EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 1
165-173
Year: 2016

DOCKING OF SHORT CHAIN PEPTIDES TEMPORINS WITH EBOLA VIRUS TARGET 4IBK

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Article Received on 19/10/2015 **Article Revised on 10/11/2015** **Article Accepted on 01/12/2015**

ABSTRACT
The needs of unexplored molecules in the treatment of emerging highly dangerous viral infections are increasing day by day. The discovery of new compounds or molecules will be of highly expensive and so we aim to screen the reported short chain peptides with antibacterial property whose susceptibility against viral pathogens are unknown. Temporins are nine different types of small peptides with a maximum of 13 aminoacids. They are chosen as ligands and the viral protein VP 35 with PDB Id 4IBK was chosen as target. The docking was performed for these 9 different combinations in ZDOCK software of Discovery Studio version 3.1 (30 days trial) and the ZScore of all the docked structures were noted. The viral target showed promising scoring of -120.50 with AP00110 4IBK and -112.45 with AP00108 4IBK.

KEYWORDS: Tamporins family, 4IBK, protein-peptide interactions, ZDOCK.

INTRODUCTION
In silico is an expression used to mean "performed on computer or via computer simulation." The phrase was coined in 1989 as an allusion to the Latin phrase *In vivo*, *in vitro*, and *in situ*, which are commonly used in biology.[1] *In silico* research in medicine is thought to have the potential to speed the rate of discovery while reducing the need for expensive lab work and clinical trials. One way to achieve this is by producing and screening drug candidates more effectively. In 2010, for example, using the protein docking algorithm EA Dock by researchers found potential inhibitors to an enzyme associated with cancer activity *in silico*.[2] Fifty percent of the molecules were later shown to be active inhibitors *in vitro*. This approach differs from use of expensive high-throughput screening (HTS) robotic labs to physically test thousands of diverse compounds a day often with an expected hit rate on the order of 1% or less with still fewer expected to be real leads following further testing drug discovery.

Antimicrobial peptides (also called host defense peptides) are part of the innate immune response and are found among all classes of life. These peptides are potent, broad spectrum antibiotics which demonstrate potential as novel therapeutic agents. Antimicrobial peptides have been demonstrated to kill Gram negative and Gram positive bacteria, enveloped viruses, fungi and even transformed or cancerous cells.[3] Antimicrobial peptides are a unique and diverse group of molecules, which are divided into subgroups on the basis of their amino acid composition and structure.[4] [5]

Antimicrobial peptides are originally identified in insects; they have subsequently been extracted from plants, crustaceans, ascidians and vertebrates.[6] Amphibian skin has proved to be an especially rich source of such peptides, which form a remarkably heterogeneous ensemble with a broad spectrum of antimicrobial activity and little sequence similarity.[7] The peptides are normally stored in the dermal glands of frogs and toads, and are released into skin secretions in a holocrine fashion upon stress or injury, acting as the first line of defence against invading pathogens.[8,9,10] Synthesis of antimicrobial peptides in the skin of *Rana esculenta* was demonstrated to be stimulated by micro-organisms, providing *in vivo* evidence for the induction of defence peptides in a vertebrate.[11]

The improving trend of biology has increasingly turned into a data-rich science, the need for storing and communicating large data sets grown tremendously. The obvious examples are the nucleotide sequences, the protein sequences, and the 3D structural data produced by X-ray crystallography and macromolecular NMR. A new field of science dealing with issues, challenges and new possibilities created by this database has emerged: bioinformatics.[12] [14]

Ebola virus one of two members of the family of filo viruses, causes a severe hemorrhagic fever with 50–90%

human mortality. That no vaccines or treatments are yet available combined with the frequent re-emergence of the virus, its high prevalence among wildlife, and ease of importation of the virus make it a significant public health concern. A team of researchers from the Scripps Research Institute, has recently determined the crystal structure of an oligomeric glycoprotein from the viral surface in complex with a rare antibody derived from a human survivor. The crystal structure also reveals that most of GP is shielded by a thick cloak of carbohydrate and identifies the very few sites left exposed and available for antibody binding, making this structure suitable as a template for vaccines and antibodies to target these newly revealed slits in Ebola virus cloak.[13]

MATERIALS AND METHODS

Ligand Peptide Collection

An Antimicrobial Peptide Database (APD) has been established based on an extensive literature search. It contains detailed information for 525 peptides (498 antibacterial, 155 antifungal, 28 antiviral and 18 antitumor). It also provides statistical data for a selected group of or all the peptides in the database. APD is a useful tool for studying the structure–function relationship of antimicrobial peptides. The database can be accessed via a web-based browser at the URL: http://aps.unmc.edu/AP/main.html.[6]

The peptides for the study were selected based on the following criteria: Short chain peptides of low molecular weight, Peptides belonging to same family, Peptide those are of easy for synthesis, Peptides without structure in PDB (Protein Data Base), Peptides whose antiviral and antifungal property is unknown.

The above criteria very well suits for the peptides belonging to Temporin family (Table:1). Nine types of Temporins were identified from the frog skin belonging to *Rana sp*. They are a family of small linear antibiotic peptides with exciting biological properties. Nine type of temporin L peptide sequence were collected from the available database.

