In-Silico Protein-Ligand Docking Studies against the NS5 Methyltransferase Protein of Dengue Virus

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Abstract

From last few decade dengue viruses is the most significant arthropod-borne human pathogen. We found that the number of cases have been increased and reported in every year. Currently vaccines and drugs against the dengue viruses are available in rare quantity and till now people are depend on the natural drugs that may or may not be beneficiary for the patient. In this study, the problem of designing the drug molecule against the dengue virus has been solved in-silico protein-ligand docking using computational method. We found some potential lead compounds which are active against dengue virus. After the molecular docking we perform the virtual screening and get thousand analogs of lead compounds. The interactions of the analogs with the active site of 1L9K and 1R6A protein were analyzed. On the basis of activity and high binding interactions to find some best compounds which suggested new drug candidate for the future plan.

Keywords - *Methyltransferase (MTase); NS5 (Nonstructural protein); DENV (Dengue virus); RTP (Ribavirin triphosphate); SAH (S-Adenosyl-L-homocysteine); PDB (Protein Data Bank).*

I. INTRODUCTION

The cause of dengue fever is *Aedes* mosquitoes (*Aedesaegyptii*). It is a viral disease and transmitted between human hosts. Every year more than 20 million cases of dengue fever recorded in whole world. The dengue viruses are members of the genus *Flavivirus* in the family *Flaviviridae*. Flavivirus includes the West Nile virus (WNV), Japanese encephalitis virus (JEV), Yellow fever virus (YEV), Murray valley encephalitis virus (MVEV), Usutu virus (USUV), West Nile virus (WNV), St. Louis encephalitis, and Kunjin virus (KUN) are member of the genus. The symptoms of dengue fever result in severe flulike, including fever, headache, and myalgia, but more severe cases can progress into a hemorrhagic fever and shock syndrome with considerable lethality. An Interesting "pandemic of dengue" (likely chikungunya) occurred in the years 1870-73, appearing first on the coast of East Africa, then on the Arabian coast. The human dengue viruses evolved as a parasite of Subhuman primates. Animal infected with ancestral dengue virus must have become separated for prolonged periods permitting the evolution of viruses whose envelop proteins differed sufficiently to escape cross- neutralization. Treatment of dengue fever is nonspecific and only based on the symptomatic with a regimen of analgesics and fluid replacement [1,2].

NS5 methyltransferase is one of the seven nonstructural proteins in the polyprotein encoded by the flavivirus genome's single open reading frame. This is non-structural proteins NS5 Methyltransferase (MTase). It is the largest and most conserved protein in a flavivirus. Methyltransferase is recently established drug target in dengue virus [5]. The nonstructural protein (NS5) has separate encoded in separate domains. The two terminal of NS5 protein are present called the C-terminal and N-terminal. The C-terminal contains 600 amino acids of NS5 RNA-dependent RNA-polymerase (RdRp) and the N-terminal contains 300 amino acids represent the NS5 methyltransferase domain. This methylates the cap structure found on the 5' end of viral RNA. Both RNA polymerase and methyltransferase activity are essential for the viral lifecycle. The methyltransferase NS5 activity includes both 2'-0 methylation and N7 methylation, which is rare since other methyltransferase typically, carry out only one type of methylation reaction [5].

Here present dengue MTase protein with their PDB IDs.

>gi|29726395|pdb|1L9K|AChainA, Dengue Methyltransferase

>gi|55669630|pdb|1R6A|A Chain A, Structure of the Dengue Virus 2'o Methyltransferase in Complex with S-AdenosylHomocysteine and Ribavirin5'Triphosphate

We performed a computer-aided virtual screen more than 20 available chemical compounds to find potential inhibitors of NS5 MTase. We are using Structure-based virtual screening using the crystal structure of NS5 MTase [PDB: 1L9K]. The structure based compounds

screening performed using the LIDAEUS program. These map the active site of the receptor and screen it against many commercially available compounds in the database. These discovered the several compounds that fit into the two binding sites of NS5 MTase with a higher binding affinity than its active ligands. Molegro Virtual Docker software was use to prepare ligands for virtual screening and docking process. The LIDAEUS (Ligand Discovery at Edinburgh University) program was using to search for potential inhibitors based on a map of the NS5 MTase binding site by screening of chemical compounds. EDULISS (EDinburgh University LIgand Selection System) is a chemical compounds database that was use throughout this study to screen for potential inhibitors based on ligand structure similarities with the known natural NS5 MTase substrates RTP and SAH [17].

