Supporting Information

Cell-Permeable Stapled Peptides Based on HIV-1 Integrase Inhibitors Derived from HIV-1 Gene Products

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Experimental Procedures General Information

For analytical HPLC, a Cosmosil 5C18-ARII column (4.6 x 250 mm, Nacalai Tesque, Inc., Kyoto, Japan) was employed with a linear gradient of CH₃CN containing 0.1% (v/v) TFA at a flow rate of 1 cm³ min⁻¹ on a JASCO PU-2089 plus (JASCO Corporation, Ltd., Tokyo, Japan) or a Pump L-2130 (Hitachi High-Technologies Corporation, Tokyo, Japan), and eluting products were detected by UV at 220 nm. Preparative HPLC was performed using a Cosmosil 5C18-AR II column (20 x 250 mm, Nacalai Tesque, Inc.) on a JASCO PU-2086 plus (JASCO Corporation, Ltd., Tokyo, Japan) in a suitable gradient mode of CH₃CN solution containing 0.1% (v/v) TFA at a flow rate of 10 cm³ min⁻¹ and a Cosmosil 5C18-AR II column (10 x 250 mm, Nacalai Tesque, Inc.) on a JASCO Corporation, Ltd., Tokyo, Japan) in a suitable gradient mode of CH₃CN solution (10 x 250 mm, Nacalai Tesque, Inc.) on a JASCO Corporation, Ltd., Tokyo, Japan) in a suitable gradient mode of CH₃CN solution (10 x 250 mm, Nacalai Tesque, Inc.) on a JASCO PU-2089 plus (JASCO Corporation, Ltd., Tokyo, Japan) in a suitable gradient mode of CH₃CN solution (10 x 250 mm, Nacalai Tesque, Inc.) on a JASCO PU-2089 plus (JASCO Corporation, Ltd., Tokyo, Japan) in a suitable gradient mode of CH₃CN solution (10 x 250 mm, Nacalai Tesque, Inc.) on a JASCO PU-2089 plus (JASCO Corporation, Ltd., Tokyo, Japan) in a suitable gradient mode of CH₃CN solution containing 0.1% (v/v) TFA at a flow rate of 3 cm³ min⁻¹.

ESI-TOFMS was recorded on a micrOTOF-2 focus (Bruker Daltonics) mass spectrometer.

General Procedure of Peptide Synthesis

Chemical reagents including Fmoc-protected amino acids for peptide synthesis were purchased from Novabiochem, Wako Pure Chemical Industries, Ltd. and Tokyo Chemical Industry Co., Ltd. Peptides were synthesized using Rink amide resin (0.60 mmol/g, 0.05 mmol scales). Fmoc solid-phase peptide synthesis was manually performed using N^{α} -Fmoc-protected staple-type amino acid derivatives: 2-(Fmoc)amino)hept-6-enoic acid and 2-((Fmoc)amino)-3-(allyloxy)propanoic acid. The following side chain protected amino acids were used: Pbf for Arg, OBu' for Glu, Trt for Gln and His. All peptides were prepared using Fmoc-based solid phase synthesis. Each cycle involves (i) 15 min deprotection (20%) piperidine/DMF) and (ii) coupling with: Fmoc-amino acid (Fmoc-AA-OH) (3 equiv), HOBt (3 equiv) and DIPCI (3 equiv) in DMF for 90 min. Coupling was efficiency checked by the Kaiser ninhydrin test. In the case of a slightly positive Kaiser test, the coupling step was repeated (double coupling) using a mixture of Fmoc amino acid (3 equiv), HOBt (3 equiv), DIPEA (3 equiv) and HBTU (2.9 equiv). If the Kaiser test was positive even after a double coupling, rest free amino groups were acetylated (capping) using a mixture of Ac₂O (100 μ L) and DMF (5 mL). After the construction of the protected peptides, for the stapled peptides, ring-closing metathesis (RCM) was performed on the resin as described below. After RCM, the Fmoc group at the N-terminus was deprotected, and Ac₂O (100 μ L) and DMF (5 mL) were treated for 20 min in the synthesis of the acetylated peptides. In the synthesis of the corresponding linear peptides with olefinic side chains, the Fmoc group at the N-terminus was deprotected without the RCM reactions, and subsequently Ac_2O (100 µL) and DMF (5 mL) were treated for 20 min. The conjugates with oligo-arginine or the quartet repeat of arginine and glutamic acid were synthesized in the same method. For the fluorescein-labeled peptides, Fmoc-GABA-OH and the subsequent fluorescein were condensed. The resin was extensively washed (DMF, DCM, methanol and diethyl ether) and then dried in vacuo (6 h) before deprotection and cleavage.

On-Resin Ring-Closing Metathesis

Ring closing metathesis of resin-bound N^{α} -Fmoc and side-chain protected peptides was performed using 20 mol% of Grubbs second generation catalyst in degassed DCE for 2 h at 60 °C. The reactions were monitored by HPLC after cleavage of the peptides from a resin aliquot. When metathesis was still incomplete, the resin was treated with fresh catalyst for additional 2 h. After the reaction solution was drained, the resin was washed with DCM.

