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Designing of Anti Dengue Drug Molecule against Insilico Modeled Target DC-Sign (CD-209)

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Abstract

The C-type lectin DC-SIGN (CD209) plays a major role in receptor on human dendritic cells, it binds to several glycoproteins of viruses that facilitate disease progression. In dengue fever, the disease targets of arbovirus infection, show dendritic and reticuloendothelial cells that may affect immune system. The phytochemical extracts of Bosenbergia rotunda (BR) have been effectively used as potential small molecular inhibitors to inhibit DC-SIGN (CD209) function. Using rational drug designing the training sets include Panduratin-A and 4-hydroxypanduratin is designed from BR derivatives could be an effective inhibitor of a DC-SIGN (CD209) binding towards the drug discovery/ therapy against dengue fever.

Keywords

Dendritic cells, Dengue, arbovirus, Flavovirida, immune response, Bosenbergia rotunda, Panduratin-A, Hydroxypanduratin

1. Introduction

Dengue fever is a mosquito-borne viral disease affecting humans, results in about 50-100 million cases and 250,000 to 500,000 cases of more severe dengue hemorrhagic fever/dengue shock syndrome and about 20,000 deaths annually [1,2]. The development of vaccine against dengue is under the process of developing. With four different serotypes of the dengue virus that can cause the disease, the drug must immunize against all four types to be effective [3]. Vaccination against only one serotype could possible lead to severe DHS when infected with another serotype due to Antibody-dependent enhancement. There is still limited information of how the disease typically behaves and how the virus interacts with the immune system.

Dengue virus is composed of four serotypes based on their antigens on the surface of the virus and their genome structure [4]. The four serotype sequences are 60-80% homology between each other [5]. The major difference for humans lies in subtle differences in the surface proteins in dengue subtypes. The dengue infection is induces a long-life protection against the infected serotypes but it gives only a short time cross protective immunity against the other types [6]. Primary infection with one DENV serotype does not confer protection to infection with a heterologous serotype [7]. Epidemiological studies have demonstrated that DHF occurs at a higher rate in secondary infection than in primary infection [8].
In primary infection the primary interaction between dengue virus and human cell shows DC-SIGN plays an important role in the early interaction of a pathogen with a dendritic cell [9] and has a key role in DC-T cell interaction, DC migration and pathogen uptake [10]. DC-SIGN protein contains three functional domains, the N-terminal cytoplasmic domain, a transmembrane domain anchors to the cytoplasmic membrane, and extracellular domain present in neck region and is formed by seven highly conserved 23 amino acid repeats and a carbohydrate domain for pathogen binding [11]. The DC-SIGN pattern recognition receptor activating T cells, initiating immune response and immune escape of pathogens and tumors [12]. It has been found that immature human DCs susceptible to Dengue infections [13], expresses DC-SIGN [14]. Therefore it is necessary to block primary infection at the level of the DV envelope interacting with DC-SIGN receptors on DCs [15].

The primary infection of dengue fever shows decreased count on platelets in patient, but to increase the platelet count patients have to take natural remedies on anti-oxidants and c vitamins [16]. Plants have been traditionally used to cure a number of human diseases. In present study of samples, few plants derivatives due to their medicinal properties have successfully been tested against viral diseases. The initial step is to DENV cycle is attached with host via host receptors. Envelop DC-SIGN (CD209) protein is involved in viral and host attachment. Thus, dengue infection can be diminish by targeting envelop protein/inhibiting host-viral interactions [17]. The GLU324 amino acid is important antiviral drug targets due to their role in viral replication and other cellular processes in DC-SIGN (CD209). Up to date, many medicinal plants have been tested against DENV and some of them showed significant inhibition effects in the DENV replication cycle [18].

