Advanced Techniques in Biology & Medicine

Rao et al., Adv Tech Biol Med 2013, 1:1
DOI: 10.4172/2379-1764.1000102

Research Article Open Access

Conserved HIV Wide Spectrum Antipeptides-A Hope for HIV Treatment

Balaji S Rao, Krishna Kant Gupta, Suchitra Kumari, Ankit Gupta and K Pujitha*

Department of Bioinformatics, NTHRYS Biotech Labs, Hyderabad, 500044, Andhra Pradesh, India

Abstract

Antipeptide or inhibiting peptide aims to prevent virus/coreceptor interaction. HIV inhibting peptide dataset collected from HIPdb database was used in this study. There are 110 HIV inhibiting peptide is present in HIPdb database. Multiple Sequence Alignment (MSA) of all total 110 antipeptide has been performed and got some best conserved antipeptides. Next, prediction of antigenicity method was used for finding the maximum antigenicity out of the 14 conserved inhibiting peptides. All peptides were screened for hydrophobicity as low hydrophobicity induces humoral mediated immunity. Afterwards, Antimicrobial Peptide Prediction (AMP) and its classification was performed. In this study, PWQGGRRKFR and KYRRFRWKFK are the promising HIV antipeptide for AIDS treatment. This study will help scientists to promote research for better understanding of HIV treatment in forms of drug and vaccine development.

Keywords: HIV; HIPdb; Multiple sequence alignment; Antigenicity; AMP

Introduction

Acquired immunodeficiency syndrome (AIDS) is caused by Human immunodeficiency virus (HIV) in which progressive failure of the human immune system causes life-threatening opportunistic infection or cancer. HIV-1 virus multiplies only inside the human body. HIV-1 entry into cells involves formation of a complex between gp120 of the viral envelope glycoprotein (Env), a host receptor (CD4), and a chemokine coreceptors usually CCR5 or CXCR4 [1]. gp120 contains the transmembrane protein gp41 and is derived from polyprotein gp160. Polyprotein gp 160 is encoded by env gene, which is found in all retrovirus [2]. Antiretroviral drugs, peptides have demonstrated potential to inhibit the Human immunodeficiency virus (HIV) [3]. Antipeptide or inhibiting peptide aim to preventing virus/coreceptor interaction by binding either virus envelope proteins or host proteins. The main cause of HIV infection starts with the interaction of exterior envelop of the viral protein gp120 with the chemokine receptors of CD4, the target cell. CD4 not only contributes in the viral attachment but also triggers some conformational changes in the HIV envelop that helps in the recognition of various chemokine receptors and leads to the membrane fusion. These conformational changes leads to the increase in the sensitivity of gp120 loops, release number of gp120 proteins and formation of chemokine receptor site along with its exposure of to gp120 molecules. It also leads to the formation of epitopes of neutralizing antibodies which blocks chemokine receptor binding [4].

Materials and Methods

Dataset for HIV antipeptide

HIV antipeptide dataset collected from HIPdb [3] database is freely available HIV antipeptide database. It is a manually curated database of experimentally validated HIV inhibitory peptides targeting various steps or proteins involved in the life cycle of HIV like fusion, integration, reverse transcription, etc. It is newly introduced database for HIV antipetides, which was used for collection of viral entry antipeptides. There are total 110 large dataset of antipeptide having different source and cell lines (Table 1) in this database. Out of 110 peptides, 47, 41, 5, 5, 4, 2, 2, 1, 1 inhibiting peptides were taken from GB virus, alpha-anti trypsin, Apelin, gp120, gp41, synthetic, RhoA, Tat; Dynein sources, respectively (Figure 1).

Multiple sequence alignment (MSA)

MSA were performed for all 110 inhibiting peptides using clustalW [5] and validate its result with T-Coffee [6] and Muscle [7]. Conserved patches of antipeptides for different cell lines and sources were collected from the clustalW result.

Prediction of antigenicity of conserved peptides and analysis

Low hydrophobic sequence evokes the production of peptide antibodies. The antigenicity analysis assists in the selection of low hydrophobic sequences from the one with the highest calculated antigenic potential. All the antipeptides were screened for their antigenicity using VaxiJen [8]. The threshold value for antigenicity is 0.4 in VaxiJen. Based on their conserved nature and antigenicity, few lists of peptides are proposed for their wide spectrum activity. The antigenicity is the ability of a compound to bind with antibodies and with cells of immune system.

Prediction of hydrophobicity of conserved peptides and analysis

Peptide property calculator [9] was used to calculate the percentage of hydrophobicity of all antipeptides. By using this tool, we got chemical formulae, molecular weight, hydrophobicity, hydrophilic, isoelectric point for analysis of inhibiting peptides. We analyzed our whole 11 inhibiting peptide with derived ID from HIPDB database inhibiting from Peptide Property Calculator.

Prediction of antiviral and antimicrobial activity of 11 peptides

Conserved Antimicrobial Peptide (CAMP) [10] is used for

***Corresponding author:** K. Pujitha, Department of Bioinformatics, NTHRYS Biotech Labs, Hyderabad, 500044, Andhra Pradesh, India, Fax: 914027610748; E-mail: coordinator@nthrys.org, bioinfo@nthrys.org

Received February 25, 2013; **Accepted** March 07, 2013; **Published** March 11, 2013

Citation: Rao BS, Gupta KK, Kumari S, Gupta A, Pujitha K (2013) Conserved HIV Wide Spectrum Antipeptides-A Hope for HIV Treatment. Adv Tech Biol Med 1: 102. doi:10.4172/2379-1764.1000102