Cleavage of Peptides from Resin and Purification

The synthetic peptides were cleaved from the resins with the deprotection of all the protecting groups by treatment with a mixture of TFA, water and triisopropylsilane (9.5/0.25/0.25, v/v) at room temperature for 90 min. The reaction mixtures were filtered, and the resins were washed with TFA (3 times). The filtrates and washed solutions were evaporated under vacuum, and the peptides were precipitated as solid powder by addition of cold Et₂O. After centrifugation, the supernatants were removed. The precipitations were washed with cold Et₂O (3 times). The obtained peptides were dried under vacuum for 6 h. Peptide purification was performed by reverse phase HPLC. The purified peptides were identified by ESI-TOF MS and lyophilized.

peptide	formula	calcd [M+H] ⁺	found	yield (%)
3S (stapled)	$C_{107}H_{177}N_{26}O_{21}$	2158.33	2158.44	2
4S (stapled)	$C_{109}H_{175}N_{29}O_{23}$	2259.35	2259.47	3
5S (stapled)	$C_{106}H_{169}N_{29}O_{23}$	2217.30	2217.42	11
6S (stapled)	$C_{107}H_{172}N_{28}O_{22}$	2202.33	2202.17	4
7S (stapled)	$C_{107}H_{171}N_{25}O_{22}$	2159.31	2159.13	1
8S (stapled)	$C_{106}H_{169}N_{29}O_{23}$	2217.30	2217.13	3
9S (stapled)	$C_{106}H_{169}N_{29}O_{23}$	2217.30	2217.12	2
4L (linear)	$C_{111}H_{179}N_{29}O_{23}$	2287.38	2287.18	3
5L (linear)	$C_{108}H_{173}N_{29}O_{23}$	2245.34	2245.15	3
6 L (linear)	$C_{109}H_{176}N_{28}O_{22}$	2230.36	2230.00	4
8L (linear)	$C_{108}H_{173}N_{29}O_{23}$	2245.34	2245.15	5
9L (linear)	$C_{108}H_{173}N_{29}O_{23}$	2245.34	2245.15	3
11S (stapled)	$C_{107}H_{171}N_{29}O_{25}$	2263.31	2263.10	3
11L (linear)	$C_{109}H_{175}N_{29}O_{25}$	2291.34	2291.13	3

Characterization Data of the Synthetic Peptides <u>Stapled Peptides 3S-9S and 11S and Linear Peptides 4L-6L, 8L, 9L and 1</u>1L

HPLC profiles

3S

1 20

CH₃CN (56%, isocratic)



CH₃CN (49%, isocratic)





CH₃CN (44%, isocratic)

6S



CH₃CN (51%, isocratic)





CH₃CN (56%, isocratic)



CH₃CN (43%, isocratic)









CH₃CN (51%, isocratic)



CH₃CN (46%, isocratic)



CH₃CN (53%, isocratic)

8L



CH₃CN (46%, isocratic)

9L



CH₃CN (45%, isocratic)





CH₃CN (44%, isocratic)



CH₃CN (48%, isocratic)

Peptides with Hydrophilic Sequences 17-19

peptide	formula	calcd [M+H] ⁺	found	yield (%)
17 (stapled)	$C_{155}H_{268}N_{60}O_{30}$	3451.14	3450.83	13
18 (stapled)	$C_{151}H_{248}N_{48}O_{38}$	3342.90	3342.86	1
19 (linear)	$C_{153}H_{252}N_{48}O_{38}$	3370.93	3371.06	9

HPLC profiles

17



CH₃CN (40%, isocratic)



11L



Peptides with Oligo-arginine Sequences 20-23

peptide	formula	calcd $[M+H]^+$	found	yield (%)
20 (stapled)	$C_{131}H_{220}N_{44}O_{26}$	2826.72	2826.89	7.3
21 (stapled)	$C_{137}H_{232}N_{48}O_{27}$	2982.83	2982.83	3.7
22 (stapled)	$C_{143}H_{244}N_{52}O_{28}$	3138.93	3138.82	3.4
23 (stapled)	$C_{149}H_{256}N_{56}O_{29}$	3295.03	3294.89	5.0

HPLC profiles





CH₃CN (43%, isocratic)





CH₃CN (44%, isocratic)









CH₃CN (43%, isocratic)