Antiviral effects of two cyclohexenyl chalcone derivatives of Boesenbergia rotunda (L.), 4-hydroxypanduratin A and panduratin A and showed inhibitory activity of both compounds against DEN-2 DC-SIGN (CD209) protein [19]. The inhibition of host-pathogen interactions of protein is an effective strategy of drug development [20]. The reference ligands are reported as competitive inhibitors on dengue fever. In our study is using bioinformatics techniques to develop Insilco models of these natural drugs are docked with DC-SIGN protein. The Insilco models of these two ligands that obeys Lipinski's rule of five were designed and docked with DC-SIGN (CD-209). Thus the ligands designed from potential Panduratin A and Hydroxy Panduratin has been identified to take good step towards the drug discovery against Dengue [21].

2. Materials and Methods

2.1 Selection of peptide sequence of DC-SIGN (CD209)

The peptide sequence of DC-SIGN (CD209) is used for drug design using bioinformatics techniques. The protein sequence of DC-SIGN (CD209) (Swiss-Prot ID: Q9NNX6) belonging to Homo sapiens was extracted from the Swiss-Prot database release 54.0 is 404 amino acids in total length. The main activity of this receptor is mediated by conserved carbohydrate-recognition domain (CRD).

2.2 Antigenic peptide prediction

The online web server gives us a pathway to predict sequences of peptides within a protein that are likely to be antigenic by eliciting an antibody response. Antigenic peptides are determined using on a table that reflects the occurrence of amino acid residues in experimentally known segmental epitopes. We enter the amino acids of DC-SIGN (CD209) of serotype-I strain to study on antigenic prediction in pathogenicity.

2.3 Templates identification and modeling of DC-SIGN

We have identified the templates using PSI-BLAST, these templates is used for build the model of protein structure of DC-SIGN209. We have built the model of the C-type lectin domain of DC-SIGN by exploiting the sequence similarity to the carbohydrate recognition domain (CRD) of the asialoglycoprotein receptor; the homology modeling was carried out using the SWISS MODEL program. The target and the template sequences were aligned using a comparative protein modeling program to generate the 3-D structures of DC-SIGN (CD209). The theoretical model was subjected to energy minimization in Swiss Pdb Viewer software for correcting the stereochemistry of the model.

2.4 Validation of the generated models

The geometric inaccuracies of the theoretical model was corrected and then submitted to SAVS which provides validation reports such as PROCHECK, VERIFY3D and ERRAT to evaluate the 3D-model of DC-SIGN (CD209) protein. This protein structure is used for identification of drug binding sites.

2.5 Generation of Lead molecules and Docking

The entry point for any chemistry program within drug discovery research is generally the identification of specifically acting low-molecular-weight modulators with an adequate activity in a suitable target assay. Panduratin A and Hydroxy Panduratin extracted from *Boesenbergia rotunda* were found to show inhibitory activity against DC-SIGN (CD209) which already showed inhibitory activity against Dengue virus-2 serine protease in a different mechanism priorly. The binding interaction between this inhibitors and DC-SIGN (CD209) were studied using AutoDock 4.0. Then the training set of ligands were designed based on the interactions of Panduratin A and Hydroxy Panduratin with DC-SIGN(CD209).These training set of derivatives were again docked with the initial target cell of Dengue virus to study their interactions and a novel compound that inhibits Dengue virus infection was screened.

3. Results

3.1 Sequence Similarity identification on DC-SIGN (CD209)

The selected protein sequences were sequences, modeled and analyzed using bioinformatics and generated 56 different peptides in a total of 4 isoform sequences. PSI-BLAST tool was used to rank the proteins in descending order such that the proteins most likely to bind to a ligand would be clustered at the top. Scores were generated by calculating the similarity between each peptide sequence as compared to the length of the protein sequence of the CD 209 peptide (table 1). The similarity threshold was selected both empirically and experimentally and corresponded to approximately three identities and one similarity for every five amino acids.