S.No.	ID	SEQUENCE	LENGTH	SOURCE	CELL LINE
1.	HIP1153	FVFLM	5	alpha1-antitrypsin	NA
2.	HIP1159	EFVFLM	6	alpha1-antitrypsin	NA
3.	HIP1158	PFVFLE	6	alpha1-antitrypsin	NA
4.	HIP1160	PEVFLM	6	alpha1-antitrypsin	NA
5.	HIP1157	PFVYLI	6	alpha1-antitrypsin	NA
6.	HIP1154	PFVFLM	6	alpha1-antitrypsin	NA
7.	HIP1161	PFVFLR	6	alpha1-antitrypsin	NA
8.	HIP1155	KPFVFLM	7	alpha1-antitrypsin	NA
9.	HIP1156	NKPFVFLM	8	alpha1-antitrypsin	NA
10.	HIP944	GRKKRRQRRR	10	Tat	P4-R5 MAG
11.	HIP177	PRLSHKGPMPF	11	Apelin	NP-2/CD4
12.	HIP189	RPRLSHKGPMPF	12	Apelin	NP-2/CD4
13.	HIP260	QRPRLSHKGPMPF	13	Apelin	NP-2/CD4
14.	HIP951	PKSSWSDHEASSGV	14	RhoA	TZM-bl
15.	HIP405	KFRRQRPRLSHKGPMPF	17	Apelin	NP-2/CD4
16.	HIP950	TDVILMCFSIDSPDSLENI	19	RhoA	TZM-bl
17.	HIP557	AEAIPMSIPPEVKFNKPFVF	20	alpha1-antitrypsin	P4-CCR5
18.	HIP661	LEAIPMSIPPEVKFNKPAVF	20	alpha1-antitrypsin	P4-CCR5
19.	HIP662	LEAIPMSIPPEVKFNKPFAF	20	alpha1-antitrypsin	P4-CCR5
20.	HIP630	LAAIPMSIPPEVKFNKPFVF	20	alpha1-antitrypsin	P4-CCR5
21.	HIP663	LEAIPMSIPPEVKFNKPFVA	20	alpha1-antitrypsin	P4-CCR5
22.	HIP632	LEAAPMSIPPEVKFNKPFVF	20	alpha1-antitrypsin	P4-CCR5
23.	HIP628	KVINPEPIVEPFMSKPFALF	20	alpha1-antitrypsin	P4-CCR5
24.	HIP638	LEAIPCSIPPCVAFNKPFVF	20	alpha1-antitrypsin	P4-CCR5
25.	HIP635	LEAIPCSIPPCFAFNKPFVF	20	alpha1-antitrypsin	P4-CCR5
26.	HIP639	LEAIPCSIPPCVFFGKPFVF	20	alpha1-antitrypsin	P4-CCR5
27.	HIP637	LEAIPCSIPPCFLFGKPFVF	20	alpha1-antitrypsin	P4-CCR5
28.	HIP656	LEAIPMSIPPEVFFNKPFVF	20	alpha1-antitrypsin	P4-CCR5
29.	HIP642	LEAIPCSIPPCVGFGKPFVF	20	alpha1-antitrypsin	P4-CCR5
30.	HIP643	LEAIPCSIPPCVLFNKPFVF	20	alpha1-antitrypsin	P4-CCR5
31.	HIP640	LEAIPCSIPPCVFFNKPFVF	20	alpha1-antitrypsin	P4-CCR5
32.	HIP646	LEAIPMCIPPECAFNKPFVF	20	alpha1-antitrypsin	P4-CCR5
33.	HIP652	LEAIPMSIPPEFLFGKPFVF	20	alpha1-antitrypsin	P4-CCR5
34.	HIP651	LEAIPMSIPPEAKFNKPFVF	20	alpha1-antitrypsin	P4-CCR5
35.	HIP659	LEAIPMSIPPEVKFNAPFVF	20	alpha1-antitrypsin	P4-CCR5
36.	HIP650	LEAIPMSIPPAVKFNKPFVF	20	alpha1-antitrypsin	P4-CCR5
37.	HIP660	LEAIPMSIPPEVKFNKAFVF	20	alpha1-antitrypsin	P4-CCR5
38.	HIP634	LEAIPASIPPEVKFNKPFVF	20	alpha1-antitrypsin	P4-CCR5
39.	HIP664	LEAIPMSIPPEVKFNKPFVF	20	alpha1-antitrypsin	P4-CCR5
40.	HIP648	LEAIPMSIAPEVKFNKPFVF	20	alpha1-antitrypsin	P4-CCR5
41.	HIP658	LEAIPMSIPPEVKFAKPFVF	20	alpha1-antitrypsin	P4-CCR5
42.	HIP647	LEAIPMSAPPEVKFNKPFVF	20	alpha1-antitrypsin	P4-CCR5
43.	HIP633	LEAIAMSIPPEVKFNKPFVF	20	alpha1-antitrypsin	P4-CCR5
44.	HIP654	LEAIPMSIPPEVAFAKPFVF	20	alpha1-antitrypsin	P4-CCR5
45.	HIP657	LEAIPMSIPPEVKANKPFVF	20	alpha1-antitrypsin	P4-CCR5
46.	HIP655	LEAIPMSIPPEVAFNKPFVF	20	alpha1-antitrypsin	P4-CCR5
47.	HIP649	LEAIPMSIPAEVKFNKPFVF	20	alpha1-antitrypsin	P4-CCR5
48.	HIP636	LEAIPCSIPPCFAFNKPFVF	20	alpha1-antitrypsin	P4-CCR5
49.	HIP645	LEAIPCSIPPEFLFGKPFVF	20	alpha1-antitrypsin	P4-CCR5
50.	HIP947	GCKKYRRFRWKFKGKFWFWG	20	Synthetic	TZM-bl
51.	HIP948	GKKYRRFRWKFKFGKWFWFG	20	Synthetic	TZM-bl
52.	HIP686	PTGERVWDRGNVTLLCDCPN	20	GB virus C gE2	TZM-bl
53.	HIP694	RIPTGERVWDRGNVTLLCDC	20	GB virus C gE2	TZM-bl
54.	HIP728	WDRGNVTLLCDCPNGPWVWV	20	GB virus C gE2	TZM-bl
55.	HIP600	GPWVWVPAFCQAVGWGDPIT	20	GB virus C gE2	TZM-bl
56.	HIP712	TLLCDCPNGPWVWVPAFCQA	20	GB virus C gE2	TZM-bl
57.	HIP631	LCDCPNGPWVWVPAFCQAVG	20	GB virus C gE2	TZM-bl
58.	HIP569	DCPNGPWVWVPAFCQAVGWG	20	GB virus C gE2	TZM-bl
59.	HIP684	PNGPWVWVPAFCQAVGWGDP	20	GB virus C gE2	TZM-bl
60.	HIP730	WVWVPAFCQAVGWGDPITHW	20	GB virus C gE2	TZM-bl