A. The Structure preparation and analysis of NS5 MTase

The three-dimensional structure of NS5 MTase S-adenosyl-L-homocysteine (SAH) retrieved from the Protein data bank [PDB: 1L9K]. Structure of NS5 MTase ribavirin (RTP) is prepared for structure-based virtual screening and molecular docking processes by removing all sulfate ions, hydrogen ions and water molecules. Ligands (SAH and RTP) removed and saved as two separate pdb files for further ligand-based virtual screening and docking DENV Methyltransferase.

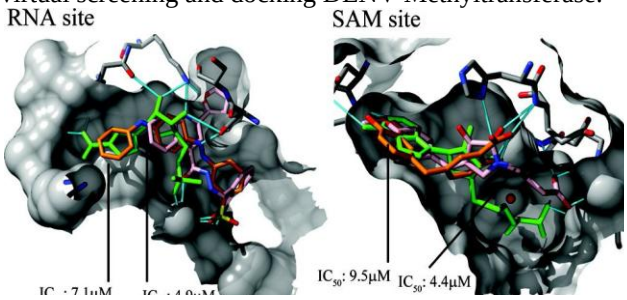


Fig 1. Structures of the MTasecomplexed with S-adenosyl-L-homocysteine (SAH) molecule dock to the SAM site and ribavirin triphosphate (RTP) molecule dock to the RNA site [11].

II. METHODOLOGY

In-silico drug discovery process is a important and beneficial way to design novel drugs by increasing the knowledge about the biological processes, biochemical activities of chemical compounds and targets. Bioinformatics tools are majorly facilitating in silico drug discovery.

A. Selection of target protein

Identifying and selecting the most appropriate drug target or receptor is the initial step in the drug designing procedure. Excellent drug targets can be identified with the help of bioinformatics. Mostly proteins act as good targets for the drugs. In some cases, enzymes can also serve as excellent drug targets [13]. NS5 methyltransferase protein of Dengue virus-2 was selected for the present study. The three dimensional structure of dengue virus NS5 methyltransferase is available in the Protein Data Bank (accession code: 1L9K and 1R6A) and is shown in Figure 2. Once the target has been is identified, the candidate ligand of drugs can be selected either by analyzing previously known drugs having potent effects against DENV or by designing the novel inhibiting compounds.

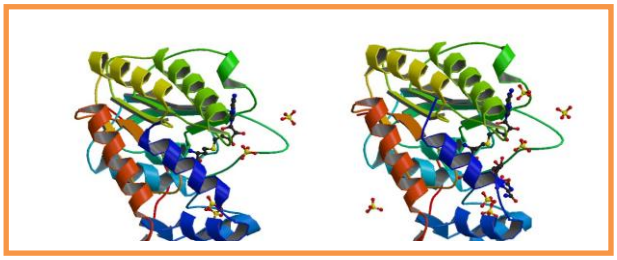


Fig 2. Crystal Structure of Dengue Virus NS5 Methyltransferase (A) 1L9K (B) 1R6A

B. Structure- based virtual screening

To utilized the search for potential inhibitors against NS5 MTase by matching the binding site map of the receptor against chemical compounds in the database. First, the NS5 MTase structure uploaded into LIDAEUS after that SAH and RTP separately. LIDAEUS generated an energy map and site points on the MTase structure based on the positions where SAH and RTP reside in their binding pockets (the SAM binding site and RNA cap-binding site, respectively). We have taken 28 compounds for molecular docking in which three compounds get best for virtual screening. After the virtual screening process, we find the two compounds are the best for drug designing purpose.

C. Ligand-based virtual screening

Using the web-based EDULISS programs (software), we have done Virtual screening based on the structure of NS5 MTase active ligands (SAH and RTP). To search similar compound we used two-dimensional structure of SAH and RTP in the EDULISS database from common chemical compounds. Take 20 chemical compounds, which have the highest structural similarity to SAH and RTP was use for further molecular docking analysis [10]. Then we get molecule which have highest energy of ligand.