Table 1: Sequence similarity of DC-SIGN (CD209) templates, the scores is calculated using length of sequence using PSI-BLAST algorithm

Template ID	Template Name	Score	Identity
NP_066978.1	CD209 antigen isoform 1	818	100%
NP_001138399.1	mDC-SIGN1A type II isoform	775	97%
AAK91854.1	mDC-SIGN1B type I isoform	789	96%
NP_001138368.1	sDC-SIGN1A type I isoform	732	94%
Q8HXZ8.1	Dendritic cell-specific ICAM-3-grabbing non-integrin 1	761	92%
AAG13848.2	probable mannose binding C-type lectin DC-SIGNR	650	83%
AAK91848.1	mDC-SIGN1A type III isoform	560	75%

3.2 Antigenicity prediction

The prediction results for antigenic peptides of the DC-SIGN (CD209) for DEN-1 are shown in figure-1. The result shows the DC-SIGN protein contains various antigenic peptides all peptides binds with skin cells and cause disease (Table 2).

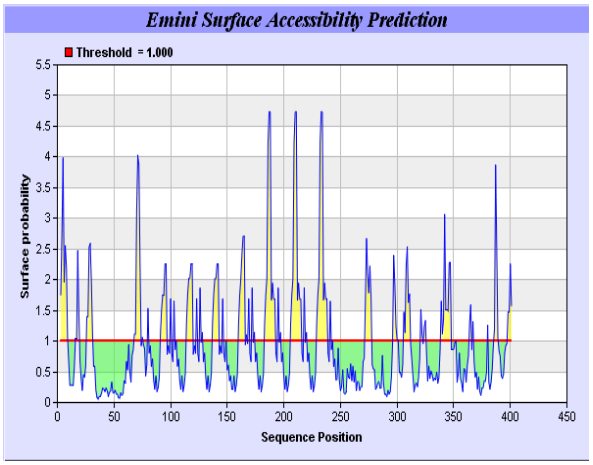


Fig.1: Antigenecity prediction on DC-SIGN (CD209). The prediction of epitopes (Table 2) of the DC-SIGN (CD209) of DEN-1 standard strain was predicted by the means of the position of amino acids with the online server respectively.

Table 2: Antigenicity prediction table, antigenic peptide epitopes present in DC-SIGN (CD-209)

Sl.No	Peptide Start	Peptide End	Peptide sequence	Score
1	3	9	DSKEPRL	7
2	26	31	RQTRGY	6
3	68	73	SQEQSR	6
4	91	96	LSEKSK	6
5	114	119	LPEKSK	6
6	137	142	LPEKSK	6
7	160	165	LPEKSK	6
8	183	192	LPEKSKQQEI	10
9	206	215	LPEKSKQQEI	10
10	229	238	LPEKSKQQEI	10

3.3 Homology modeling

The homology modeling was carried out using the SWISS MODEL program. The target and the template sequences (PDB ID: 1xph) were aligned using a comparative protein modeling program to generate the 3-D structures of DC-SIGN (CD209) (See Homology Modeled Sequence). The theoretical model was subjected to energy minimization in Swiss PDB Viewer software for correcting the stereochemistry of the model. Figure 2 shows the 3D model of DC-SIGN (CD209) in cartoon view with Molegro Molecule viewer software.

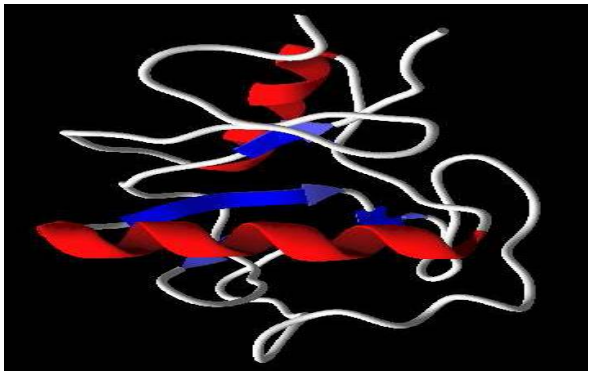


Fig 2a: Modeled DC-SIGN (CD-209) protein structure visualized using Molegro Visual Software.