61.	HIP592	GAPASVLGSRPFDYGLKWQS	20	GB virus C gE2	TZM-bl
62.	HIP724	VSVTCVWGSVSWFASTGGRD	20	GB virus C gE2	TZM-bl
63.	HIP702	SWFASTGGRDSKIDVWSLVP	20	GB virus C gE2	TZM-bl
64.	HIP699	SKIDVWSLVPVGSASCTIAA	20	GB virus C gE2	TZM-bl
65.	HIP717	VGSASCTIAALGSSDRDTVV	20	GB virus C gE2	TZM-bl
66.	HIP665	LGSSDRDTVVELSEWGVPCV	20	GB virus C gE2	TZM-bl
67.	HIP580	ELSEWGVPCVTCILDRRPAS	20	GB virus C gE2	TZM-bl
68.	HIP704	TCILDRRPASCGTCVRDCWP	20	GB virus C gE2	TZM-bl
69.	HIP563	CGTCVRDCWPETGSVRFPFH	20	GB virus C gE2	TZM-bl
70.	HIP585	ETGSVRFPFHRCGTGPRLTK	20	GB virus C gE2	TZM-bl
71.	HIP691	RCGTGPRLTKDLEAVPFVNR	20	GB virus C gE2	TZM-bl
72.	HIP681	PFDYGLKWQSCSCRANGSRI	20	GB virus C gE2	TZM-bl
73.	HIP575	DLEAVPFVNRTTPFTIRGPL	20	GB virus C gE2	TZM-bl
74.	HIP716	TTPFTIRGPLGNQGRGNPVR	20	GB virus C gE2	TZM-bl
75.	HIP599	GNQGRGNPVRSPLGFGSYTM	20	GB virus C gE2	TZM-bl
76.	HIP701	SPLGFGSYTMTKIRDSLHLV	20	GB virus C gE2	TZM-bl
77.	HIP711	TKIRDSLHLVKCPTPAIEPP	20	GB virus C gE2	TZM-bl
78.	HIP614	KCPTPAIEPPTGTFGFFPGV	20	GB virus C gE2	TZM-bl
79.	HIP708	TGTFGFFPGVPPINNCMPLG	20	GB virus C gE2	TZM-bl
80.	HIP685	PPINNCMPLGTEVSEALGGA	20	GB virus C gE2	TZM-bl
81.	HIP707	TEVSEALGGAGLTGGFYEPL	20	GB virus C gE2	TZM-bl
82.	HIP598	GLTGGFYEPLVRRCSELMGR	20	GB virus C gE2	TZM-bl
83.	HIP566	CSCRANGSRIPTGERVWDRG	20	GB virus C gE2	TZM-bl
84.	HIP723	VRRCSELMGRRNPVCPGYAW	20	GB virus C gE2	TZM-bl
85.	HIP696	RNPVCPGYAWLSSGRPDGFI	20	GB virus C gE2	TZM-bl
86.	HIP669	LSSGRPDGFIHVQGHLQEVD	20	GB virus C gE2	TZM-bl
87.	HIP565	CRANGSRIPTGERVWDRGNV	20	GB virus C gE2	TZM-bl
88.	HIP560	ANGSRIPTGERVWDRGNVTL	20	GB virus C gE2	TZM-bl
89.	HIP603	GSRIPTGERVWDRGNVTLLC	20	GB virus C gE2	TZM-bl
90.	HIP593	GERVWDRGNVTLLCDCPNGP	20	GB virus C gE2	TZM-bl
91.	HIP698	RVWDRGNVTLLCDCPNGPWV	20	GB virus C gE2	TZM-bl
92.	HIP676	NVTLLCDCPNGPWVWVPAFC	20	GB virus C gE2	TZM-bl
93.	HIP729	WVPAFCQAVGWGDPITHWSH	20	GB virus C gE2	TZM-bl
94.	HIP677	PAFCQAVGWGDPITHWSHGQ	20	GB virus C gE2	TZM-bl
95.	HIP588	FCQAVGWGDPITHWSHGQNQ	20	GB virus C gE2	TZM-bl
96.	HIP687	QAVGWGDPITHWSHGQNQWP	20	GB virus C gE2	TZM-bl
97.	HIP608	HWSHGQNQWPLSCPQYVYGS	20	GB virus C gE2	TZM-bl
98.	HIP668	LSCPQYVYGSVSVTCVWGSV	20	GB virus C gE2	TZM-bl
99.	HIP693	RGNVTLLCDCPNGPWVWVPA	20	GB virus C gE2	TZM-bl
100.	HIP946	PKDGPSPGGTLMDLSERQEVS SVRSLSST	29	Dynein	HeLa
101.	HIP824	LVQPRGPRSGPGPWQGGRRK FRRQRPRLSHKGPMPF	36	Apelin	NP-2/CD4
102.	HIP963	CTRPNNNTRKSIRIQRGPGRAF VTIGKIGNMRQAHC	36	gp120	JY
103.	HIP958	YTSLIHSLIEESQNQQEKNEQEL LELDKWASLWNWF	36	gp41	PM-1
104.	HIP959	YTSLIHSLIEESQNQQEKNEQEL LELDKWASLANAA	36	gp41	PM-1
105.	HIP965	EINCTRPNNNTRKSIHIGPGRAF YTTGEIIGDIRQAHCNIS	41	gp120	JY
106.	HIP962	EINCTRPNNNTRKSIRIQRGPG RAFVTIGKIGNMRQAHCNIS	42	gp120	JY
107.	HIP964	ESVKITCARPYQNTRQRTPIGL GQSLYTTRSRSIIGQAHCNIS	43	gp120	JY
108.	HIP966	ESVVINCTRPNNNTRRRLSIGP GRAFYARRNIIGDIRQAHCNIS	44	gp120	JY
109.	HIP1016	WMEWDREINNYTSLIHSLIEES QNQQEKNEQELLELDKWASLWNWFR S	48	gp41	PM-1
110.	HIP953	WMEWDREINNYTSLIHSLIEESQ NQQEKNEQELLELDKWASLWNWFRS	48	gp41	PM-1

Table 1: HIV inhibiting peptide dataset in HIPdb database.

prediction of antimicrobial activity of our 11 peptides using the Support Vector Machine (SVM) [11] and Random Forest (RF) [12] algorithm. The category of AMPs has been analyzed from class AMP [13].

Results

The results of MSA of 14 antipeptides were visualized in Jalview. Figure 2 is showing the decamers antipeptides in jalview. Then we predicted antigenicity of all 14 inhibiting peptides through VaxiJen. All the 14 HIV antipeptides' antigenicity is shown in table 2. The final step is to predict the best HIV antipeptide among these 14 as shown in table 3. In our result, DRRPASCGTC, EESQNQQEKN, KYRRFRWKFK, SDRDTVVELS and KIRPSLHLVKC are showing hydrophobicity below 60%. These antipeptides have potential to inhibit HIV virus entry and they are able to induce humoral mediated immunity. In this study, DRRPASCGTC is showing minimum hydrophobicity with 20% and also having high antigenicity with 0.7480.