Table: 1 List of Temporins with Physicochemical and Functional Properties

APD ID	NAME AND CLASS	SEQUENCE	SOURCE	PROPERTIES			ACTIVITY
				NET CHARGE	HYDROPHOBIC RATIO	LENGTH	
AP00104	Temporin-1Ca	FLPFLAKITGVL	Green frog *Rana clamitan*, North America.	2	69%	13	
AP00105	Temporin-1Cb	FLPLFASLIGKLL	Green frog *Rana clamitan*, North America.	2	69%	13	Anti-gram+
AP00106	Temporin-1Cc	FLPFLASLLTKVL	Green frog *Rana clamitan*, North America.	2	69%	13	_
AP00107	Temporin-1Cd	FLPFLASLLSKVL	Green frog *Rana clamitan*, North America.	2	69%	13	Anti-gram+
AP00108	Temporin-1Ce	FLPFLATLLSKVL	Green frog *Rana clamitan*, North America.	2	69%	13	Anti-gram+
AP00109	Temporin-1La	VLPLISMALGLL	*Rana luteiventris*, North America.	2	69%	13	Anti-gram+
AP00110	Temporin-1Lb	NFLGTLINAKKIM	*Rana luteiventris*, North America.	3	57%	14	Anti-gram+
AP00111	Temporin-1Lc	FLPILINLIHKLL	*Rana luteiventris*, North America.	2	64%	14	Anti-gram+
AP00112	Temporin-1P	FLPIVGKLLSGLL	*Rana pipiens*, North America.	2	61%	13	Anti-gram+

Target Protein Collection

The Protein Data Bank (PDB) is a database containing experimentally determined three-dimensional structures of proteins, nucleic acids and other biological macromolecules, with approximately 8000 entries. The protein data bank or (PDB) is primary database (www.rcsb.org/pdb**).**[14] The protein crystal structure with appropriate physiological properties (Table:2) VP35 (Fig: 1) with PDB entry 4IBK (Viral) was collected from PDB. The viral protein 4IBK is multifunctional in nature and is responsible for pathogenesis. The proteins were chosen as the target so as to prove the efficiency of the antibacterial peptides with antifungal and antiviral property.

Table: 2 Properties of Target

PDB ID	NAME	LENGTH	RESOLUTION	MOLECULAR WEIGHT	ORGANISM	FRAGMENT
4IBK (viral protein)	Ebola Virus VP35 Bound to small Molecule	129	1.85(Å)	29706.20	Ebola virus	Unp residues 215-340

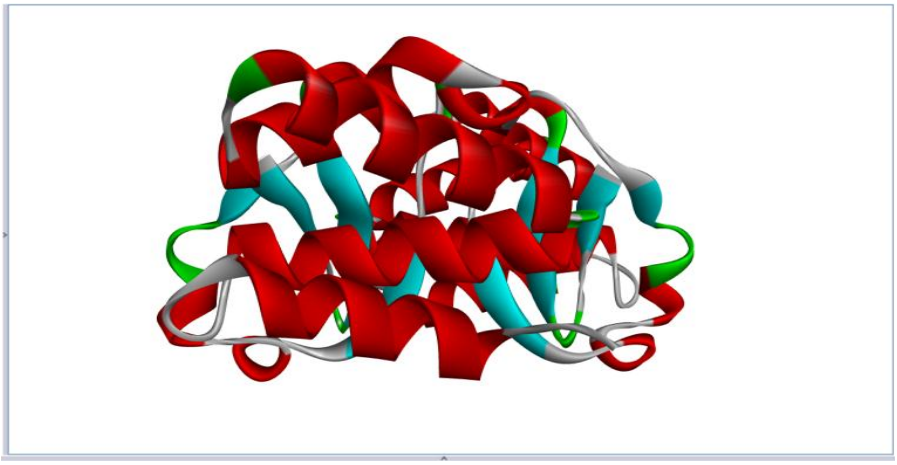

Figure: 1 Viral Target 4IBK

Construction of Ligand Structure

Temporins of nine types Temporin 1 Ca, 1Cb, 1 Cc, 1 Cd, 1 Ce, 1 La, 1 Lb, 1 Lc, 1P lacks structure in PDB and so the structure was constructed in Discovery Studio 3.5 demo version using the macromolecules tool kit options build and edit protein.

Preparation of Targets and Ligands

The X-ray crystal structure of viral target 4IBK was prepared for docking studies by applying forcefield and minimization.