Fluorescein-labeled Peptides 3S-F, 6S-F, 8S-F, 6L-F, 1-F and 2-F

peptide		sequence		
3S-F (stapled)	Fluorescein-GAB	A- <u>c</u> aii <u>c</u> ilqqllf	IHFRIG-NH	I_2
6S-F (stapled)	Fluorescein-GAB	A-EAI <u>c</u> RIL <u>c</u> QLLI	FIHFRIG-N	H_2
8S-F (stapled)	Fluorescein-GAB	A-EAIIR <u>c</u> LQQ <u>c</u> L	FIHFRIG-N	H_2
6L-F (linear)	Fluorescein-GAB	A-EAI <u>c</u> RIL <u>c</u> QLLI	FIHFRIG-N	H_2
1-F	Fluorescein-GAB	A-EAIIRILQQLL	FIHFRIG-N	H_2
2-F	Fluorescein-GAB	A-EAIIRILQQLL	FIHFRIG-R	$_{8}$ -NH ₂
peptide	formula	calcd $[M+H]^+$	found	yield (%)
3S-F (stapled)	$C_{129}H_{187}N_{27}O_{25}$	2515.43	2515.30	0.1
6S-F (stapled)	$C_{129}H_{187}N_{29}O_{26}$	2559.43	2559.41	0.3
8S-F (stapled)	$C_{128}H_{184}N_{30}O_{27}$	2574.40	2574.38	0.2
6L-F (linear)	$C_{131}H_{191}N_{29}O_{26}$	2587.46	2587.45	0.2
1-F	$C_{128}H_{188}N_{30}O_{27}$	2578.43	2578.43	0.3
2-F	$C_{176}H_{284}N_{62}O_{35}$	3827.24	3827.12	0.7

HPLC profiles

3S-F

CH₃CN (56%, isocratic)

6S-F



CH₃CN (51%, isocratic)

8S-F



CH₃CN (46%, isocratic)





CH₃CN (53%, isocratic)





CH₃CN (48%, isocratic)



CH₃CN (40%, isocratic)

IC50 (µM) strand transfer																						
Table 4																						
peptide conc. (µM)			pepti	ie 20				pe	ptide 2	1			р	eptide 2	2		pe	eptide 2	3			
0.00188						5			0	0	15					13			5			
0.00565					10	7			3	6	13				0	21		0	15			
0.0169					13	11			27	19	25				0	25		14	21			
0.0508	16	13	8	20	17	3			7	33	22				9	36		37	43			
0.152	29	32	12	46	33	42	12	33	26	43	20	51	48	57	45	54	83	78	79			
0.457	87	91	54	- 90	82	70	88	89	74	92	72	74	104	64	71	97	87	80	95			
1.37	100	105	91	100	98	87	100	94	85	88	91	105	92	87	84	96	90	102	96			
4.12	98	93	95	100	99	100	99	98	87	97	99	94	96	90	91	99	99	103	99			
12.35	103	97	100	100	103		102	98	97	99		92	92	91	93		100	- 99				
37.04	97	98		111	100		96	100		100		94	92	91	100		101					
111.1				111			100	100				102	105	91			100					
333.3				110								106	104	90			102					
peptide conc. (µM)			pepti	ie 17					peptie	ie 6S				pept	ide 1				pept	ide 2		
0.00188	-4		4			-4					0	13				4		0	0			9
0.00565	4		23		24	7					10	10		0	7	5		2	2	0	11	10
0.0169	15		17		28	10			11		15	9		0	3	6	10	0	10	13	14	15
0.0508	23	30	55	21	20	22			12	11	19	5	0	-9	-9	12	41	67	36	67	46	16
0.152	96	85	102	78	39	79	22	10	10	20	19	27	1	-3	5	3	80	103	96	98	54	40
0.457	- 99	96		92	83	90	25	33	49	23	28	39	11	13	11	-1	100		100	100	90	54
1.37	98	100		93	78	101	71	65	61	77	67	59	29	13	36	26	100			101	93	85
4.12	100	99		97	100	100	97	73	100	101	91	92	86	75	75	78				104	92	99
12.35		93		100	107		107	99		104	100		100	94	90	104				106		
37.04				100			94	100		105			100	97						102		
111.1							100	109		106			102	100								
333.3							102	98					100									