Discussion

To explore AMPs as drugs, it is essential to understand sequence-specificity relationship of Anti Microbial Peptides (AMPs). Two algorithms have been used for prediction of antibacterial, antifungal

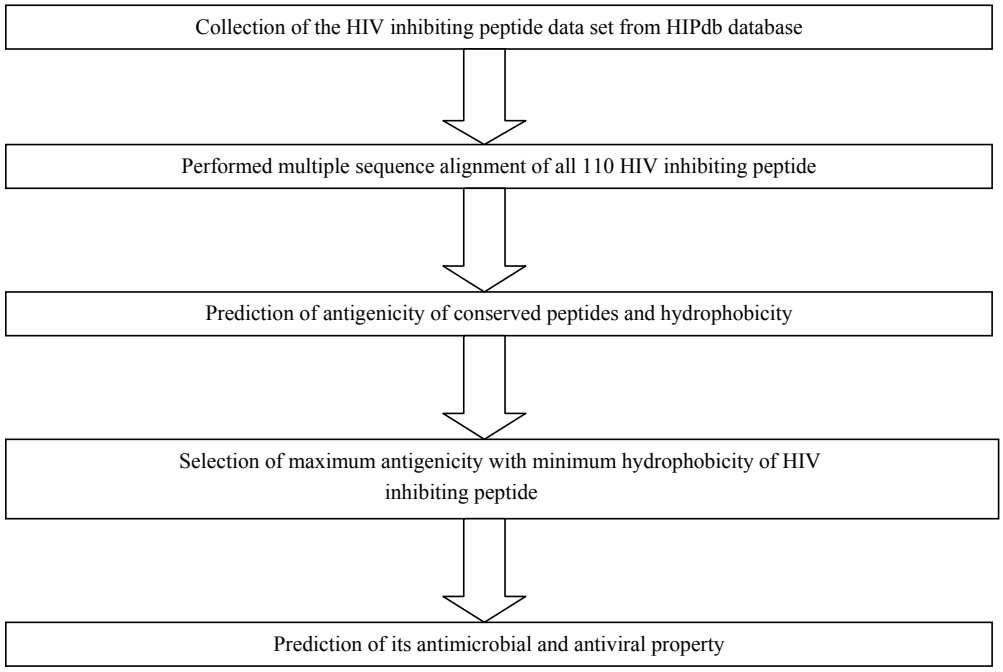

Figure 1: Overall block diagram of the methodology used.

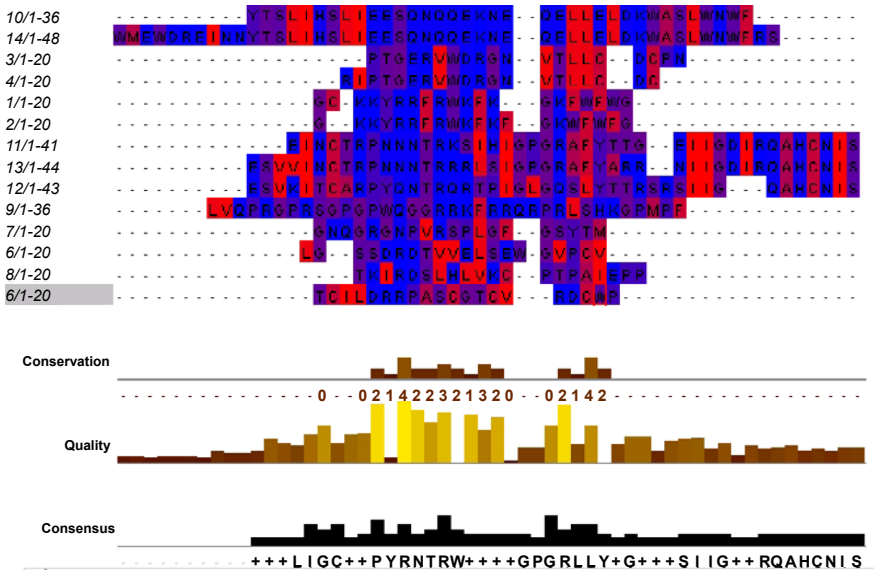

Figure 2: Jalview image of decamers inhibiting peptides.

and antiviral peptides based on their sequence composition (Tables 5-7). The individual inference about each 11 peptides is as follows:

EESQNQQEKN

For a peptide to be antigenic, it must be less hydrophobic and VaxiJen score should be more than 0.4. To our surprise, this peptide is predicted to be 0% hydrophobicity and has antigenic score of 0.5418. This peptide has a good probability of immunogenicity because it has molecular weight 1233.21 D, as per the rule the molecular weight has to be greater than 500D. As per CAMP analysis, this peptide is predicted to be AMP from both algorithms, SVM and Random Forest with probability of 0.69 and 0.55, respectively. This peptide has AMP category of antifungal activity from both algorithms predicted from classAMP and Random Forest with probability of 0.72 and 0.58 respectively. After AVBlast analysis of this peptide, it was found that this sequence is a part of "WQEWERKVDFLEENITALLEEAQIQQEKNMYELQK" sequence which has antiviral activity to Simian immunodeficiency Virus with 80% identity & E value of 1.3. Thus this conserved peptide has derived from gp41 sequence which is working as a potent viral entry inhibitor of HIV-1, SIVmac251 and SHIV89.6P [14]. This

S.No.	ID	Sequence	SOURCE	CELL LINE
1.	HIP947	GCKKYRRFRWKFKGKFWFWG	Synthetic	TZM-bl
2.	HIP948	GKKYRRFRWKFKFGKWFWFG	Synthetic	TZM-bl
3.	HIP686	PTGERVWDRGNVTLLCDCPN	GB virus C gE2	TZM-bl
4.	HIP694	RIPTGERVWDRGNVTLLCDC	GB virus C gE2	TZM-bl
5.	HIP665	LGSSDRDTVVELSEWGVPCV	GB virus C gE2	TZM-bl
6.	HIP704	TCILDRRPASCGTCVRDCWP	GB virus C gE2	TZM-bl
7.	HIP599	GNQGRGNPVRSPLGFGSYTM	GB virus C gE2	TZM-bl
8.	HIP711	TKIRDSLHLVKCPTPAIEPP	GB virus C gE2	TZM-bl
9.	HIP824	LVQPRGPRSGPGPWQGGRRKFRRQRPRLSHKGPMPF	Apelin	NP 2/CD4
10.	HIP958	YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF	gp41	PM-1
11.	HIP965	EINCTRPNNNTRKSIHIGPGRAFYTTGEIIGDIRQAHCNIS	gp120	JY
12.	HIP964	ESVKITCARPYQNTRQRTPIGLGQSLYTTRSRSIIGQAHCNIS	gp120	JY
13.	HIP966	ESVVINCTRPNNNTRRRLSIGPGRAFYARRNIIGDIRQAHCN IS	gp120	JY
14.	HIP1016	WMEWDREINNYTSLIHSLIEESQNQQEKNEQELLELDKWA SLWNWFRS	gp41	PM-1

Table 2: All the 14 HIV antipeptides' antigenicity.