Simulation by Force field

The viral target protein VP35 with PDB entry 4IBK containing 129 amino acid residues at a resolution of 1.85 was taken and molecular simulation was done using forcefield application. Also all the nine peptide ligand structures were also applied with forcefield and prepared for docking.[15]

Energy Minimization

The selected peptides and target protein structures were carried out with energy minimization by applying steepest descent method followed by conjugant gradient method until the convergence gradient was satisfied.[15]

Protein – Peptide Docking

For protein-peptide docking studies, we employed the dock proteins protocol (Discovery Studio) to generate a set of possible configurations for the Temporin peptides and targets complexes. The ZDOCK protocol is used for docking the Temporin peptides and targets. Zdock is a rigid-body docking algorithm using a Fast Fourier Transform (FFT) to perform an exhaustive six-dimensional search in the translational and rotational space between the two molecules. Each protein is projected into a three-dimensional grid and different values are assigned to the cells of the grid, representing the surface or the interior of the molecules. The rotational search sampling grid can use a 15 degree grid which samples a total of 2000 docked poses, or a 6 degree grid which samples a total of 54,000 poses for more accurate results.[15] Zdock searches orientational space by rotating the ligand around its geometric center with the receptor protein kept fixed in space. For each sampled angle only the ligand translation corresponding to the best geometric match between the two proteins is retained. As part of the ZDOCK protocol, the ZRANK function is used to rerank the docked poses. The obtained configurations for the complex are ranked based on a scoring function combining shape complementarity, desolvation energy, and electrostatics.

In the initial stage of ZDOCK, the Temporin peptides and the targets were treated as rigid bodies and all six rotational and translational degrees of freedom are fully explored with scoring functions that are tolerant to conformational changes. No contact information was used, which can filter hits or block residues during the search. An evenly distributed Euler angular step of 15 degree was used for rotational search, which results in 2000 poses.

RESULTS AND DISCUSSION

Construction of Ligand Structure of Temporins Peptide

The structure of temporins collected from the Antimicrobial Peptide Database was constructed using the edit protein tool available with the Discovery studio software. All the bonds and functional groups were represented in stick format. The drawn structures of all the peptides were shown below (Fig: 2 -10)

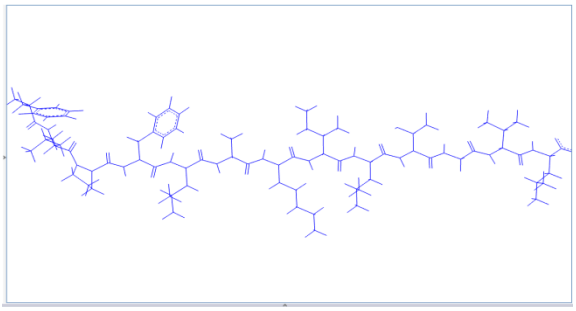

Fig: 2 AP00104 (TEMPORIN 1Ca)

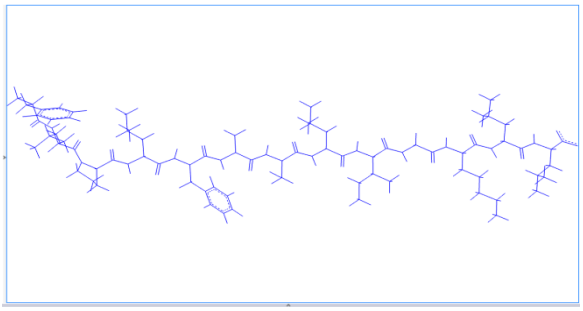

Fig: 3 AP00105 (TEMPORIN1Cb)

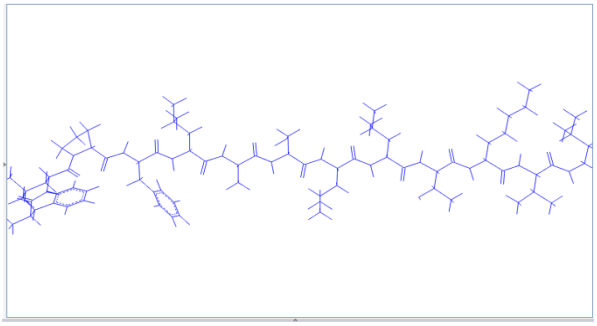

Fig: 4 AP00106 (TEMPORIN 1Cc)

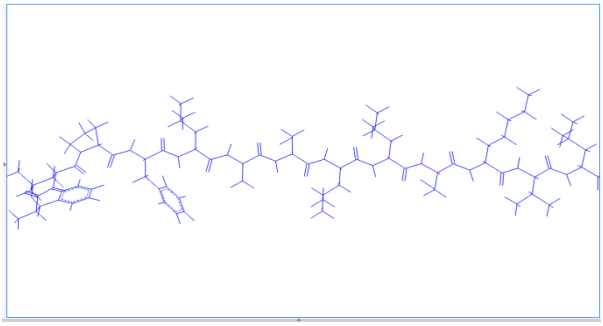

Fig: 5 AP00107 (TEMPORIN 1Cd)

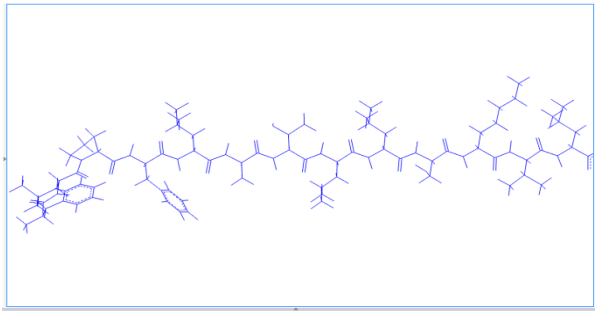

Fig: 6 AP00108 (TEMPORIN 1Ce)