D. Protein-ligand based Molecular docking

Molecular docking performed using the Molegro Virtual Docker program. This protein-ligand software is integrated software for predicting protein-ligand interactions. It is deals with high quality docking

based on an optimization technique. Software also has a user interface that will focus on usability and productivity. It also handles maximum aspects of the process, from preparing the molecules to determining the potential binding site of the target protein.

E. Inhibitors bound to SAM binding site in (1L9K Protein)

1. Aurintricarboxylic acid

Influenza viruses cause serious infections that can be prevent or treated using vaccines or antiviral agents, respectively. While vaccines are effective, they have a number of limitations. Influenza strains resistant to currently available anti-influenza drugs are increasingly isolated. Aurintricarboxylic acid (ATA) is a polyromantic carboxylic acid derivative that inhibits nucleases and nucleic acid processing enzymes. Aurintricarboxylic acid (ATA) inhibit the serve acute respiratory syndrome associated coronavirus (SARS-CoV) and vaccinia virus. ATA can substantially inhibit the replication of several strains of cultures with moderate cytotoxicity [11].

2. Dehydrosinefungin

Dehydrosinefungin is novel inhibitors of NS5 Methyltransferase. It consists of 4, 5- dehydroadenosine and ornithylvaline.

3. Sinefungin

Sinefungin, a known inhibitor of protein methylation, inhibited the myelin basic protein (arginine) methyltransferase activity in homogenates of cultured cerebral cells from embryonic mice. Fifty percent inhibition achieved with 25-microM sinefungin. Strong competitive inhibitor of methyltransferases which uses S-adenosyl-L-methionine as the methyl group donor to yield methylated products such as 5-methylcytosine or N6- methyl adenosine on DNA and RNA. In addition, sinefungin is involved in a number of physiological processes [12].

4. Inhibitors bound to RNA cap site in (1R6A)

We performed molecular docking of 20 compounds obtained from the ligand-based EDULISS screen. These 20 compounds ranked based on their binding affinities. The top five with binding affinities for the RNA cap site that are having more negative affinity than that of RTP which are listed in the table1. The five compounds that showed the strongest affinities for the RNA cap site of NS5 MTase were SPH1-103-799, SPH1-101-102, 28SPH1-115-917, 35SPH1-021-288 and 28SPH1-185-015 each with a binding affinity of -7.8 and -7.9 kcal/mol respectively.

5. Database used

The three dimensional structure of dengue virus NS5 methyltransferase is available in the Protein Data Bank (accession code: 1L9K and 1R6A). For the *in silico* design and molecular docking studies a number of inhibitors of NS5 methyltransferase were identified and selected. There were 28 compounds [11-17] which were selected for molecular studies having drug like properties against dengue virus. These drugs were selected through the analysis of previous and current research literature on the dengue virus. Performing the virtual screening process to fetched the analogues from zinc database.

III. RESULTS AND DISCUSSION

A. Molecular Docking

On the basis of molecular docking, a usually smaller molecule which binds with the larger molecule such as enzyme or protein initiates the replication process. On the basis of docking of anti-Dengue drugs with the receptor protein, the 1L9K and 1R6A was searched for its active site. There after we perform the virtual screening of lead compounds to against the dengue virus NS5 MTase. Once all the 28 compounds were docked against the receptor protein 1L9K and the 19 compounds were docked against the receptor protein 1R6A, these docked complexes were visualized in VMD. From these selected compounds screening for further molecule docking experiments using docking software. These structure and results shown in the form of the figures and tables.

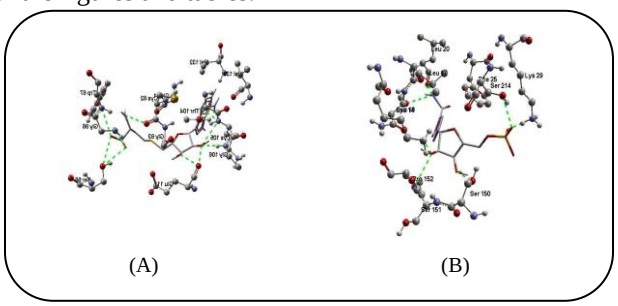


Fig 3.Redocking of RTP and SAH into RNA cap site and SAM binding site with accession code: 1L9k and 1R6A protein.

B. Lead Identification

Protein binding interactions 1L9K protein with the 28 chemical compounds and 1R6A protein with the 19 chemical compounds have shown in table 1.