The geometric inaccuracies of the theoretical model was corrected and then submitted to SAVS which provides validation reports such as PROCHECK, a verification program on Ramachandran plot (Figure 2B) to evaluate the 3D-model of DC-SIGN (CD209) protein. The predicted structure by assessing various parameters such as lengths, angles and planarity of the peptide bonds, geometry of the hydrogen bonds,

and side chain conformations of protein structures as a function of atomic resolution. The Verify3D determines the compatibility of an atomic model (3D) with its own amino acid sequence (1D) by assigning a structural class based on its location and environment (alpha, beta, loop, polar, nonpolar etc.,) and comparing the results to valid structures.

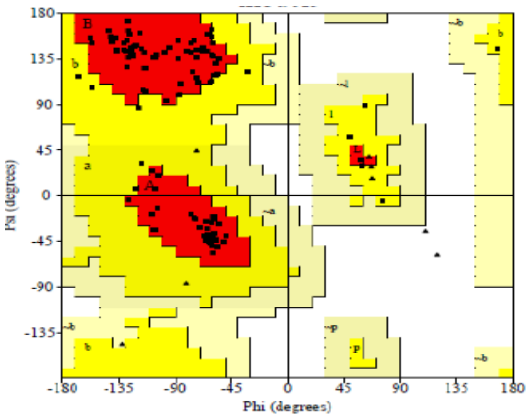


Fig 2b: DC-SIGN (CD209) Modeled protein and visualized using Ramachandran Plot

3.4 Active site Prediction

The modeled DC-SIGN CD-209 active sites was revealed using q-Site finder in which ASP320, GLU324, GLY325, PRO348, ASN349, ASN350, GLU354 and ASP366 amino acid residues were found to be the best binding site (Figure 3).

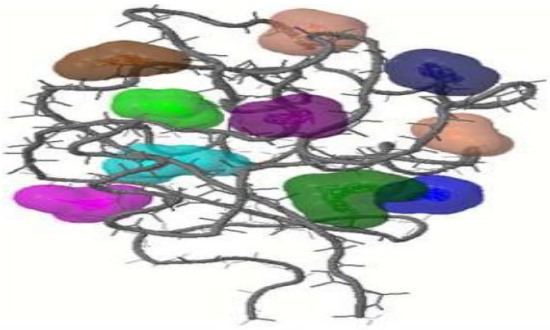


Fig 3: DC-SIGN drug target sites identified using Qsite Finder

3.5 Generation of Novel Ligand Molecules

The structure of the fragments i.e., "the training set of ligands" was designed based on the basis of docking studies of Panduratin A and Hydroxy Panduratin with DC-SIGN (CD209). The fragments were identified on the basis of "Lipinski's Rule of Five" and may therefore represent suitable starting point for evolution of good quality lead compounds. We developed 100 novel ligands for the inhibitory site in DC-SIGN (CD209) protein. Out of 100 novel ligands generated, 12 ligands were selected on the basis of maximum binding affinity measured in kcal/mol. The selected 12 ligands were then analyzed for drug relevant properties based on "Lipinski's rule of five" using Molinspiration property explorer (http://www.molinspiration.com/cgibin/properties) in TABLE 3.