Representative Primary Data for Values of IC50, EC50 and CC50

$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	EC50 (µM) p24 ELISA												
peptide cnc. (µM) 100 55 1.3 100 55 2.5 1.3 100 55 2.5 1.3 200 100 55 2.5 Op<	Table 3												
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$		peptide 17				peptide 18				peptide 19			
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	peptide conc. (µM)	10	5	2.5	1.3	10	5	2.5	1.3	20	10	5	2.5
$ \begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	OD	-232448	366519.8	2000279	2112148	611234.1	2445426	1972312	1790524	666694	608354.4	474516.4	432763.6
	% of control	-16	25	137	145	42	168	135	123	107	98	76	70
peptide conc. (µM)peptide de(N)peptide cb(N)(Peptide cBS)(N)(Peptide cBS)peptide conc. (µM)-23011712941031541148143860116428813522661398981167399315601161433941429279% of control-1680006991061008029961151089898% inhibitionPeptide Control-16800.69910610087744-151089898% inhibitionPeptide Control11611862.51.31052.51.31052.51.310052.51.31.310052.51.3<	% inhibition	116	75	-37	-45	58	-68	-35	-23	-7	2	24	30
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $		peptide 6S				peptide 6L				peptide 8S			
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	peptide conc. (µM)	10	5	2.5	1.3	10	5	2.5	1.3	10	5	2.5	1.3
$ \begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	OD	-230117	1294103	1541148	1438601	1545810	1464238	1352369	1398981	1673993	1569116	1433940	1429279
	% of control	-16	89	106	99	106	100	93	96	115	108	98	98
peptide RL peptide one. (µM)peptide RL iipeptide ipeptide fS ipeptide ipeptide ipe	% inhibition	116	11	-6	1	-6	0	7	4	-15	-8	2	2
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$		peptide 8L				peptide 5S				peptide 1			
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	peptide conc. (µM)	10	5	2.5	1.3	10	5	2.5	1.3	10	5	2.5	1.3
	OD	1545810	1457246	1387328	1326732	1480552	1412965	1301095	1510850	1916377	1368683	1347708	1492206
	% of control	106	100	95	91	101	97	89	104	131	94	92	102
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	% inhibition	-6	0	5	9	-1	3	11	-4	-31	6	8	-2
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$		peptide 2				AZT							
OD -300035 -104264 1531826 1550470 -297705 -66974.1 1860442 % of control -21 -7 105 106 -21 -20 -5 127 % inhibition 121 107 -5 -6 121 120 105 -277	peptide conc. (uM)	10	5	2.5	1.3	0.8	0.16	0.032	0.0064				
% of control -21 -7 105 106 -21 -20 -5 127 (105 106 -21 % inhibition 121 107 -5 -6 121 120 105 -27 (105 -27 Table 4 -0 -10 -5 -6 121 120 105 -27 (105 -27 Table 4 -0	OD	-300035	-104264	1531826	1550471	-307027	-297705	-66974.1	1860442				
% inhibition 121 107 -5 6 121 120 100 -2.7 Table 4	% of control	-21	-7	105	106	-21	-20	-5	127				
Table 4 peptide 20 peptide 21 peptide 21 peptide 22	% inhibition	121	107	-5	-6	121	120	105	-27				
Table 4 peptide 20 peptide 23 Peptide 24 Peptide 24 Peptide 24 Peptide 23 Peptide 24 Peptide 24 Peptide 23 Peptide 24 Peptide 2													
peptide 20 loc peptide 21 peptide 21 peptide 22 peptide 22 peptide 23 peptide 24 peptide 24 peptide 25 peptide 26 peptide 26 peptide 26 peptide 27 peptide 28 peptide 28 <td>Table 4</td> <td></td>	Table 4												
peptide conc. (μM) 10 5 2.5 1.25 10 5 2.5 1.25		peptide 20				peptide 21				peptide 22			
OD -132399-244 666563.0004 1439595.436 1050648.299 -258807.064 35341.847 58007.0075 82583.0815 -159949.67 1078198.721 1220812.671 1031200.942 % of control -17.821662 89.72302311 193.777141 141.4230036 -34.8368454 4.5191593 78.16142411 111.1010741 -21.5300994 45.131441 164.3280582 138.805283 peptide 20 peptide 23 peptide 17 5 2.5 1.1010741 peptide 23 -64.3280582 38.805283 OD -257186.451 4850543362 506122.302 70869.8402 1096025.465 969617.6449 92.8861.082 -2.239359.707 23.8853.31 76179.1716 901551.8959 % of control -34.618072 65.20906484 68.12682874 95.39475092 147.5310181 30.5158347 124.8529635 -32.2191249 81.47871434 102.5394824 123.538184 OD -34.618070 65.20906484 68.12682874 95.39475092 147.5310181 30.5158347 124.6259055 -32.2191249 81.47871434 <t< td=""><td>peptide conc. (µM)</td><td>10</td><td>5</td><td>2.5</td><td>1.25</td><td>10</td><td>5</td><td>2.5</td><td>1.25</td><td>10</td><td>5</td><td>2.5</td><td>1.25</td></t<>	peptide conc. (µM)	10	5	2.5	1.25	10	5	2.5	1.25	10	5	2.5	1.25
