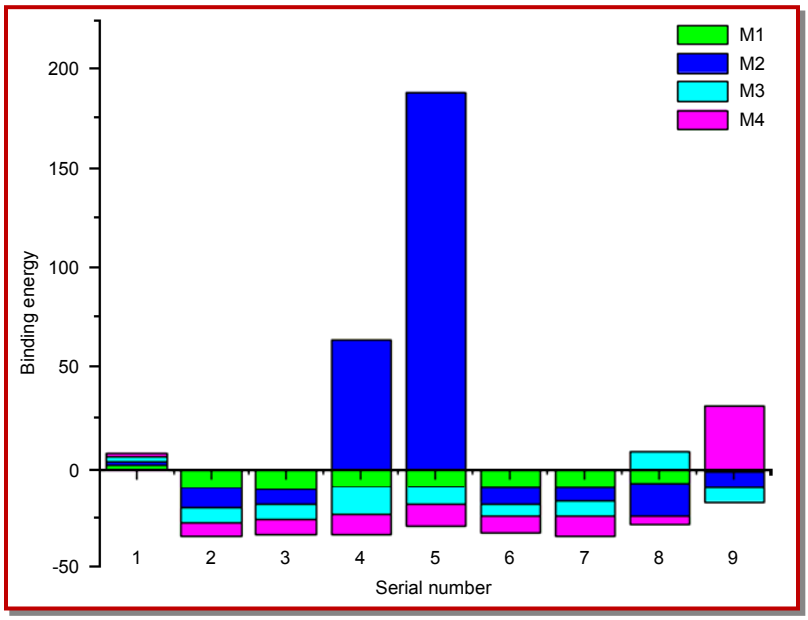


Figure 5: Binding enegry analysis from the interaction study of the ligands it was observed that the ligands 3, 8, 4 and 5 are the best inhibitors for DEN-I, DEN-II, DEN-III and DEN-IV respectively. These ligands showed the binding energy of -9.18, -16.52, -15.87 and -10.66 Kcal/mol respectively for the corresponding serotypes