3.6 Docking experiment using homology model

The docking of competitive bioactive molecules Panduratin A, Hydroxy Panduratin A and its training set of ligands onto the conserved carbohydrate-recognition domain region of DCSIGN (CD209) were performed using Autodock4.0 software package. The homology model of DC-SIGN (CD209) was added polar hydrogen atoms and its non-polar hydrogen atoms were merged. For the ligands, non-polar hydrogen atoms were merged with Gustier charges assigned. All rotatable bonds of ligands were set to be rotatable. Docking was performed using genetic algorithm and local search methods. A population size of 150 and 10 millions energy evaluations were used for 100 times searches, with a 80 x 80 x 80 dimension of grid box size and 0.375 Å grid spacing around the domain. Clustering histogram analyses were performed after the docking searches. The best conformations were chosen from the lowest docked energy that populated in the highest number of molecules in a particular cluster with not more than 1.5 Å root-mean-square deviations (RMSD). The H-bond interactions and its binding energy were evaluated for the best affinity by using Molegro Molecule Viewer software in Figure: 4a, 4b and Table: 4

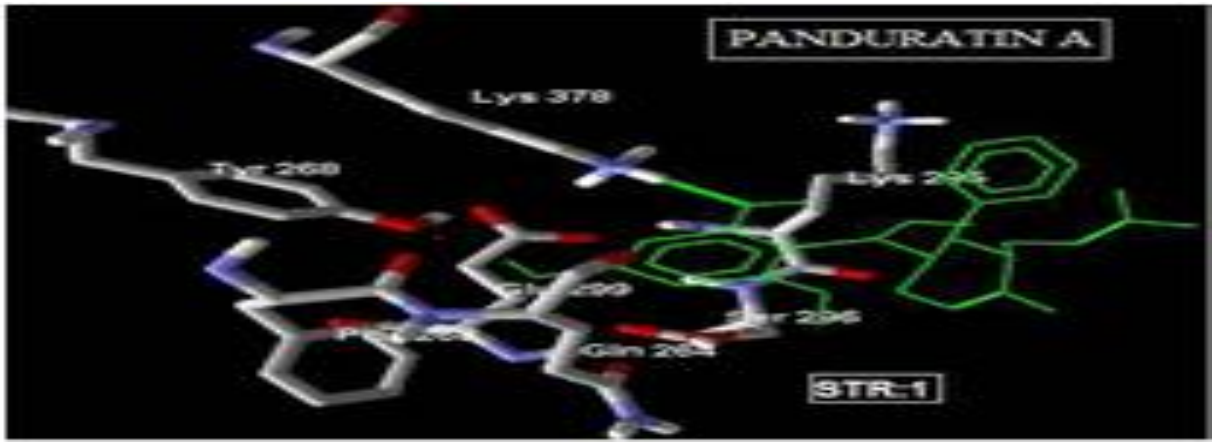


Fig 4a: Protein DC-SIGN CD209 interacts with Panduratin A Compound showing 3 hydrogen bonds with an amino acids Lys 379, Gln 264 and Lys 298 with binding energy -3.62 and 1.79