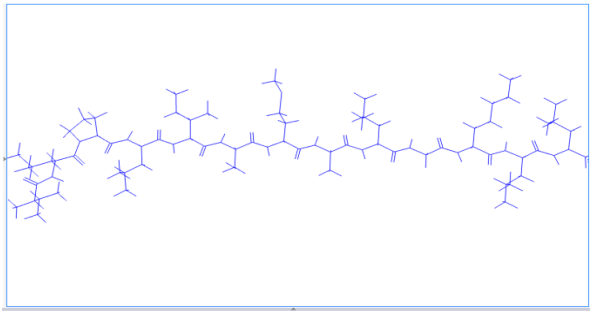

Fig: 7 AP00109 (TEMPORIN 1La)

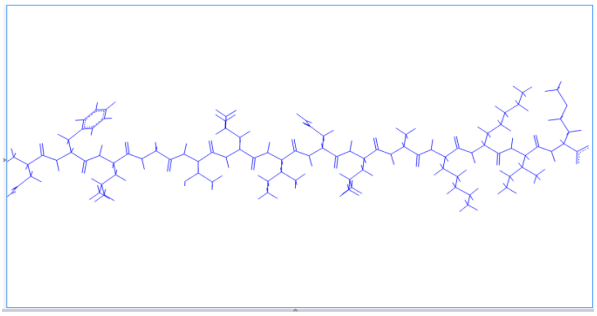

Fig: 8 AP00110 (TEMPORIN 1Lb)

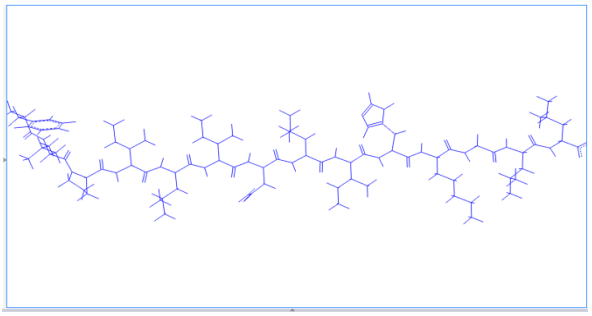

Fig: 9 AP00111 (TEMPORIN 1Lc)

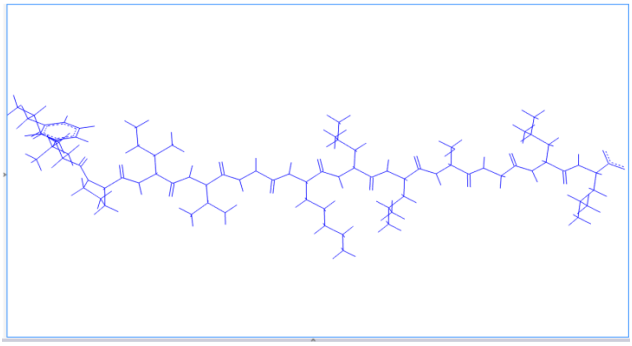

Fig: 10 AP00112 (TEMPORIN 1P)

Force field and Minimization

The ligands and targets were applied with forcefield and minimization for perfect docking poses. The results of forcefield and minimization applications of ligands and target were shown in Table: 3 respectively. The applied forcefield was calculated from the initial RMS gradient to final RMS gradient.

Table: 3 Force Field and Minimization Results of Ligands and Targets

NAME	FORCE FIELD	INITIAL POTENTIAL ENERGY (kcal/mol)	POTENTIAL ENERGY (kcal/mol)	VANDER WAALS ENERGY (kcal/mol)	INITIAL RMS GRADIENT (kcal/(mol x Angstrom))	FINAL RMS GRADIENT (kcal/(mol x Angstrom))	MINIMIZATION CYCLES	EXECUTION TIME
MINIMIZATION RESULTS OF LIGANDS TEMPORINS								
AP00104	CHARMm	2539.11477	-367.94342	-53.116559	498.31311	0.09650	849	00.00.21
AP00105	CHARMm	2552.22174	-468.49646	-43.71824	505.57530	0.09315	1496	00.00.18
AP00106	CHARMm	2145.67480	-458.54569	-46.07867	422.03055	0.09734	1194	00.00.17
AP00107	CHARMm	2102.87457	-452.36437	-41.50110	424.75403	0.09575	1195	00.00.17
AP00108	CHARMm	2140.87938	-469.55602	-43.31476	422.04550	0.09807	1328	00.00.17
AP00109	CHARMm	2555.53448	-463.34254	-42.91614	512.15410	0.09847	1444	00.00.18
AP00110	CHARMm	1941.72042	-640.20826	-49.24204	419.42118	0.09913	1482	00.00.18
AP00111	CHARMm	91505.79759	-464.27398	-64.41367	57136.26165	0.09840	1106	00.00.18
AP00112	CHARMm	2763.984220	-381.45146	-50.70555	518.48023	0.09998	903	00.00.17
MINIMIZATION RESULTS OF TARGET 4IBK								
4IBK	CHARMm	-5599.39539	-14871.16739	-1723.9215	319.68858	0.28390	500	00.01.25

Zdock (Protein-Protein Docking Of Viral Target With Ligands)

The protein peptide docking was performed for the 4IBK with all the ligands. This docking approach generated 2000 protein poses. The best protein pose was selected for analyzing the binding mode, aminoacid residues, Zrank and Zscore and the results were illustrated in Table 4.