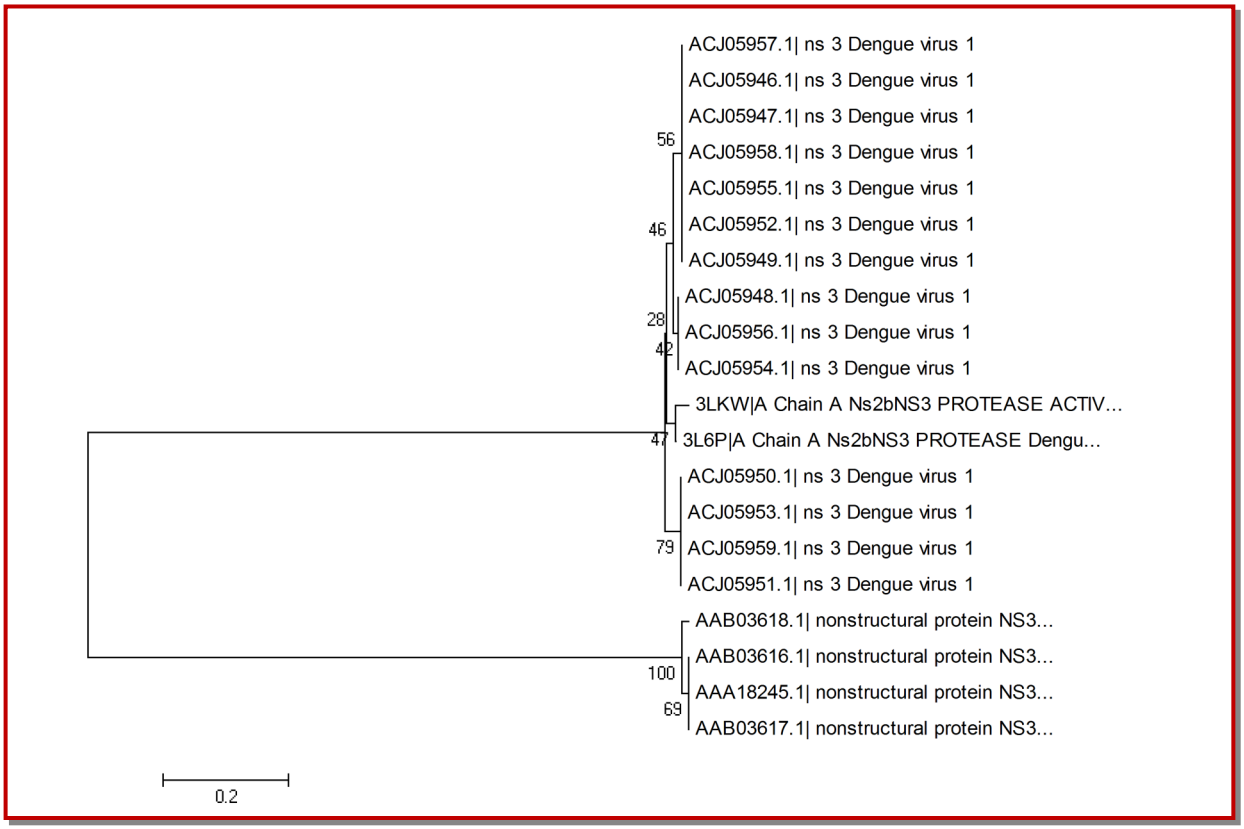


Figure 6: Phylogenetic analysis of DENV I

tapeptide libraries in a positional scanning format, each containing 130,321 substrates (Guzman et al., 2010). Development of new genomic and proteomic studies coupled with computational sciences could provide the discovery of various target proteins and potential inhibitor to be developed as drugs (Li et al., 2005; Tambunan et al., 2011). The NS3 enzyme of dengue is responsible for replication of the virus. The replication complex include the NS3 nucleotide the NS3 protease and with its NS2B cofactor, the NS3 nucleotide triphosphatase. This protein serves as the potential inhibitory targets for antiviral agents since they are required for virus replication. The multifunctional C-terminal domain of NS3 encodes NTPase, helicase and RTPase activities. NTP hydrolysis is thought to provide the chemical energy required for helicase activity. There is currently no antiviral therapy available against dengue virus. In this study we generated some antiviral ligands of mycophenolic acid and ribavirin. We predicted the binding activity of the ligands against NS3 protein of Dengue virus. From the interaction study of the ligands it was observed that the ligands 3, 8, 4 and 5 are the best inhibitors for DEN-1, DEN-2, DEN-3 and DEN-4 respectively. These ligands showed the binding energy of -9.2, -16.5, -15.9 and -10.7 Kcal/mol respectively for the corresponding serotypes. To study the interaction of ligands at the inhibition point, it is necessary to know the antigenic sites of the disease causing protein. It is observed that, there is great variation in the antigenic site of the proteins. However the most conserved antigenic site found to be the aminoacid, Proline almost in every serotype. The ligands were docked into each antigenic site of the protein and the binding energy was reported. Phylogenetic analysis also suggested the typical variations in all the NS3 sequences of NCBI. Finding antigenic site of a mutated protein is the primary aim of any computer aided drug design. The current research discovered several variations in the original sequences as well as antigenic site in the NS3 protein of the 4 serotypes. The antigenic sites were targeted to block the replication activity of the virus. The antigenic sites were targeted by the derivative compounds of mycophenolic acid and ribavirin to block the replication process of the virus. Hence the current study may be useful in designing the drugs that may be synthesized in wet lab and can be used as antiviral drugs against dengue serotypes. The docking of two groups of inhibitors from mycophenolic acid and ribavirin again NS3 were carried out. In this work, the complexation energy of the docking was used as the descriptors for selecting new candidates for competitive dengue inhibitors. The antigenic sites were highly variable. The phylogenetics studies were also evaluated for finding the interspecies variation in NS3. Homologies of protein were constructed for all the serotypes. AutoDock4.2 helped in carrying out the

interaction of the drugs with the protein models. Complexation energies for all the new ligand-enzyme complexes were evaluated. Detailed structural information is becoming increasingly available for the dengue NS3 proteins. Since these proteins are requested for virus infectivity and replication. Structure based computational approaches offer an attractive strategy for the discovery and optimization of dengue antiviral drugs. Moreover, these computational approaches promise and improve the effectiveness of current structure based calculations.

Conclusion

Based on the complexation energies calculated, the 3rd compound of the mycophenolic was found to be the best inhibitor for DENV I. Similarly drug4 of ribavirin, drug4 of mycophenolic acid and drug1 of ribavirin were found to be best drugs against DENV II, DENV III and DENV IV respectively the lowest and closest energies to the reference compounds.

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References

Allison AC, Eugui EM. Immunosuppressive and other effects of mycophenolic acid and an ester prodrug, mycophenolate mofetil. Immunol Rev. 1993; 136: 5-28.

Bera AK, Kuhn RJ, Smith JL. Functional characterization of cis and trans activity of the flavivirus NS2B-NS3 protease. J Biol Chem. 2007; 282: 12883-92.

Bhattacharya A, Wunderlich Z, Monleon D, Tejero R, Montelione GT. Assessing model accuracy using the homology modeling automatically software. Proteins 2008; 70: 105-18.

Brinkworth RI, Fairlie DP, Leung D, Young PR. Homology model of the dengue 2 virus NS3 protease: Putative interactions with both substrate and NS2B cofactor. J Gen Vir. 1999; 80: 1167-77.

Conner CS. Ribavirin. Drug Intell Clin Pharm. 1984; 18: 137-38.

Diamond MS, Zachariah M, Harris E. Mycophenolic acid inhibits dengue virus infection by preventing replication of viral RNA. Virology 2012; 304: 211-21.

Gentry MK, Henchal EA, McCOWN LM, Brandt WE, Dalrymple JM. Identification of distinct antigenic determinants on dengue-2 virus by using monoclonal antibodies. Am J Trop Med Hyg. 1982; 31: 548-55.