1L9K protein with crystallized conformation of SAH shown in yellow carbons. The best-redocked conformation of SAH has shown in dotted lines carbons. Second 1R6A protein with Crystallized conformation of RTP shown in yellow carbons. The best-redocked pose of RTP shown in green carbons. Residues with hydrophobic contacts with RTP and SAH

labeled in red and hydrogen bonds drawn as dashed lines show in above figure 3.

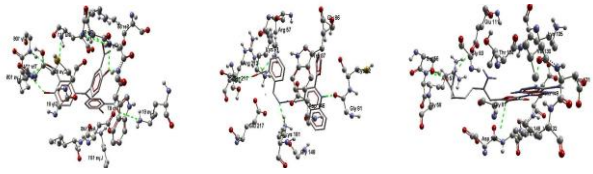
Table 1- List of NS5 MTase inhibitors (1L9K and 1R6A) Protein.(This table is showing at the end of paper)

In table 2, show the molecular docking result, docking validation was performed by redocking co-crystallized into SAH into their respective binding sites. The co-crystallized ligand with binding affinities of -164 (kcal/mol) for SAH respectively. There after performing the molecular docking result and we find the best molecule name as Aurintricarboxylic acid (ATA)hashigh Moldockscore-187(kcal/mol).

Table 2- Docking Result (1L9K Protein)(This table are showing at the end of paper)

C. Lead Compounds

After the molecular docking, we found the three best potential lead compounds. This shows the high protein ligand binding energy.Although mostly compounds show good interactions with the target protein, but high binding affinity of compounds is shown in figure 4 (A,B,C). (a) AurinTricarboxylic Acid (b) ZINC03369470 and (C) Dehydrosinefungin**.**



(A) (B) (C)

Fig 4.Docking views of (A) AurinTricarboxylic Acid (B) ZINC03369470 and (C) Dehydrosinefungin.

Table 3- List of best five compounds showing the high Moldock energy. (This table is showing at the end of paper)

D. Analogs Searched

After that the molecular docking process we search the thousand analogs of these chemical compounds from Zinc database and thus allowed to the Virtual Screening. On the bases of compound similarity we searched the analogs: Twenty three analogs of Aurintricarboxylic acid, five hundred forty six analogs of Sinefungin, twenty analogs of Dehydrosinefungin and five hundred seven analogs of ZINC03369470 searched from the zinc database. All the analogs were docked within the active site of 1L9K with the same procedure as discussed earlier. After that, the virtual screening of 1L9K we find the several analogs, which have the high energy binding conformation for SAH, respectively. The best confirmation was selected up on the least binding energy of analog in 5 conformations. Binding energy of analogs is shown in Table in 4. Each selected analog conformation was saved and then analyzed.

Table 4- Virtual Screening Results of (1L9K Protein)(This table are showing at the end of paper)

On the basis of protein-ligand interactions with the target protein 1L9K, analog 1,2,3 of sinefungin and analog 1,2,3 of ZINC03369470 has showed the maximum binding affinity. Although mostly analogs of lead compounds show good interactions with the target protein, but high binding affinity is shown in above table 4 with these analogs. Therefore, these analogs showed the good interactions with the target protein 1L9K.

Table 5- Docking Result (1R6A Protein)(This table is showing at the end of paper)

Same procedure we follow the target protein 1R6A. To obtained the protein structure from Protein Data Bank and removes the water molecules before the docking experiments. Then for the ligand preparation, input the ligand and Gasteiger charges were merged with non-polar hydrogens of ligand. Performing the molecular docking process we find the active conformation of ligand and the binding modes of 19 selected compounds with the active site of the target protein 1R6A. After the docked compounds then we found only one of the compound SPH1-013-271 which showing the high Moldock Score -177 (kcal/mol) for RTP molecule. Show the above table 5, we search the thousands of similar compounds of SPH1-013-271 and analogs from zinc database on 60% similarity and thus allowed to the Virtual Screening. In figure 5 shows the, maximum interactions with the target protein 1R6A to the lead compound (SPH1-013-271). On the bases of virtual screening result to find the maximum binding energy conformation of analogs with the target protein 1R6A (shown in table 6).