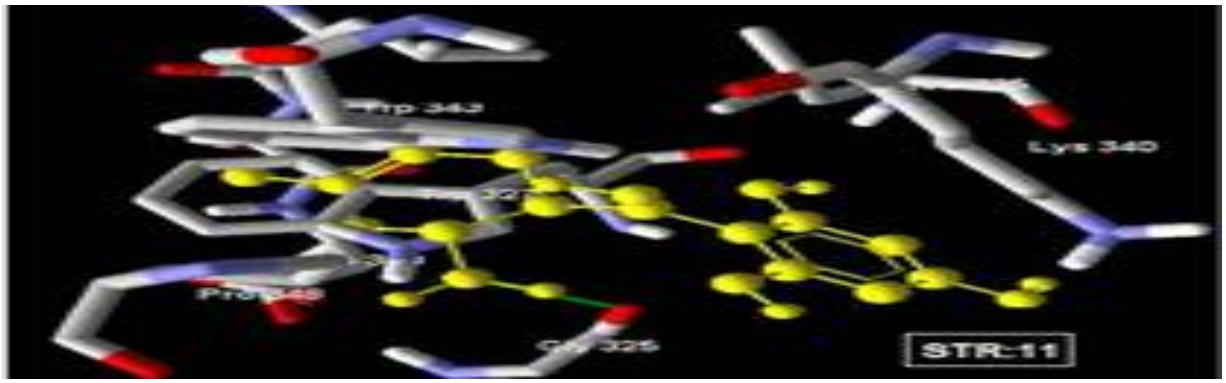
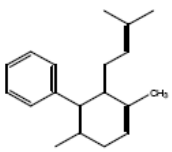
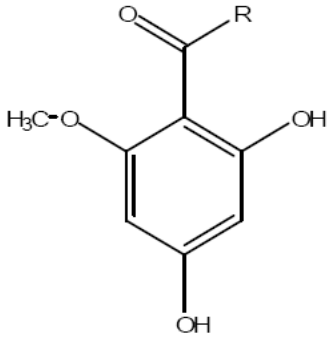
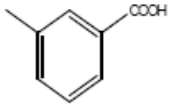
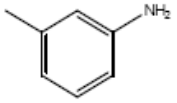
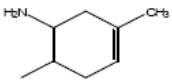
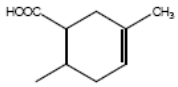
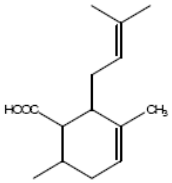
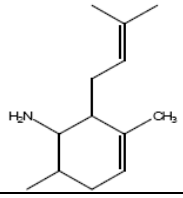
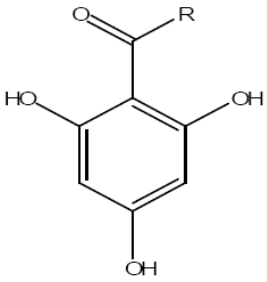
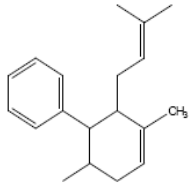
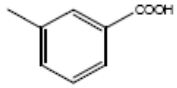
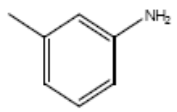


Fig 4b: Protein DC-SIGN CD209 interacts with Hydroxy Panduratin A Compound showing 4 hydrogen bonds with an amino acids Lys 379, Gly 325 and Lys 298 with Binding energy -4.57 and 1.19

Our results showed hydroxypanduratin A and Panduratin A exhibited Van der Waals interactions with Lys 379, Gln 264 and Lys 298. In addition, hydrogen bonding interaction was also observed between these two ligands and the residues Gly 325 and Lys 298, indicating another possible mode of interaction between these ligands and the DEN2 DC-SIGN CD-209 protein.

Table 3: Anti Dengue drug obeying "Lipinski's Rule of Five"

DC-SIGN (CD209) Inhibitor	R=	mLogP	MW	nON	nOHNH	nrotb
	Structure 1 	6.0836	406.522	4	2	6
	Structure 2	2.525	255.255	6	3	4

 **R= STR** 1-Panduratin						
	Structure 3 	1.69	259.261	5	4	3
	Structure 4 	1.408	277.32	5	4	3
	Structure 5 	2.161	306.314	6	3	4
	Structure 6 	4.207	374.433	6	3	6
	Structure 7 	3.455	345.439	5	4	5
 **R= STR** 8- Hydroxy Panduratin	Structure 8 	6.767	392.495	4	3	5
	Structure 9 	2.457	274.228	6	4	3
	Structure 10 	1.622	245.234	5	5	2
	Structure 11	1.34	263.293	5	5	2

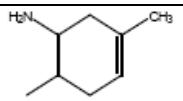
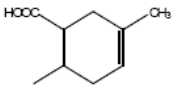
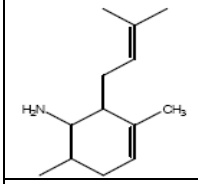
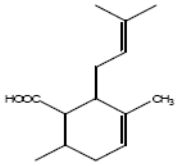
						