% of control -17.821662 89.72302311 193.7774141 141.4230036 -34.8368454 4.51491593 78.16142411 111.1010741 -21.5300994 145.131411 164.3280582 138.805283 % inhibition 117.821662 10.27697689 93.7774141 41.4230036 134.8368454 10.519159 21.83857589 -11.1010741 -21.5300994 45.13141 164.3280582 38.805283 peptide conc. (μM) 10 5 2.5 1.25 9 21.83857589 -11.010741 21.5300994 45.13141 164.3280582 38.805283 Oppide conc. (μM) 10 5 2.5 1.25 0.625 2.0 0.62 2.0 0.0 5 2.5 2.5 0.62 2.0 10 5 2.5 0.62 2.0 10 5 2.5 0.55 2.5 0.62 2.0 10 5 2.5 0.55 2.5 0.62 2.0 10 5 2.5 2.5 0.62 2.0 0.0 3.17871414 10.533817	OD	-132399.244	666563.0004	1439595.436	1050648.299	-258807.064	-33541.847	580670.5075	825383.0815	-159949.667	1078198.721	1220812.671	1031200.942
% inhibition 117.821662 10.27697689 -93.7774141 -41.4230036 134.836845 104.5149159 21.8385789 -11.1010741 121.530094 -64.5131441 -64.3280582 -38.805283 peptide conc. (μM) 10 5 2.5 1.25 5 2.5 1.25 0.625 20 10 5 2.5 0.55 2.5 0.25 0.25 0.25 90.151.8395 90.151.8395 90.151.8395 90.151.8395 90.151.8395 90.151.8395 90.151.8395 90.151.8395 90.151.8395 121.530141 10.2534824 102.534824 <	% of control	-17.821662	89.72302311	193.7774141	141.4230036	-34.8368454	-4.51491593	78.16142411	111.1010741	-21.5300994	145.131441	164.3280582	138.805283
peptide 23 peptide 23 peptide 24 peptide 17 peptide 17 peptide 25 peptide 23 peptide	% inhibition	117.821662	10.27697689	-93.7774141	-41.4230036	134.8368454	104.5149159	21.83857589	-11.1010741	121.5300994	-45.131441	-64.3280582	-38.805283
peptide conc. (μM) 10 5 2.5 1.25 5 2.5 1.25 0.625 20 10 5 2.5 OD -257186451 485054362 506122.3062 708698.9402 1096025.465 969617.6449 925861.092 -239359.707 233859.31 762179.1716 901551.8959 % of control -34.618702 65.29096484 68.12682874 95.39475092 147.5310181 130.5158347 124.6259635 -32.2191249 31.47874134 102.5934824 121.5358128 // inihibition 134.618702 34.79073163 137112/6 40.6904097 47.310181 -30.5158347 24.6259635 -32.2191249 85.125866.2 -59348238 21.3538128		peptide 23				peptide 17				peptide 6S			
OD -257186.451 485054.3362 506122.3062 708698.9402 1096025.465 969617.6449 925861.092 -239359.707 233859.31 762179.1716 901551.8959 % of control -34618702 65.20906484 68.12682874 95.39475092 147.5310181 130.5158347 124.6259635 -32.2191249 31.47574134 102.5394824 121.5358124 % inhibition 134.618702 34.71712/6 40/540079 47.5310181 30.5158347 124.6259635 -32.2191249 53.9284238 121.5358124 14.618702 34.07005163 137.7112/6 40/540079 47.5310181 -30.5158347 124.6259635 132.2191249 58.21286.6 29.9348238 121.5358124	peptide conc. (uM)	10	5	2.5	1.25	5	2.5	1.25	0.625	20	10	5	2.5
% of control -34.618702 65.29096484 68.12682874 95.39475092 147.5310181 130.5158347 124.6259635 -32.2191249 31.47874134 102.5934824 121.3538128 % inhibition 134.618707 34.70903516 31.87317126 4.60540079 -47.5310181 -30.5158347 -24.6259635 132.2191249 68.52125866 -2.9934828 -21.3538128	OD	-257186.451	485054.3362	506122.3062	708698,9402	1096025.465	969617.6449	925861.092		-239359.707	233859.31	762179.1716	901551.8959
% inhibition 134 618702 34 70903516 31 87317126 4 605249079 -47 5310181 -30 5158347 -24 6259635 132 2191249 68 52125866 -2 59348238 -21 3538128	% of control	-34.618702	65,29096484	68,12682874	95,39475092	147.5310181	130.5158347	124.6259635		-32.2191249	31.47874134	102.5934824	121.3538128
	% inhibition	134.618702	34,70903516	31.87317126	4.605249079	-47.5310181	-30.5158347	-24.6259635		132.2191249	68.52125866	-2.59348238	-21.3538128
pentide 1 pentide 2 AZT		peptide 1				neptide 2				AZT			
pentide conc. (µM) 20 10 5 25 20 10 5 25 08 0.16 0.032 0.0064	peptide conc. (uM)	20	10	5	2.5	20	10	5	2.5	0.8	0.16	0.032	0.0064
OD 596915.2 609498.3 642099.8 667837.9 -81997.7 -65982.9 528280.4 722745.8 -140502.31 671424.8396 1493075.667 773523.4631	OD	596915.2	609498.3	642099.8	667837.9	-81997.7	-65982.9	528280.4	722745.8	-140502.31	671424.8396	1493075.667	773523.4631
% of control 96 98 103 107 -13 -11 85 116 -17.9137287 85.60515812 190.3637921 98.62250317	% of control	96	98	103	107	-13	-11	85	116	-17.9137287	85.60515812	190.3637921	98.62250317
% inhibition 4 2 -3 -7 113 111 15 -16 1179137287 14 39484188 -00 3637921 1 377496831	% inhibition	4	2	-3	-7	113	111	15	-16	117,9137287	14,39484188	-90,3637921	1.377496831