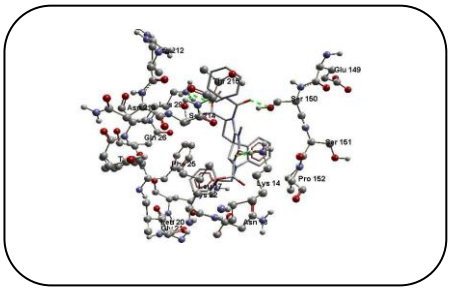


Figure 5.Docking views of (SPH1-013-271) compound.

Table 6- Virtual Screening Results of (1R6A Protein)(This table are showing at the end of paper)

In this study, we have done molecular docking then performing the screening of 1thousand similar compounds which searched from a zinc database and then we find some lead compounds which are the best for the anti-dengue drug.

We discovered several compounds that fit into the two binding sites of NS5 MTase with a higher binding affinity than its active ligands. These compounds can be further tested to confirm their biological activities against the DV. Dengue and DHF remain pharmacologically neglected diseases, especially in developing countries. Because these countries have healthcare facilities is limited. Therefore, research group has taken an initiative to be developed drug for the poor and developed countries [14,15].

Binding affinities used for the selection of potential compounds. Compounds with stronger binding affinities for the RNA site and SAM binding sites than that of SAH and RTP molecule (NS5 MTase active ligands) selected as potential inhibitors. In addition, the selected compounds must interact with residues that are catalytically important for the function of NS5 MTase. In contrast to the RNA cap site, the SAM binding site is rather closed and long. These compounds are thought to specifically bind to dengue NS5 MTase and do not interfere with related human enzymes [19]. It has been suggested that the guanosine moiety is important for promoting the activity of inhibitors that bind to the SAM binding site due to the strong hydrophobic contacts between the guanosine ring and Lys105 and Ile147 [16]. Nevertheless, Podvinec et al. reported that two compounds with no guanosine moiety bound to the SAM binding site (IC50 of 4.4
and 9.5 μM) [18]. In further studies we analyze the all proteins of dengue virus with their resolution power with the help of machine learning approaches to find the best protein sequence.

IV. CONCLUSION

In this work, we find the novel drug molecules as anti-dengue compounds using the structure-based drug design technique. 1L9K and 1R6A protein was used as a target for the purpose of finding novel drugs. About 28 drugs (1L9K) and 19 drugs (1R6A) were selected as ligands for this study that showed activity against the dengue virus. Each ligand with the target, the complexes formed was analyzed based on their energy values and their binding affinities with the target. Based on the results of this analysis, a lead compound was identified. It is finding that two lead compounds such as Aurintricarboxylic acid and ZINC03369470 compound get after docking and screening process. It is above show in table 4 molecule that show the highest energy of Aurintricarboxylic acid and ZINC03369470 compound and its analogs are (ZINC00669666, ZINC00668589, ZINC0444329, and ZINC15907608) to after virtual screening. For the RNA cap site in (1R6A) protein, also show the moldock score of only one compound SPH1-013-271 and its more than five hundred analogs compounds. Therefore, we suggest that these two compounds (Aurintricarboxylic and ZINC03369470) the best out of all compounds or analogs can used for developing an effective dengue drug for future research.

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Table 1- List of NS5 MTase inhibitors (1L9K and 1R6A) Protein.