	Structure 12 	2.093	292.287	6	4	3
	Structure 13 	3.386	331.412	5	5	4
	Structure 14 	4.139	360.406	6	4	5

Table 4: Energies in kcal/mol calculated using Auto Dock 4.0.
DC-SIGN CD209 protein interactions with Panduratin A and Hydroxy Panduratin A

Novel Ligands	Free Energy Binding	Ki µm	Intermolecular Energy	Vdw Energy	Electrostatic Energy	Torsional Free Energy
STR:1	-3.62	2.21	-5.41	-5.30	-0.12	1.79
STR:2	-6.29	24.38	-6.29	-6.09	-0.21	0.0
STR:3	-6.53	16.41	-6.53	-6.48	-0.05	0.0
STR:4	-6.91	8.63	-6.91	-6.74	0.17	0.0
STR:5	-6.59	14.66	-6.59	-6.61	0.01	0.0
STR:6	-4.5	499.51	-5.1	-4.94	0.16	0.6
STR:7	-4.15	909.94	-5.64	-6.14	0.5	1.49
STR:8	-4.57	450.34	-5.76	-5.75	-0.01	1.19
STR:9	-7.13	5.98	-7.14	-7.18	-0.06	0.0
STR:10	-6.38	20.95	-6.38	-6.38	0.0	0.0
STR:11	-6.44	19.12	-6.74	-6.44	0.29	0.3
STR:12	-5.73	62.93	-6.33	-4.99	-1.33	0.6
STR:13	-5.37	115.58	-6.27	-5.37	-0.9	0.89
STR:14	-5.51	90.78	-6.71	-5.58	-1.32	1.19

4. Discussion

The crystal structure of mDC-SIGN1B type I isoform [Homo sapiens] (PDB ID: 1S14) that shows 100% sequence identity was used as a template to predict the structure of protein and the predicted 3D structure of DC-SIGN (CD209) protein was generated by Swiss Model and energy minimized by Swiss PDB viewer, the structure with the lowest energy scores were Table: 2 selected. Then qualities of the 3D models were evaluated using the PROCHECK program and assessed using the Ramachandran plot. It is evident from the Ramachandran plot that the predicted models have most favorable regions, the allowed regions, the generic regions and the disallowed regions. Such a percentage distribution of the protein residues determined by Ramachandran plot shows that the predicted models are of good quality. The models show all the main chain and side chain parameters to be in the 'better' region.

The Protein-ligand interaction plays a significant role in structure based drug designing. Overall, the best confirmation shows the free energy of binding (Δ Gbind kcal/mol) for the designed ligand from Panduratin A and Hydroxy Panduratin A with DC-SIGN (CD209) (Table:4). The negative and low value of ΔGbind indicates strong favorable bonds between NS3 protein and the novel ligand indicating that the ligand was in its most favorable conformations. The information about the number of hydrogen bonds formed and catalytic site residues involved in protein-ligand complex are shown in Figure: 4.

5. Conclusion

In this study, we designed a novel ligands that obeys "Lipinski's Rule of Five" against DC-SIGN (DC-Specific Intracellular adhesion molecule (ICAM) 3–Grabbing Non integrin) (CD209), a member of C-type lectin receptors (CLR).The molecular docking was applied to explore the binding mechanism and studies on the novel ligand against the DC-SIGN (CD209) receptors showed that the free binding energy for the inhibitor was small, indicating that the ligand binds favorably to the binding site. The training set of ligand was observed as the best inhibitors than Panduratin A and Hydroxy Panduratin A, which may be considered as potential ligands for the treatment of Dengue fever caused by flavivirus. The ligand thus developed is likely to inhibit viral infections, which share high sequence similarity with the mDC-SIGN1B type I isoform [Homo sapiens] (PDB ID: 1S14) from same family. We have planned to undergo ADME/T (Absorption, Distribution, Metabolism, Excretion /Toxicity) studies on the designed ligand using the available commercial ADME/T tools in future.

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