Table: 4 The docking results of temporin ligands against 4IBK

PEPTIDE AND PROTEIN	ZDOCK SCORE	ZRANK SCORE	HBONDS FORMATION AMINOACID	TIME
AP00104 & 4IBK	40.04	-108.911	A:THR335,B:THR335	06:43:22
AP00105 & 4IBK	39.83	-123.328	-	06:22:22
AP00106 & 4IBK	40.67	-105.73	A:SER253	13:30:53
AP00107 & 4IBK	42.93	-103.917	PRO292	07:01:21
AP00108 & 4IBK	46.54	-112.451	A:SER253,B:ASP252, GLY333,ALA291,B:SER317	07:12:28
AP00109 & 4IBK	42.09	-99.484	THR335,THR335.	06:59:30
AP00110 & 4IBK	42.93	-111.108	B:LYS248,B:VAL294, B:SER253.	06:41:15
AP00111 & 4IBK	45.17	-120.508	PRO316,LYS319,LEU249.	20:42:10
AP00112 & 4IBK	38.65	-103.189	ASP252	06:51:05

From the above table the highest Zrank score of -123.32 was observed with the peptide AP00105, but the nature of bond formation and the aminoacids involved in bonding is unknown. The efficient interaction between the complex was observed with the AP00108 and 4IBK. The 13 length aminoacid peptide forms a complex with their five aminoacids serine, asparagine, glycine, alanine and again another serine in the peptide chain. The next highest energy interaction was observed with the peptide AP00110. All the studied peptides were shown to have good interaction with the viral target and apart from antibacterial property they also showed to have very good antiviral property. This type of temporins are reported from the work as potent promising antiviral agents.

When receptor-ligand pharmacophores based on the analogs of these molecules and the protein structures were constructed, the molecular features partially overlapped with the common features of solely ligand-based pharmacophores models based on FDA approved drugs. These previously identified FDA approved drugs with activity against Ebola were therefore docked into this protein. The antimalarial chloroquine and amodiaquine docked favorably in VP35.

The study of Raj.U *et al* deals with the evaluation of the inhibitory activity of flavonoids against the four selected Ebola virus receptor proteins, using *Insilco* studies. The viral proteins VP40, VP35, VP30 and VP24 were docked with small molecules obtained from flavonoid class and its derivatives, evaluated on the basis of energetics, stereo chemical considerations and pharmacokinetic properties to identify potential lead compounds. The results showed that both top ranking screened flavonoids i.e., Gossypetin and Taxifolin showed better docking scores of -81.2 and -85.67 respectively and binding energies in all the EBOV receptors when compared with that of the reported compound.

The binding efficiency of such pharmacophores are very much similar to that of the docked temporin ligands with the target. The chemical compounds available in market for the treatment of infection caused by Ebola virus was also evaluated for its docking efficiency against the viral protein VP35 and the promising interaction was reported.

So far most of the literatures review only the molecular docking approach of synthetic and natural compounds, and also many of the market available drugs. Peptides are small molecules that are emerging in the field of pharmacy with many potent applications. One of the major vision about peptides is in proving them as drugs with multiple applications. This part of the work helps the researchers and scientist of pharmaceutical field to better understand the peptide applications as antimicrobial drugs.

Compared to the reported literatures the docking score of the studied ligands were reported with high binding efficiency compared to the other compounds. This reveals the successful docking efficiency of peptides.

The docking pose and the hydrogen bond formation between the ligand and target was well shown from the below docked figures (Fig: 11 – 28).