NS5 methyltransferase protein 1L9K				NS5 methyltransferase protein 1R6A		
S.No	**Ligand Name**	**IC50**	**References**	**Ligand Name**	**IC50**	**References**
1.	5-aza-2-deoxycytidine		(stock, 2005)	28SPH1-021-288	4.9 -to- 7.1µM	(Lim, 2011)
2.	Dimethylated arginine		J Leiper ; 2011	28SPH1-024-902		
3.	Monomethylated arginine		J Leiper ; 2011	28SPH1-026-800		
4.	Cantharidin		E Lissina ;2011	28SPH1-081-432		
5.	Procarnamide		(Lim, 2011)	28SPH1-115-917		
6.	Catechol-o		(Palma P. N., January 2012)	SPH1-103-799		
7.	Azacitidine		(Lyko, 2008)	28SPH1-149-718		
8.	Decitabine		(Lyko, 2008)	28SPH1-185-015		
9.	Chactocin			28SPH1-348-781		
10.	Thymidylated			28SPH1-63-033		
11.	Tricostatin			SPH1-014-180		
12.	Zebularin			SPH1-000-259		
13.	Sinefungin		(Podvinec, January, 2010)	SPH1-013-271		
14.	Dehydrosinefungin	2.3µM	(Podvinec, January, 2010)	SPH1-013-272		
15.	Aurintricarboxylic acid (ATA)		(M.Hashem, 2007)	SPH1-013-273		
16.	Ribavirin triphosphate		(Lim, 2011)	SPH1-013-274		
17.	Prolglhydroxylase			SPH1-027-074		
18.	NSC15765	14.3µM	(Podvinec, January, 2010)	SPH1-047-692		
19.	NSC140047	8.78µM	(Podvinec, January, 2010)	SPH1-101-102		
20.	NSC14778	1.52µM	(Podvinec, January, 2010)			
21.	ZINC03369470	4.80µM	(Podvinec, January, 2010)			
22.	ZINC02911543	7.56µM	(Podvinec, January, 2010)			
23.	ZINC01078518	9.28µM	(Podvinec, January, 2010)			
24.	SPH1-103-799	4.9 and7.1 µM	(Lim, 2011)			
25.	SPH1-007-088		(Lim, 2011)			
26.	SPH1-101-102	4.9 and7.1 µM	(Lim, 2011)			
27.	28SPH1-115-917	4.9 and7.1 µM	(Lim, 2011)			
28.	25SPH1-103-433		(Lim, 2011)			

Table 2- Docking Result (1L9K Protein)

		SAM Site	
S. No	**Ligand Name**	**MoldockScore (kcal/mol)**	**HBond**
	SAH molecule	-164.224	-16.9123
1.	Aurintricarboxylic acid (ATA)	-187.769	-15.067
2.	ZINC03369470	-172.542	-10.2867
3.	Dehydrosinefungin	-163.99	-6.65296
4.	Sinefungin	-158.197	-15.8751
5.	ZINC02911543	-145.884	-6.44874
6.	SPH1-007-088	-144.558	-4.35874
7.	NSC140047	-141.083	-9.48148
8.	NSC14778	-139.009	-9.29705
9.	NSC15765	-137.073	-13.1919
10.	SPH1-101-102	-121.837	-2.89083
11.	Prolglhydroxylase	-118.668	-7.82975
12.	Ribavirin triphosphate	-114.918	-10.1049
13.	Monomethylated arginine	-112.163	-15.5086
14.	Tricostatin	-108.424	-12.1929
15.	Azacitidine	-105.293	-7.01151
16.	Decitabine	-104.357	-11.2344
17.	Zebularine	-104.087	-4.41083
18.	Procarnamide	-103.684	-1.09283
19.	Dimethylated arginine	-96.3692	-14.3438
20.	5-aza-2-deoxycytidine	-93.6301	-12.7013
21.	catechol-o	-93.3611	-8.43426
22.	thymidylated	-66.1526	-3.89358
23.	25SPH1-103-433	872.129	-5.26063
24.	28SPH1-115-917	893.693	-3.83338
25.	Cantharidin	929.524	-2.61824
26.	SPH1-103-799	4847.89	-12.4371
27.	Chactocin	4851.39	-15.4786
28.	ZINC01078518	5869.31	-3.8606

Table 3- List of best five compounds showing the high Moldock energy

S. No	Ligand Name	Moldock Score kcal/mol	HBond	Common residues	Searched Similar Compounds (Zinc Id)
1.	Aurintricarboxylic Acid	-187.769	-15.067	Thr, Lys, Gly, Trp,	ATA (23) analogs
2.	ZINC03369470	-172.542	-10.2867	Lys, Gly	SF (546)analogs
3.	Dehydrosinefungin	-163.99	-6.65296	Gly,	DSF(20) analogs
4.	Sinefungin	-158.197	-15.8751		ZINCO3369470(507) analogs
5.	ZINC02911543	-145.884	-6.44874		

Table 4- Virtual Screening Results of (1L9K Protein)

S.No	Inhibitor Name	Moldoc Score	S.No	Analogs Id	Analogs Moldock Score (kcal/mol)	HBond
1.	Aurintricarboxylic acid (23)	-187.769	1)	ZINC04974299	-179.745	-21.3507
			2)	ZINC02358769	-170.08	-119.655
			3)	ZINC02546473	-168.786	-19.5128
			4)	ZINC32113592	-155.442	-6.29071
			5)	ZINC04726498	-154.764	-18.1523
2.	Dehydrosinefungin(20)	-163.99	1)	ZINC38555871	-175.236	-21.7835
			2)	ZINC3855587_1	-174.574	-19.4225