*These are rough estimates using four concentrations of compounds based on their EC_{50} values in the MTT assays.

EC50 (µM) MTT assay									
Table 3									
10000	peptide 17								
peptide conc. (uM)	10	5	2.5	1.3	0.6	0.3	0.2	0.1	0
OD	-0.0015	1.7735	0.2305	0.1135	0.0985	0.0895	0.0825	0.0685	0.0595
% of control	0	128	17	8	7	6	6	5	4
% inhibition	100	-28	83	92	93	94	94	95	96
	nentide 18		0.5						,,,
peptide.conc. (uM)	10	5	2.5	13	0.6	0.3	0.2	0.1	0
OD	1 2735	0.2295	0.0695	0.0475	0.0475	0.0455	0.0505	0.0425	0.0485
% of control	92	17	5	3	3	3	4	3	3
% inhibition	2	92	05	07	07	07	96	07	07
76 InfiloRion	nantida 10	60	35	51	51	51	30	31	21
pantida conc. (uM)	10	5	2.5	1.3	0.6	0.3	0.2	0.1	0
OD	0.1365	0.1095	0.0565	0.0465	0.0445	0.0465	0.0515	0.0565	0.0545
0D % af aantral	0.1505	0.1095	0.0505	0.0403	0.0445	0.0403	0.0515	0.0505	0.0045
76 OF CONTION	10	0	4	07	07	07	4	4	4
% innibition	90	92	90	97	97	97	90	90	90
and the second (AO)	peptide 65	5	2.5	1.2	0.0	0.2	0.2	0.1	0
op	10	1 225	2.3	1.3	0.0	0.3	0.2	0.1	0.045
	1	1.225	0.084	0.04	0.041	0.04	0.038	0.047	0.045
% of control	/5	92	6	3	3	3	3	4	3
% inhibition	25	8	94	97	97	9/	9/	96	9/
	peptide 6L								
peptide conc. (µM)	10	5	2.5	1.3	0.6	0.3	0.2	0.1	0
OD	0.0385	0.0415	0.0455	0.0395	0.0395	0.0355	0.0355	0.0385	0.0385
% of control	4	4	4	4	4	3	3	4	4
% inhibition	96	96	96	96	96	97	97	96	96
	peptide 8S								
peptide conc. (µM)	10	5	2.5	1.3	0.6	0.3	0.2	0.1	0
OD	0.7685	0.0555	0.0445	0.0485	0.0415	0.0395	0.0385	0.0405	0.0365
% of control	70	5	4	4	4	4	4	4	3
% inhibition	30	95	96	96	96	96	96	96	97
	peptide 8L								
peptide conc. (µM)	10	5	2.5	1.3	0.6	0.3	0.2	0.1	0
OD	0.0575	0.0455	0.0455	0.0455	0.0405	0.0385	0.0345	0.0365	0.0395
% of control	5	4	4	4	4	4	3	3	4
% inhibition	95	96	96	96	96	96	97	97	96
	peptide 58								
peptide conc. (µM)	10	5	2.5	1.3	0.6	0.3	0.2	0.1	0
OD	0.3085	0.0495	0.0335	0.0415	0.0435	0.0485	0.0455	0.0425	0.0445
% of control	19	3	2	3	3	3	3	3	3
% inhibition	81	97	98	97	97	97	97	97	97
	peptide 1								
nentide conc. (uM)	10	5	2.5	1.3	0.6	0.3	0.2	0.1	0
OD	0.083	0.059	0.05	0.051	0.048	0.043	0.044	0.046	0.046
% of control	6	4	4	4	4	3	3	3	3
% inhibition	94	96	96	96	96	97	97	97	97
/ minoration	nentide 2	50	50	50	50				
pantida conc. (uM)	10	5	25	1.3	0.6	0.3	0.2	0.1	0
OD	0.000	0.714	0.348	0.101	0.05	0.042	0.043	0.047	0.049
% of control	0.009	5.7.14	0.346	0.101	0.05	0.045	0.045	0.047	0.048
/s or control	00	34	20	0	4	07	07	4	4
76 mm10m	99 4.7T	40	/4	92	90	9/	97	90	96
	AZT	20	-	0.0	0.16	0.022	0.00/	0.001	0.0003
pepude conc. (µM)	100	20	4	0.8	0.16	0.032	0,006	100.0	0.0003
OD	1.1865	1.4545	1.1555	1.3885	1.4175	0.2075	0.0795	0.0475	0.0465
% of control	74	91	72	87	89	13	5	3	3
% inhibition	26	9	28	13	11	87	95	97	97