Conserve Hiv Anti-Peptide Patches	Antigen (Threshold=0.4)	H*	HIPdb antipeptide	Antigen (Threshold=0.4)	H*	Source	Cell-Line
EESQNQQEKN (2times)	Overall Antigen Prediction=0.5418 (Probable Antigen)	0%	YTSLIHSLIEESQNQQEKN EQELLELDKWASLWNWF	-0.0022	36	gp41	PM-1
PTGERVWDRG (2 times)	Overall Antigen Prediction=0.2602 (Probable NON-Antigen)	30%	PTGERVWDRGNVTLLCDCPN	0.3447	35	GB Virus C gE2	TZM-bl
KYRRFRWKFK (2 times)	Overall Antigen Prediction=1.6162 (Probable Antigen)	60%	GCKKYRRFRWKFKGKFWFWG	1.2550	35	Synthetic	TZM-bl
PNNNTRKSIH	Overall Antigen Prediction=0.1556 (Probable NON-ANTIGEN)	20%	EINCTRPNNNTRKSIRIQR GPGRAFVTIGKIGNMRQAHCNIS	0.6019	31	gp120	JY
PNNNTRRRLS	Overall Antigen Prediction=0.3115 (Probable NON-ANTIGEN)	20%	ESVKITCARPYQNTRQRTP IGLGQSLYTTRSRSIIGQAHCNIS	0.613	34	gp120	JY
PYQNTRQRTP	Overall Antigen Prediction=0.1124 (Probable NON- Antigen)	20%	ESVKITCARPYQNTRQRTP IGLGQSLYTTRSRSIIGQAH CNIS	0.7910	28	gp120	JY
PWQGGRRKFR	Overall Antigen Prediction=0.1293	30%	LVQPRGPRSGPGPWQGGR RKFRRQRPRLSHKGPMPF	0.3389	39	Apelin	NP-2/CD4
RGNPVRSPLG	Overall Antigen Prediction=-0.2856 (Probable NON-ANTIGEN)	40%	GNQGRGNPVRSPLGFGSY TM	0.6627	30	GB Virus C gE2	TZM-bl
SDRDTVVELS	Overall Antigen Prediction=0.5728 (Probable Antigen)	30%	LGSSDRDTVVELSEWGVP CV	0.7667	40	GB Virus C gE2	TZM-bl
KIRDSLHLVKC	Overall Antigen Prediction=0.5728 (Probable Antigen)	45%	TKIRDSLHLVKCPTPAIEPP	0.1542	50	GB Virus C gE2	TZM-bl
DRRPASCGTC	Overall Antigen Prediction=0.7480 (Probable ANTIGEN)	20%	TCILDRRPASCGTCVRDC WP	1.3830	35	GB Virus C gE2	TZM-bl

Note: H* indicates hydrophobocity.

Table 3: The best HIV antipeptide.

Peptide Sequence	H**(%)	H*(%)	Others (%)	Chemical Formula	MW(Da)	pI	Piechart
KIRDSLHLVKC	45	36	18	C57H102N18O15S1	1311.60	9.67	
KYRRFRWKFK	60	30	10	C74H111N23O12	1514.83	12.27	
PNNNTRKSIH	30	20	50	C48H81N19O16	1180.28	11.66	
PNNNTRRRLS	30	20	50	C48H86N22O16	1227.34	12.80	
PTGERVWDRG	40	30	30	C50H77N17O16	1172.26	6.51	
PWQGGRRKFR	40	30	30	C58H90N22O12	1287.48	12.81	
RGNPVRSPLG	20	40	40	C44H77N17O13	1052.19	12.50	
SDRDTVVELS	40	30	30	C45H77N13O20	1120.17	3.88	
EESQNQQEKN	40	0	60	C47H76N16O23	1233.21	3.98	
DRRPASCGTC	30	20	50	C39H68N16O15S2	1065.19	8.23	
PYQNTRQRTP	20	20	60	C53H85N19O17	1260.36	11.15	

Note: H* indicates hydrophobicity; pI indicates isoelectric point; H** indicates hydrophilicity; MW indicates molecular weight. Piechart: Red indicates +vely charged hydrophilic residues; Blue indicates -vely charged hydrophilic residues;
Green indicates hydrophobic residues; Grey indicates others.

Table 4: Hydrophobicity of 11 conserved anti-peptides.

S. No.	Antipeptides	Class (SVM)	Probability (SVM)	Class (RF)	Probabilty (RF)
1	EESQNQQEKN	AMP	0.696	AMP	0.55
2	PTGERVWDRG	Non-AMP	0.706	AMP	0.706
3	KYRRFRWKFK	AMP	1.000	AMP	0.892
4	PNNNTRKSIH	AMP	0.913	Non-AMP	0.586
5	PNNNTRRRLS	AMP	0.589	Non-AMP	0.598
6	PYQNTRQRTP	Non-AMP	0.993	AMP	0.528
7	PWQGGRRKFR	AMP	0.981	AMP	0.81
8	RGNPVRSPLG	AMP	0.625	AMP	0.632
9	SDRDTVVELS	Non-AMP	0.959	AMP	0.624
10	KIRDSLHLVKC	AMP	0.588	AMP	0.712
11	DRRPASCGTC	AMP	0.678	AMP	0.604

Table 5: Conserved antimicrobial peptides.

peptide might work against either CD4/coreceptor or gp120 to inhibit viral entry (Table 4).

PTGERVWDRG

This peptide is predicted to be 30% hydrophobicity and has antigenic score of 0.2602. This peptide has no probability of immunogenicity because VaxiJen score is less than threshold level i.e., 0.4 and it has molecular weight of 1172.26 D. As per CAMP analysis, this peptide is predicted to be non-AMP from SVM and AMP from random forest algorithms, with probability of 0.70 and 0.70 respectively. This peptide has AMP category of antiviral activity from SVM and antibacterial activity from random forest algorithms predicted from classAMP, with probability of 0.75 and 0.49 respectively. After AVBlast analysis of this peptide, it was found that this sequence is a part of "GLRKKFRKTRKRIQKLGRKIGKTGRKBWKAWREYGQIEYPCRI" sequence which has antibacterial activity to *E.coli* and *S. aureus* with 66% identity and E value of 24. This peptide is predicted to be antiviral and antibacterial activity with no immunogenicity. This conserved peptide has been derived from GB Virus C gE2, the peptides P6-2 and P4762 inhibits HIV-1 replication via interaction with HIV-1 particle and avoid the entry of virions [15]. This peptide is showing antiviral activity with no immunogenicity.