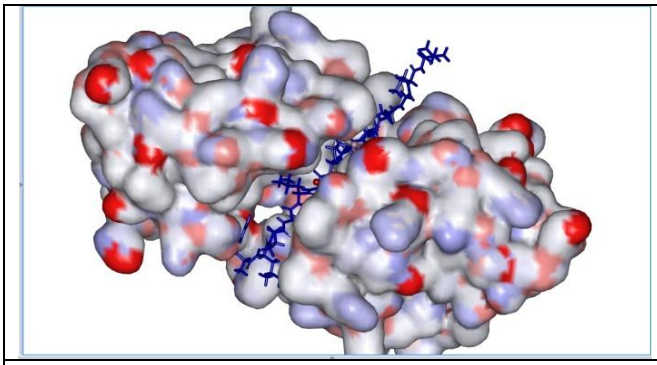

Fig: 11 FDocking pose of 4IBK - AP00104 complex

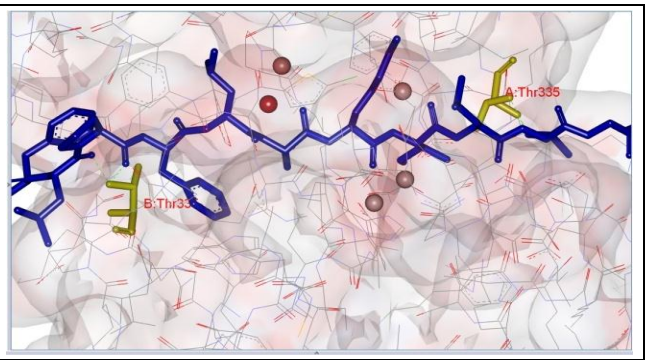

Fig: 12 Hydrogen Bond Formation of 4IBK-AP00104

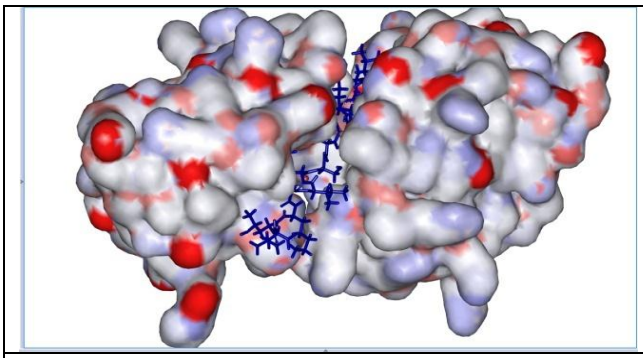

Fig:13 Docking pose of 4IBK - AP00105 complex

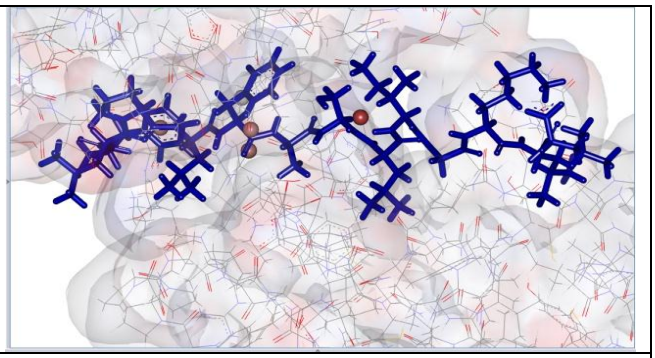

Fig:14 Hydrogen Bond Formation of 4IBK-AP00105

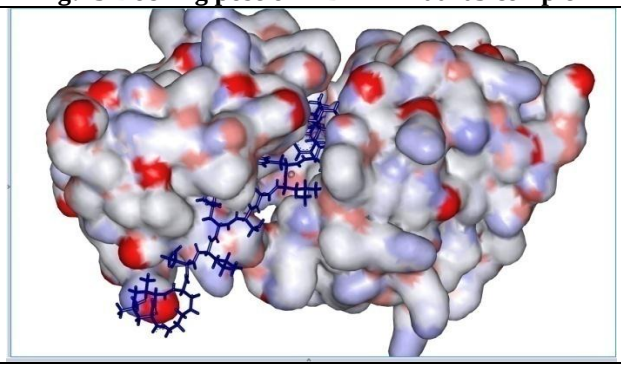

Fig:15 Docking pose of 4IBK - AP00106 complex

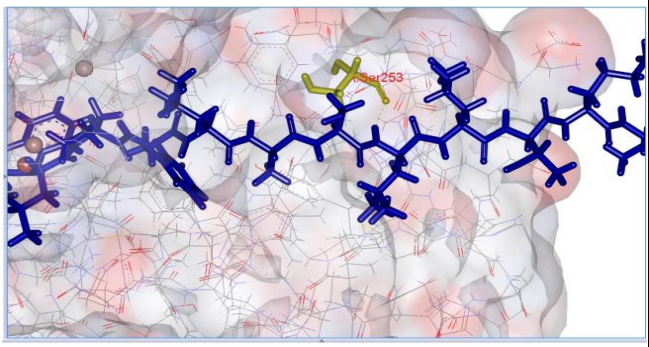

Fig:16 Hydrogen Bond Formation of 4IBK-AP00106

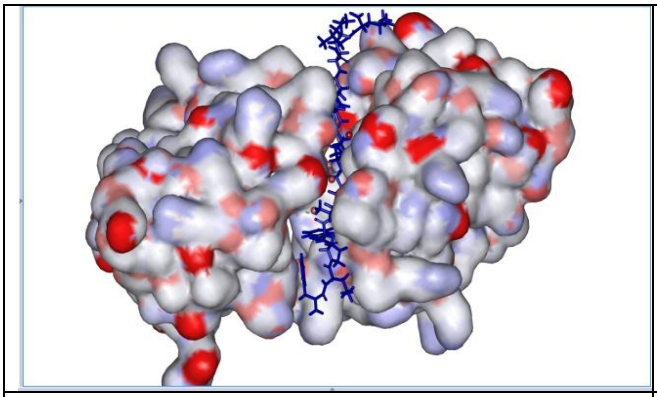

Fig:17 Docking pose of 4IBK - AP00107 complex

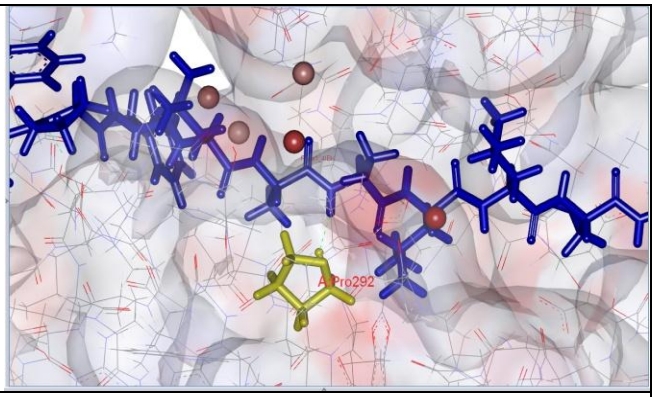

Fig:18 Hydrogen Bond Formation of 4IBK - AP00107 complex

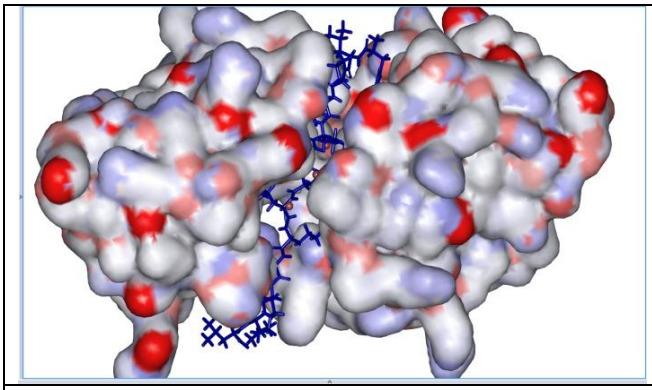

Fig:19 Docking pose of 4IBK - AP00108 complex