EC50 (µM) MTT assay									
Table 4									
Table 4	nentide 20								
nentide.conc. (uM)	20	10	5	2.5	1.25	0.625	0.3125	0 15625	0.078125
OD	0.0045	1 0355	0.8425	0.1085	0.0315	0.0235	0.0205	0.15025	0.070122
% of control	-2 0398482	144 686907	117 2201139	12 76091082	1 802656546	0.664136622	0.237191651	0.09487666	0.237191651
% inhibition	102 0398482	-44 686907	-17 2201139	87 23908918	98 19734345	99 33586338	99.76280835	99 90512334	99.76280834
, o mino kion	nentide 21	111000507	11122011255	07120500510	50.1575 15 15	55125266556	55170200055	55150512551	55170200005
nentide conc. (uM)	20	10	5	2.5	1.25	0.625	0.3125	0 15625	0.078124
OD	0.0025	0.4495	0.9015	0.1135	0.0385	0.0275	0.0235	0.0225	0.0174
% of control	-2 32447818	61 29032258	125 6166983	13 47248577	2 79886148	1 233396584	0.664136622	0.521821632	-0 18975333
% inhibition	102 3244782	38 70967742	-25.6166983	86 52751423	97 20113852	98 76660342	00 33586338	00.021021002	100 1807537
70 IIIIID RIOII	nentide 22	56.70507742	25.0100905	00.02701420	57.20115052	50.70000542	77.55566556	JJ.47017057	100.107755.
nentide conc. (uM)	20	10	5	2.5	1.25	0.625	0.3125	0.15625	0.078124
OD	0.0005	0.0995	0.4255	0.0655	0.0355	0.025	0.0145	0.0145	0.0184
% of control	J 94623656	11 82795699	53 89247312	7 440860215	3 569892473	2 537634409	0.860215054	0.860215054	1 37634408
% inhibition	100 9462366	88 17204301	46 10752688	92 55913978	96.43010753	97 46236559	99 13978495	99 13978495	98 6236559
, v mino kion	nentide 23	0011/201001	10.107520.00	72.00710710	90.15010755	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	<i>mayrolys</i>	90.0250559
nentide.conc. (uM)	20	10	5	2.5	1.25	0.625	0.3125	0.15625	0.07812
OD	0.0005	0.2595	0.2145	0.0245	0.0205	0.0215	0.0105	0.0115	0.0144
% of control	-0.94623656	32 47311828	26 66666667	2 150537634	1 634408602	1 76344086	0.344086022	0.47311828	0.860215054
% inhibition	100 9462366	67 52688172	73 333333333	97 84946237	98 3655914	98 23655914	99.65591398	99 52688172	99 1397849
/ minoxion	nentide 17	07.02000172	10.000000000	57101510457	50.5055711	50.25055711	77.05571570	77.52000172	<i>))</i> .15)/101).
nentide conc. (uM)	20	10	5	25	1.25	0.625	0.3125	0.15625	0.078124
	0.0005	0.0045	0.2285	0.0335	0.0225	0.025	0.0085	0.0115	0.0134
% of control	-0.94623656	-0.43010753	28 47311828	3 311827957	1 892473118	1 505376344	0.086021505	0.47311828	0.731182796
% inhibition	100 9462366	100.43010755	71 52688172	96 68817204	98 10752688	98 49462366	99 91 397849	99 52688172	99 268817
/•	nentide 65	100.1001075	71.52000172	70.00017204	70.10752000	70.17402500	77.71377047	77.52000172	>>.2000172
nentide conc. (uM)	20	10	5	2.5	1.25	0.625	0 3125	0.15625	0.078124
OD	1.0155	0.9175	0.0725	0.0265	0.0175	0.0165	0.0125	0.0175	0.016
% of control	141 8406072	127 8937381	7 637571157	1 091081594	-0 18975332	-0.33206831	-0 18975332	-0 18975332	-0 3320683
% inhibition	-41 8406072	-27 8937381	92.36242884	98 90891841	100 1897533	100 3320683	100 1897533	100 1897533	100 332068
/o minowion	AZT	21.0957501	52.50212001	70.70071011	100.1077555	100.5520005	100.1097955	100.1077555	100.552000.
nentide.conc. (uM)	100	20	4	0.8	0.16	0.032	0.0064	0.00128	0.00025
OD	0.6065	0.9595	0.9005	0.9285	0.10	0.0515	0.0185	0.00126	0.0002.5
% of control	70 24661893	112 3707239	105 3301512	108 6714399	91 96499602	4 017501989	0.079554495	-1 23309467	-0 3977774
% inhibition	20 75238107	12 3707230	5 33015115	8 671/200/	8 035003078	05.08240801	00.02044551	101 2220047	100 2077724