Peptide Sequence	Prediction method-SVM		Prediction method-RF	
	Prediction	Probability	Prediction	Probability
KIRDSLHLVKC	Antifungal	0.98007525738161	Antibacterial	0.858
KYRRFRWKFK	Antibacterial	0.997191821246762	Antibacterial	0.564
PNNNTRKSIH	Antifungal	0.716303607753123	Antifungal	0.562
PNNNTRRRLS	Antiviral	0.708622545527062	Antifungal	0.63
PTGERVWDRG	Antiviral	0.757892210674696	Antibacterial	0.498
PWQGGRRKFR	Antiviral	0.95167536018741	Antibacterial	0.654
RGNPVRSPLG	Antiviral	0.953364870196997	Antibacterial	0.772
SDRDTVVELS	Antifungal	0.789076193791002	Antibacterial	0.372
EESQNQQEKN	Antifungal	0.727395582704812	Antifungal	0.586
DRRPASCGTC	Antiviral	0.890473933052895	Antibacterial	0.532
PYQNTRQRTP	Antiviral	0.858966097668196	Antibacterial	0.636

Table 6: AMP analysis of Conserved antipeptides.

KYRRFRWKFK

This peptide is predicted to be 30% hydrophobicity and has antigenic score of 1.61. This peptide has a good probability of immunogenicity because it has molecular weight of 1514.83D. As per CAMP analysis, this peptide is predicted to be AMP from both algorithms, SVM and Random Forest with probability of 1 and 0.89 respectively. This peptide has AMP category of antibacterial activity from both algorithms predicted from classAMP and Random Forest with probability of 0.99 and 0.56 respectively. After AVBlast analysis of this peptide, it was found that this sequence is a part of "GRFKRFRKKFKKLFKKLS" sequence which has antibacterial and antifungal activity to *E. coli*, S. typhimurium, *T. aeruginosa*, *S. marcescens*, *S. aureus*, S. epidermidis, *B. megaterium*, *C. albicans*, *C. neoformans* with 60% identity & E value of 2.8. This conserved peptide is derived from synthetic peptides (SALPs-Synthetic anti-lipopolysaccharides peptides), bind to heparin sulphate moeties on the cell surface and inhibit the entry of HIV-1, HSV-1 and 2 both, HBV and HCV [16]. These peptides have high antiviral efficiency, no toxicity and adverse effects. This peptide is best as it is showing antibacterial as well as antifungal activity with good score of antigenicity and immunogenicity. This peptide has wide spectrum future prospective in HIV treatment.

PNNNTRKSIH

This peptide is predicted to be 20% hydrophobicity and has antigenic score of -0.155. This peptide has no probability of immunogenicity because VaxiJen score is less than threshold; it has molecular weight of 1180.28 D. As per CAMP analysis, this peptide is predicted to be AMP from SVM and non-AMP from random forest algorithms, with probability of 0.913 and 0.586 respectively. This peptide has AMP category of antifungal activity from both algorithms predicted from classAMP, and Random Forest with probability of 0.716 and 0.562 respectively. After AVBlast analysis of this peptide, it was found that this sequence is a part of "MSRSLKKGPFVFYSLIK KVDQMNSNRFKSVILTWSRSCTIIPIMIGNTIGVYNGKEHIPVL VSDQMIGHKLGEFVQPRNYRGHKKHDKKTKTKR" sequence which has antimicrobial activity to Simian immunodeficiency Virus with 50% identity and E value of 155. Similar to 2nd peptide, this peptide is predicted to be antifungal and antimicrobial activity with no immunogenicity. This conserved peptide has derived from gp120, V3 region of gp120 of T cell line trophic directly interact with CXCR4, a chemokine receptor of CD4+ cells, hence inhibiting T trophic HIV-1 infection [17]. This conserved peptide may be act as a good candidate to inhibit viral entry.

PNNNTRRRLS

This peptide is predicted to be 20% hydrophobicity and has antigenic score of -0.3115. This peptide has no probability of immunogenicity because VaxiJen score less than threshold; it has molecular weight of 1227.34. As per CAMP analysis, this peptide is predicted to be AMP from SVM and non-AMP from random forest algorithms, with probability of 0.589 and 0.598 respectively. This peptide has AMP category of antiviral activity from SVM and antifungal activity from random forest algorithms predicted from classAMP, with probability of 0.708 and 0.630, respectively. After AVBlast analysis of this peptide, it was found that this sequence is a part of "SLSVEAKAKIVADFGRDANDTGSSEV QVALLTAQINHLQGHFSEHKKDHHSRRGLLRMVSTRRKLL DYLKRKDVASYVSLIERLGLRR" sequence which has antimicrobial activity with 60% identity and E value of 119. Similar to 4th peptide, this peptide is predicted to be antifungal and antimicrobial activity with no immunogenicity. This conserved peptide has been derived from gp120, V3 region of gp120 of T cell line trophic directly interact with CXCR4, a chemokine receptor of CD4+ cells, hence inhibiting T trophic HIV-1 infection .This conserved peptide may act as a good candidate to inhibit viral entry.

PYQNTRQRTP

This peptide is predicted to be 20% hydrophobicity and has antigenic score of 0.112. This peptide has no probability of immunogenicity because VaxiJen score is less than threshold; it has molecular weight of 1260.36. As per CAMP analysis, this peptide is predicted to be non-AMP from SVM and AMP from random forest algorithms, with probability of 0.993 and 0.528 respectively. This peptide has AMP category of Antiviral activity from SVM and antibacterial activity from random forest algorithms predicted from classAMP, with probability of 0.858 and 0.636 respectively. After AVBlast analysis of this peptide, it was found that this sequence is a part of "DEKPKLILPTPAPPNLPQLVGGGGGNR KDGFGVSVDAHQKVWTSDNGGHSIGVSPGYSQHLPGPYGNS RPDYRIGAGYSYNF" sequence which has antibacterial activity to *E. coli* with 66% identity & E value of 70. This peptide sequence also has the capability to act as a AMP without having the property of inducing immune response. This peptide is predicted to be antiviral and antibacterial activity with no immunogenicity. This conserved peptide has derived from gp120, V3 region of gp120 of T cell line trophic directly interact with CXCR4, a chemokine receptor of CD4+ cells, hence inhibiting T trophic HIV-1 infection .This conserved peptide may be act as a good candidate to inhibit viral entry.