Gratz NG. Emerging and resurging vector-borne diseases. Annu Rev Entomol. 1999; 44: 51-75.

Gubler DJ. Dengue and dengue hemorrhagic fever. Clin Microbiol Rev. 1998; 11: 480-96.

Guzman MG, Halstead sb, Artsob H, Buchy P, Farrar J, Gubler DJ, Hunsperger E, Kroeger A, Margolis HS, Martínez E, Nathan MB, Pelegrino JS, Simmons C, Yoksan S, Peeling RW. Dengue: A continuing global threat. Nature Rev Micro. 2010; S7-S16.

Halstead SB. Dengue. Lancet 2007; 370: 1644-52.

Henchal EA, Gentry MK, McCowN JM, Brandt WE. Dengue virus-specific and flavivirus group determinants identified with monoclonal antibodies by indirect immunofluorescence. Am J Trop Med Hyg. 1982; 31: 830-36.

Henikoff S, Henikoff JG. Amino acid substitution matrices from protein blocks. Proc Natl Acad Sci. 1992; 15: 10915-19.

Hetényi C, van der Spoel D. Efficient docking of peptides to proteins without prior knowledge of the binding site. Protein Sci. 2002; 11: 1729-37.

Koff WC, Elm JL Jr, Halstead SB. Antiviral effects of ribavirin and 6-mercapto-9-tetrahydro-2-furylpurine against dengue viruses *in vitro*. Antivir Res. 1982; 2: 69-79.

Li J, Lim SP, Beer D, Patel V, Wen D, Tumanut C, Tully DC, Williams JA, Jiricek J, Priestle JP, Harris JL, Vasudevan SG. Functional profiling of recombinant NS3 proteases from all four serotypes of dengue virus using tetrapeptide and octapeptide substrate libraries. J Bio Chem. 2005; 280: 28766-74.

Ligon BL. Dengue fever and dengue hemorrhagic fever: A review of the history, transmission, treatment, and prevention. Semin Pediatr Infect Dis. 2005; 16: 60-65.

Luo D, Xu T, Hunke C, Grüber G, Vasudevan SG, Lescar J. Crystal Structure of the NS3. J Virol. 2008; 82: 173-83.

Malachowska-Ugarte M, Cholewinski G, Dzierzbicka K, Trzonkowski P. Synthesis and biological activity of novel mycophenolic acid conjugates containing nitroacridine/ acridone derivatives. Euro J Med Chem. 2012; 54: 197-201.

Mason PW, Zfigel MU, Semproni AR, Fournier MJ, Mason L T. The antigenic structure of dengue type 1 virus envelope and NS1 proteins expressed in Escherichia coil. J Gen Vir. 1990; 71: 2107-14.

Monath TP. Pathobiology of the flaviviruses. In: The Togaviridae and Flaviviridae. Schlesinger S, Schlesinger MJ (eds). New York, Plenum Press, 1986, 275-440.

Muhamad M, Kee LY, Rahman NA, Yusof R. Antiviral actions of flavanoid-derived compounds on dengue virus type-2. Int J Biol Sci. 2010; 6: 294-302.

Noble CG, Chen YL, Dong H, Gu F, Lim SP, Schul W, Wang QY, Shi PY. Strategies for development of dengue virus inhibitors. Antiviral Res. 2010; 85: 450-62.

Qi RF, Zhang L, Chi CW. Biological characteristics of dengue virus and potential targets for drug design. Acta Biochim Biophys Sin. 2008; 40: 91-101.

Russell PK, Nisalak A. Dengue virus identification by the plaque reduction neutralization test. J Immun. 1967; 99: 291-96.

Sousa SF, Fernandes PA, Ramos MJ. Protein-ligand docking:

Current status and future challenges. Proteins 2006; 65: 15-26.

Tambunan USF, Apriyanti N, Parikesit AA, Chua W, Wuryani K. Computational design of disulfide cyclic peptide as potential inhibitor of complex NS2B-NS3 dengue virus protease. Afr J Biotechnol. 2011; 10: 12281-90.

Wang QY, Patel SJ, Vangrevelinghe E, Xu HY, Rao R, Jaber D, Schul W, Gu F, Heudi O, Ma NL, Poh MK, Phong WY, Keller TH, Jacoby E, Vasudevan SG. A small-molecule dengue virus entry inhibitor. Antimicrob Agents Chemother. 2009; 53: 1823-31.

Wheeler DL, Barrett T, Benson DA, Bryant SH, Canese K, Chetvernin V, Church DM, Dicuccio M, Edgar R, Federhen S, Feolo M, Geer LY, Helmberg W, Kapustin Y, Khovayko O, Landsman D, Lipman DJ, Madden TL, Maglott DR, Miller V, Ostell J, Pruitt KD, Schuler GD, Shumway M, Sequeira E, Sherry ST, Sirotkin K, Souvorov A, Starchenko G, Tatusov RL, Tatusova TA, Wagner L, Yaschenko E. Database resources of the National Center for Biotechnology Information. Nucleic Acids Res. 2008; 36: D13-21.

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