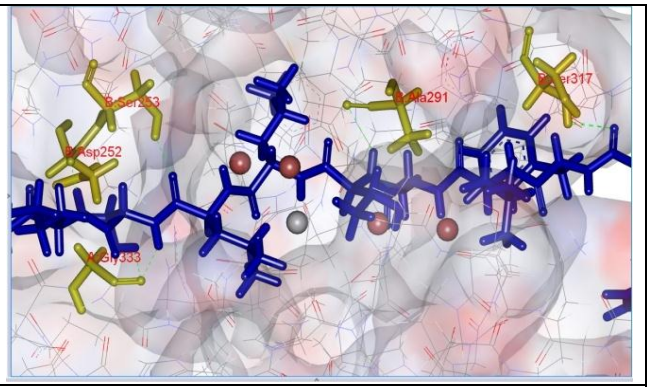

Fig:20 Hydrogen Bond Formation of 4IBK - AP00108 complex

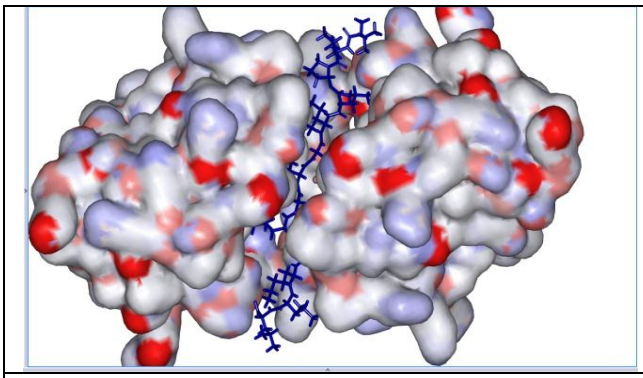

Fig:21 Docking pose of 4IBK - AP00109 complex

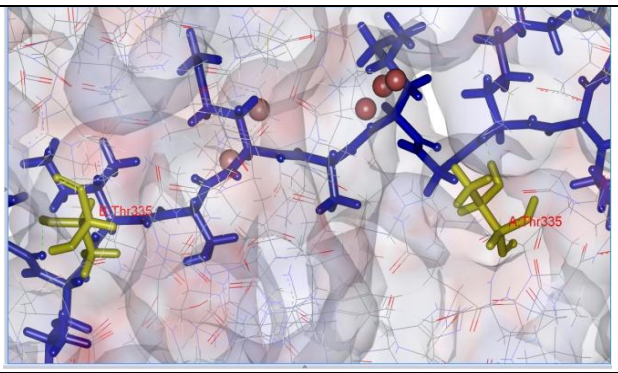

Fig:22 Hydrogen Bond Formation of 4IBK - AP00109 complex

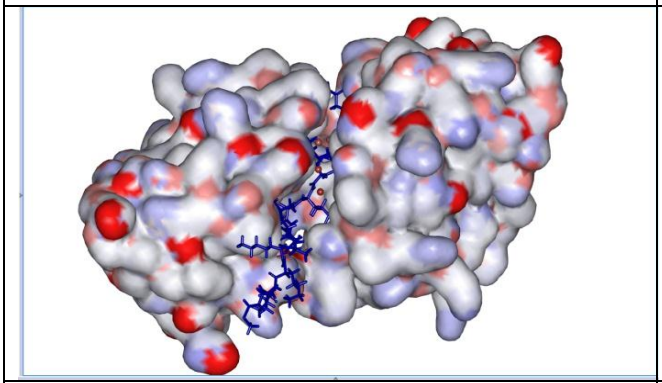

Fig:23 Docking pose of 4IBK - AP00110 complex

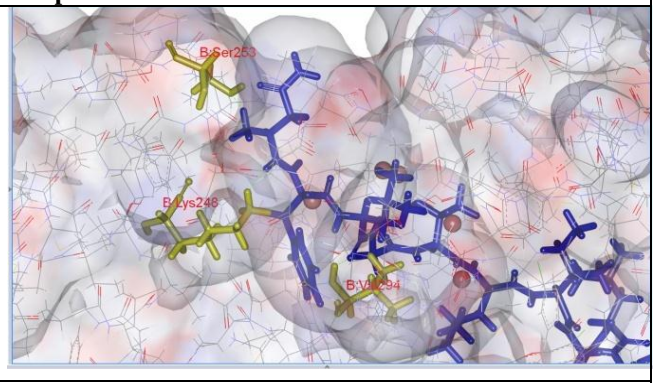

Fig:24 Hydrogen Bond Formation of 4IBK - AP00110 complex

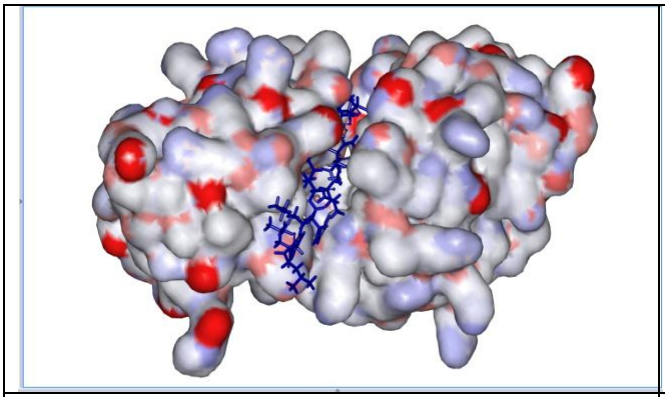

Fig:25 Docking pose of 4IBK - AP00111 complex

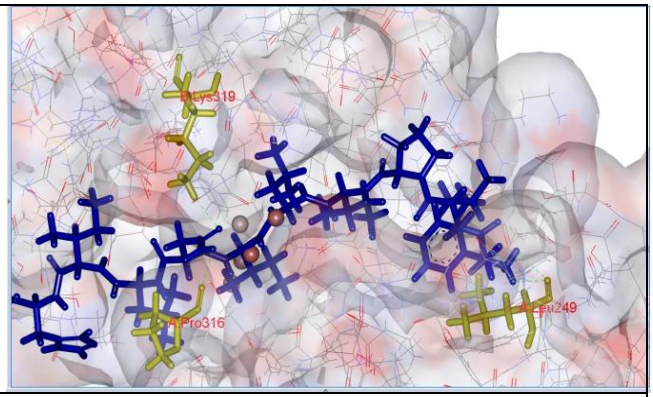

Fig:26 Hydrogen Bond Formation of 4IBK - AP00111 complex

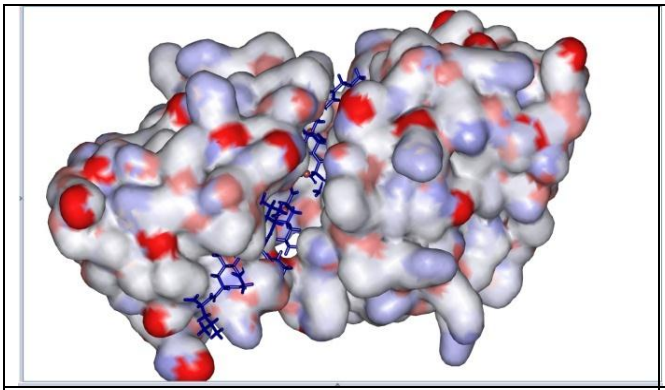

Fig:27 Docking pose of 4IBK - AP00112 complex

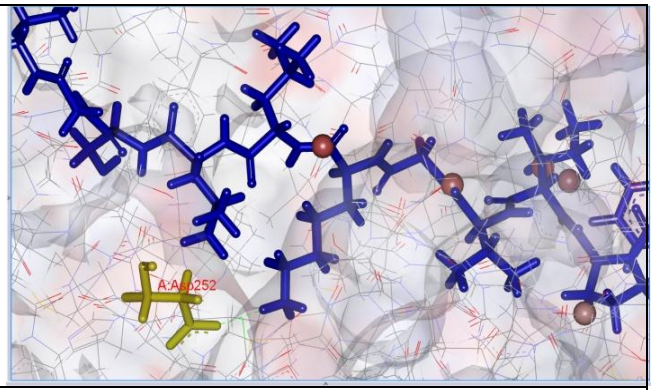

Fig:28 Hydrogen Bond Formation of 4IBK - AP00112 complex

CONCLUSION

In our present study, protein – peptide docking studies were performed to know the binding orientations of the 4IBK viral target from Ebola virus originated from *Trypanosoma cruzei* with the peptide ligands temporins of nine different types. These docking studies also provide in depth understanding of the interaction at their binding sites of peptide groups and receptor sites. Both the targets were tightly bound to the nine different types of temporins with good score. The interaction was very much higher to the target than the previously reported docking studies. This study paves a way for the wet lab studies of temporins with antiviral property.

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