PWQGGRRKFR

This peptide is predicted to be 30% hydrophobicity and has antigenic score of 0.129. This peptide has no probability of immunogenicity

Antipeptides	Target Organism	Sequences	Activity	Identity %	E-value
EESQNQQEKN	Simian Immunodeficiency virus	WQEWERKVDFLEE NITALLEEAQIQQE KNMYELQK	Antiviral	80	1.3
PTGERVWDRG	*E. coli* (ED50=30-35 nM), *S. aureus* (ED50=90-120 nM)	GLRKKFRKTRKRI QKLGRKIGKTGRK VWKAWREYGQIPY PCRI	Antibacterial	66	24
KYRRFRWKFK	*E. coli* ATCC 25922 (MIC=2μM), *E. coli* ML35 (MIC=4 μM), *E. coli* D21 MIC=4 μM), *S. typhimurium* ATCC 14028 (MIC=4 μM), *P. aeruginosa* ATCC 27853 (MIC=1 μM), *S. marcescens* ATCC 8100 (MIC=2 μM), *S. aureus* ATCC 25923 (MIC=2 μM), *S. aureus* Cowan 1 (MIC=2 μM), *S. aureus* MRSA (MIC=4μM), *S. epidermidis* ATCC 12228 (MIC=1 μM), *B. megaterium* Bm11 (MIC=2 μM), *C. albicans* (MIC=16 μM), *C. neoformans* (MIC=4 μM)	GRFKRFRKKFKKL FKKLS	Antibacterial, Antifungal	60	2.8
PNNNTRKSIH	Unknown	MSRSLKKGPFVFY SLIKKVDQMNSNR FKSVILTWSRSCTII PIMIGNTIGVYNGK EHIPVLVSDQMIGH KLGEFVQTRNYRG HKKHDKKTKTKR	Antimicrobial	50	155
PNNNTRRRLS	Unknown	SLSVEAKAKIVADF GRDANDTGSSEVQ VALLTAQINHLQG HFSEHKKDHHSRR GLLRMVSTRRKLL DYLKRKDVASYVS LIERLGLRR	Antimicrobial	60	119
PYQNTRQRTP	*E. coli*	DEKPKLILPTPAPP NLPQLVGGGGGNR KDGFGVSVDAHQK VWTSDNGGHSIGV SPGYSQHLPGPYG NSRPDYRIGAGYS YNF	Antibacterial	66	70
PWQGGRRKFR	Unknown	RIRRPIALIWRGGR RLTEWL	Antimicrobial	83	4.8
RGNPVRSPLG	Unknown	RFRPPIRRPPIRPPF RPPFRPPVRPPIRPP FRPPFRPPIGPFP	Antimicrobial	57	53
SDRDTVVELS	Unknown	MKRNQRKQLIGTV VSTKNAKTATVKV TSRFKHPLYHKSVI RHKKYHVHNFGEL VANDGDRVQIIETR PLSALKRWRIVKIIE RAK	Antimicrobial	42	346
KIRDSLHLVKC	Odorrana grahami (Yunnanfu frog)	GLLSGILGAGKHIV CGLSGPCQSLNRKS SDVEYHLAKC	Antibacterial, Antifungal	80	45
DRRPASCGTC	Unknown	EQKQGQYGEGSLR PSECGQRCSYRCSA TSHKKPCMFFCQK CCAKCLCVPPGTF GNKQVCPCYNNW KTQQGGPKCP	Antimicrobial	66	24

Table 7: Antipeptide and target organism.

because VaxiJen score is less than threshold; it has molecular weight of 1287.48. As per CAMP analysis, this peptide is predicted to be AMP from both algorithms, SVM and Random Forest with probability of 0.981 and 0.810 respectively. This peptide has AMP category of Antiviral activity from SVM and antibacterial activity from random forest algorithms predicted from classAMP, with probability of 0.951 and 0.654 respectively. After AVBlast analysis of this peptide, it was found that this sequence is a part of "RIRRPIALIWRGGRRLTEWL" sequence which has antimicrobial activity with 83% identity and E value of 4.8. This conserved peptide has derived from Apelin, inhibits the entry of some HIV-1 and 2 into CD4+ cells APJ receptor [18]. This is another best peptide candidate having antiviral activity with no immunogenic response.

RGNPVRSPLG

This peptide is predicted to be 40% hydrophobicity and has antigenic score of -0.2856. This peptide has no probability of immunogenicity because VaxiJen score is less than threshold; it has molecular weight of 1052.19. As per CAMP analysis, this peptide is predicted to be AMP from both algorithms, SVM and Random Forest with probability of 0.625 and 0.632 respectively. This peptide has AMP category of Antiviral activity from SVM and antibacterial activity from random forest algorithms predicted from classAMP, with probability of 0.953 and 0.772 respectively. After AVBlast analysis of this peptide, it was found that this sequence is a part of "RFRPPIRRPPIRPPFRPPFRP PVRPPIRPPFRPPFRPPIGPFP" sequence which has antimicrobial activity with 57% identity and E value of 53. This conserved peptide has derived from GB Virus C gE2, the peptides P6-2 and P4762 inhibits HIV-1 replication via interaction with HIV-1 particle and avoid the entry of virions. This peptide is showing high antiviral activity with no immunogenicity.

SDRDTVVELS

This peptide is predicted to be 30% hydrophobicity and has antigenic score of 0.572. This peptide has probability of immunogenicity because it has molecular weight of 1120.17. As per CAMP analysis, this peptide is predicted to be non-AMP from SVM and AMP from random forest algorithms, with probability of 0.959 and 0.624 respectively. This peptide has non AMP category of Antifungal activity from SVM and AMP antibacterial activity from random forest algorithms predicted from classAMP, with probability of 0.789 and 0.372 respectively. After AVBlast analysis of this peptide, it was found that this sequence is a part of "MKRNQRKQLIGTVVSTKNAKTATVKVT SRFKHPLYHKSVIRHKKYHVHNFGELVANDGDRVQIIETRPLSA LKRWRIVKIIERAK" sequence which has antimicrobial activity with 42% identity and E value of 346. This conserved peptide has derived from GB Virus C gE2, the peptides P6-2 and P4762 inhibits HIV-1 replication via interaction with HIV-1 particle and avoid the entry of virions. This peptide is showing low antiviral activity with immunogenicity.

KIRDSLHLVKC

This peptide is predicted to be 36% hydrophobicity and has antigenic score of 0.572. This peptide has a probability of immunogenicity because it has molecular weight of 1311.60. As per CAMP analysis, this peptide is predicted to be AMP from both algorithms, SVM and Random Forest with probability of 0.588 and 0.712 respectively. This peptide has AMP category of antifungal activity from SVM and antibacterial activity from Random forest, predicted from classAMP, and Random Forest with probability of 0.980 and 0.858 respectively. After AVBlast analysis of this peptide, it was found that this sequence is a part of "GLLSGILGAGKHIVCGLSGPCQSLNRKSSDVEYHLAKC" sequence which has antibacterial activity to Odorrana grahami with 80% identity and E value of 45. This conserved peptide has derived from GB Virus C gE2, the peptides P6-2 and P4762 inhibits HIV-1 replication via interaction with HIV-1 particle and avoid the entry of virions. This peptide is showing high antiviral activity with immunogenicity.