			3)	ZINC38555870	-173.745	-23.1811
			4)	ZINC3855587	-168.432	-16.7849
			5)	ZINC17111254	-162.2	-7.51562
3.	Sinefungin (546)	-158.197	1)	ZINC03869249	-204.594	-23.3822
			2)	ZINC04228234	-198.761	-20.0403
			3)	ZINC03869240	-197.221	-26.4742
			4)	ZINC03869250	-194.709	-25.721
			5)	ZINC13518964	-175.605	-28.8811
			6)	ZINC01842158	-167.275	-24.201
			7)	ZINC01571045	-165.254	-29.0619
			8)	ZINC1358982	-169.024	-25.5744
			9)	ZINC1713574	-166.989	-25.0689
			10)	ZINC03869815	-162.369	-14.6262
			11)	ZINC03870702	-162.247	-19.1449
			12)	ZINC01532626	-162.199	-21.5453
			13)	ZINC03869814	-160.866	-14.4104
4.	ZINC03369470 (507)	-172.542	1)	ZINC00669666	-205.507	-1.84742
			2)	ZINC00668589	-194.447	-4.74314
			3)	ZINC0444329	-179.4	-1.303
			4)	ZINC15907608	-176.549	-9.08478
			5)	ZINC17409522	-170.514	-7.45826
			6)	ZINC02091089	-169.594	-8.16742

Table 5- Docking Result (1R6A Protein)

		RNA Site			
S.No	**Ligand Name**	**Moldock Score (kcal/mol)**	**HBond**	**Similarity%**	**Analogs Id**
	RTP molecule	-153.323	-18.2607		
1.	SPH1-013-271	-177.417	-6.90699	(60%)	596 Analogs
2.	28SPH1-081-432	-146.201	-1.74436	(80%)	270 Analogs
3.	28SPH1-63-033	-135.636	-10.9112		
4.	SPH1-103-799	-126.554	-9.70314		
5.	28SPH1-026-800	-122.308	0		
6.	SPH1-027-074	-122.272	-10.6789		
7.	SPH1-047-692	-119.721	-10.4141		
8.	28SPH1-348-781	-115.477	-11.3199		
9.	SPH1-014-180	-111.596	-10.9081		
10.	SPH1-013-274	-104.832	-15.2752		
11.	28SPH1-115-917	-100.596	-3.34326		
12.	28SPH1-185-015	-94.7596	-17.6787		
13.	SPH1-101-102	-91.5418	-17.4868		
14.	SPH1-013-272	-83.2589	-19.478		
15.	28SPH1-149-718	-82.2765	-7.1879		
16.	28SPH1-021-288	-81.915	-18.5785		
17.	SPH1-000-259	-81.68	-1.33649		
18.	28SPH1-024-902	-75.7888	-648934		
19.	SPH1-013-273	-68.7674	-6.57661		

Table 6- Virtual Screening Results of (1R6A Protein)

S. No	Inhibitor Name	Moldock Score	S. No	Analogs Id	Analogs Moldock Score (kcal/mol)	HBond
1.	SPH1-013-271 (596)	-177.417	1)	ZINC08430514	-170.241	-8.47353
			2)	ZINC09557581	-167.623	-11.8234
			3)	ZINC09557582	-167.432	-13.4492
			4)	ZINC08430386	-167.107	-8.16354
			5)	ZINC08430379	-160.892	-10.9542
2.	28SPH1-081-432 (270)	-146.201	1)	ZINC7164225	-161.35	-10
			2)	ZINC71164227	-158.462	-10
			3)	ZINC2039310	-157.222	-9.88252
			4)	ZINC71164226	-156.382	-9.9845
			5)	ZINC71164228	-156.145	-9.91116
			6)	ZINC71164216	-154.079	-9.91116
			7)	ZINC13764232	-153.296	-7.19322
			8)	ZINC0430160	-153.022	-10
3.	28SPH1-63-033 (508)	-135.636	1)	ZINC03048499	-154.125	-5
			2)	ZINC34984187	-142.997	-13.064