DRRPASCGTC

This peptide is predicted to be 20% hydrophobicity and has antigenic score of 0.748. This peptide has a good probability of immunogenicity because it has molecular weight of 1065.19 D. As per CAMP analysis, this peptide is predicted to be AMP from both algorithms, SVM and Random Forest with probability of 0.678 and 0.604 respectively. This peptide has AMP category of antiviral activity from SVM and antibacterial activity from Random forest, predicted from classAMP, with probability of 0.890 and 0.532 respectively. After AVBlast analysis of this peptide, it was found that this sequence is a part of "EQKQGQYGEGSLRPSECGQRCSYRCSATSHKKPCMFFCQKCC AKCLCVPPGTFGNKQVCPCYNNWKTQQGGPKCP" sequence which has antimicrobial activity to Simian immunodeficiency Virus with 66% identity & E value of 24. This conserved peptide has derived from GB Virus C gE2, the peptides P6-2 and P4762 inhibits HIV-1 replication via interaction with HIV-1 particle and avoid the entry of virions. This peptide is showing moderate antiviral activity with high immunogenicity.

In general, peptide sequences between 10 and 20 amino acids in length are recommended for ideal antigen [19]. So, our conserved antipeptide having 10 amino acid length which can be used as an inhibitor. In general, most ideal antigenic epitopes are hydrophilic, surface orientated and flexible. Hydrophilic residues are surface exposed so it has better affinity to bind with paratope as compared to hydrophobic residues.

In this study, we also compare hydrophobicity and antigenicity of our conserved antipeptide with antipeptide from HIPdb databases. Low hydrophobicity shows low affinity binding characteristics.

Conclusion

Many efforts are being made for the inhibition of HIV virus entry block by in silico, in vivo, in situ and *in vitro* approaches. We came to conclusion that PWQGGRRKFR is showing hydrophobicity of 30% and antigenicity of 0.129 with a molecular weight of 1287.48. So, according to this data it will definitely work against all the pathogens without interfering our immune system. Therefore, it acts as a broad spectrum antipeptide. KYRRFRWKFK is showing hydrophobicity of 30% and antigencity of 1.61 with a molecular weight of 1514.83D. So, all the above parameters prove that this peptide will inhibit the CD4-gp120 interaction with invoking immune system. Out of 11, we got 10 having less hydrophobicity percentage from derived HIPDB database, which proves that our inhibiting peptides will be effective inhibitor peptides than HIPdb antipeptides. These antipeptides have potential to inhibit HIV virus entry and they are able to induce humoral mediated immunity. These are the conserved patches taken from different sources and effective against different cell lines so these are wide spectrum antipeptides inhibiting the primary interaction of gp120-CD4 that is the major culprit for HIV pathogenesis.

Acknowledgement

We are very thankful to the Nthrys Biotech Labs, 1-8-747/11, Bagh

Lingampally, near RTC Road, Hyderabad-44, where this work was performed. We again acknowledge the Nthrys Biotech Labs. as well as the scientific staff for providing infrastructural facilities.

References

1. Xiao X, Wu L, Stantchev TS, Feng YR, Ugolini S, et al. (1999) Constitutive cell surface association between CD4 and CCR5. Proc Natl Acad Sci U S A 96: 7496-7501.
2. Sirois S, Sing T, Chou KC (2005) HIV-1 gp120 V3 loop for structure-based drug design. Curr Protein Pept Sci 6: 413-422.
3. Qureshi A, Thakur N, Kumar M (2013) HIPdb: A Database of Experimentally Validated HIV Inhibiting Peptides. PLoS One 8: e54908.
4. Myszka DG, Sweet RW, Hensley P, Brigham-Burke M, Kwong PD, et al. (2000) Energetics of the HIV gp120-CD4 binding reaction. Proc Natl Acad Sci U S A 97: 9026-9031.
5. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, et al. (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947-2948.
6. Notredame C, Higgins DG, Heringa J (2000) T-Coffee: A novel method for fast and accurate multiple sequence alignment. J Mol Biol 302: 205-217.
7. Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32: 1792-1797.
8. Doytchinova IA, Flower DR (2007) VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC Bioinformatics 8: 4.
9. Zibaee S, Makin OS, Goedert M, Serpell LC (2007) A simple algorithm locates beta-strands in the amyloid fibril core of alpha-synuclein, Abeta, and tau using the amino acid sequence alone. Protein Sci 16: 906-918.
10. Thomas S, Karnik S, Barai RS, Jayaraman VK, Idicula-Thomas S (2010) CAMP: a useful resource for research on antimicrobial peptides. Nucleic Acids Res 38: D774-780.
11. Joachims T (1999) Making Large-Scale SVM Learning Practical. In: Advances in Kernel Methods - Support Vector Learning, Schölkopf B, Burges CJC, Smola AJ (eds.). MIT Press, Cambridge, USA.
12. Karrer B, Newman ME (2009) Random acyclic networks. Phys Rev Lett 102.
13. Joseph S, Karnik S, Nilawe P, Jayaraman VK, Idicula-Thomas S (2012) ClassAMP: a prediction tool for classification of antimicrobial peptides. IEEE/ACM Trans Comput Biol Bioinform 9: 1535-1538.
14. Hildinger M, Dittmar MT, Schult-Dietrich P, Fehse B, Schnierle BS, et al. (2001) Membrane-anchored peptide inhibits human immunodeficiency virus entry. J Virol 75: 3038-3042.
15. Koedel Y, Eissmann K, Wend H, Fleckenstein B, Reil H (2011) Peptides derived from a distinct region of GB virus C glycoprotein E2 mediate strain-specific HIV-1 entry inhibition. J Virol 85: 7037-7047.
16. Krepstakies M, Lucifora J, Nagel CH, Zeisel MB, Holstermann B, et al. (2012) A new class of synthetic peptide inhibitors blocks attachment and entry of human pathogenic viruses. J Infect Dis 205: 1654-1664.
17. Sakaida H, Hori T, Yonezawa A, Sato A, Isaka Y, et al. (1998) T-tropic human immunodeficiency virus type 1 (HIV-1)-derived V3 loop peptides directly bind to CXCR-4 and inhibit T-tropic HIV-1 infection. J Virol 72: 9763-9770.
18. Zou MX, Liu HY, Haraguchi Y, Soda Y, Tatemoto K, et al. (2000) Apelin peptides block the entry of human immunodeficiency virus (HIV). FEBS Lett 473: 15-18.
19. Van Regenmortel MHV (1986) Trends in Biochemistry 11: 36-39.