Table 3 peptide conc. (µM) OD W of control w inhibition peptide conc. (µM) OD % of control % inhibition peptide conc. (µM) OD % of control % inhibition peptide conc. (µM) % of control % inhibition % of control % inhibition %	peptide 17 20 0.006 1 99 peptide 18 10 1.0015 79 21 peptide 19 10 1.0705 77 23 peptide 65	10 0.061 6 94 5 1.2785 92 8 5 1.1125 80	5 0.968 93 7 2.5 1.3395 97 3 2.5 1.6235	2.5 1.072 103 -3 1.3 1.3935 100 0 1.3	1.3 1.036 99 1 0.6 1.3155 95 5	0.6 0.981 94 6 0.3 1.1325 82 18	0.3 1.093 105 -5 0.2 1.1285 81 19	0.2 1.336 128 -28 0.1 1.2435 90	0.1 0.808 78 22 0 0.8595 62
peptide conc. (µM) OD % of control % inhibition peptide conc. (µM) OD % of control % inhibition OD % of control % inhibition peptide conc. (µM)	peptide 17 20 0.006 1 99 peptide 18 10 1.0015 79 21 peptide 19 10 1.0705 777 23 peptide 65	10 0.061 6 94 5 1.2785 92 8 5 1.1125 80 0	5 0.968 93 7 2.5 1.3395 97 3 2.5 1.6235	2.5 1.072 103 -3 1.3 1.3935 100 0 1.3	1.3 1.036 99 1 0.6 1.3155 95 5	0.6 0.981 94 6 0.3 1.1325 82 18	0.3 1.093 105 -5 0.2 1.1285 81 19	0.2 1.336 128 -28 0.1 1.2435 90	0.1 0.808 78 22 0 0 0.8595 62
peptide conc. (µM) OD % of control % inhibition Peptide conc. (µM) OD Peptide conc. (µM) OD OD % of control % inhibition peptide conc. (µM)	20 0.006 1 99 peptide 18 10 1.0915 79 21 peptide 19 10 1.0705 77 23 peptide 65	10 0.061 6 94 5 1.2785 92 8 5 1.1125 80 0	5 0.968 93 7 2.5 1.3395 97 3 2.5 1.6235	2.5 1.072 103 -3 1.3 1.3935 100 0 1.3 1.3955	1.3 1.036 99 1 0.6 1.3155 95 5 0.6	0.6 0.981 94 6 0.3 1.1325 82 18	0.3 1.093 105 -5 0.2 1.1285 81 19	0.2 1.336 128 -28 0.1 1.2435 90 10	0.1 0.808 78 22 0 0 0.8595 62
OD % of control % inhibition peptide conc. (µM) OD % of control % inhibition peptide conc. (µM) OD % of control % inhibition peptide conc. (µM)	0.006 1 99 peptide 18 10 1.0915 79 21 peptide 19 10 1.0705 77 23 peptide 68	0.061 6 94 5 1.2785 92 8 5 1.1125 80	0.968 93 7 2.5 1.3395 97 3 2.5 1.6235	1.072 103 -3 1.3 1.3935 100 0 1.3 1.3955	1.036 99 1 0.6 1.3155 95 5	0.981 94 6 0.3 1.1325 82 18	1.093 105 -5 0.2 1.1285 81 19	1.336 128 -28 0.1 1.2435 90	0.808 78 22 0 0 0.8595 62
% of control % inhibition DD W of control % inhibition peptide conc. (µM) OD % of control % inhibition peptide conc. (µM)	1 99 10 10 1.0915 79 21 peptide 19 10 1.0705 77 23 peptide 65	6 94 5 1.2785 92 8 5 1.1125 80	93 7 2.5 1.3395 97 3 2.5 1.6235	103 -3 1.3 1.3935 100 0 1.3	99 1 0.6 1.3155 95 5	94 6 0.3 1.1325 82 18	105 -5 0.2 1.1285 81 19	128 -28 0.1 1.2435 90	78 22 0 0.8595 62
% inhibition peptide conc. (µM) OD % of control % inhibition Peptide conc. (µM) OD % of control % inhibition peptide conc. (µM)	99 peptide 18 10 1.0915 79 21 peptide 19 10 1.0705 77 23 peptide 68	94 5 1.2785 92 8 5 1.1125 80	7 2.5 1.3395 97 3 2.5 1.6235	-3 1.3 1.3935 100 0 1.3 1.3	1 0.6 1.3155 95 5	6 0.3 1.1325 82 18	-5 0.2 1.1285 81 19	-28 0.1 1.2435 90	22 0 0.8595 62
peptide conc. (µM) OD % of control % inhibition peptide conc. (µM) OD % of control % inhibition peptide conc. (µM)	peptide 18 10 1.0915 79 21 peptide 19 10 1.0705 77 23 peptide 6S	5 1.2785 92 8 5 1.1125 80	2.5 1.3395 97 3 2.5 1.6235	1.3 1.3935 100 0 1.3	0.6 1.3155 95 5	0.3 1.1325 82 18	0.2 1.1285 81 19	0.1 1.2435 90	0.8595 62
peptide conc. (µM) DD % of control % inhibition peptide conc. (µM) DD % of control % inhibition peptide conc. (µM)	10 1.0915 79 21 peptide 19 10 1.0705 77 23 peptide 68	5 1.2785 92 8 5 1.1125 80	2.5 1.3395 97 3 2.5 1.6235	1.3 1.3935 100 0 1.3	0.6 1.3155 95 5	0.3 1.1325 82 18	0.2 1.1285 81 19	0.1 1.2435 90	0.8595 62
DD % of control % inhibition peptide conc. (µM) DD % of control % inhibition peptide conc. (µM)	1.0915 79 21 peptide 19 10 1.0705 77 23 peptide 68	1.2785 92 8 5 1.1125 80	1.3395 97 3 2.5 1.6235	1.3935 100 0 1.3	1.3155 95 5	1.1325 82 18	1.1285 81 19	1.2435 90	0.8595
% of control % inhibition peptide conc. (µM) OD % of control % inhibition peptide conc. (µM)	79 21 peptide 19 10 1.0705 77 23 peptide 68	92 8 5 1.1125 80	97 3 2.5 1.6235	100 0 1.3	95 5	82 18	81 19	90 10	62
% inhibition peptide conc. (µM) DD % of control % inhibition peptide conc. (µM)	21 peptide 19 10 1.0705 77 23 peptide 68	8 5 1.1125 80	3 2.5 1.6235	0	5	18	19	10	
peptide conc. (μM) DD % of control % inhibition peptide conc. (μM)	peptide 19 10 1.0705 77 23 peptide 68	5 1.1125 80	2.5 1.6235	1.3	0.6			10	
peptide conc. (μM) DD % of control % inhibition peptide conc. (μM)	10 1.0705 77 23 peptide 6S	5 1.1125 80	2.5 1.6235	1.3	0.6				
DD % of control % inhibition peptide conc. (μM)	1.0705 77 23 peptide 6S	1.1125	1.6235	1 2205	0.6	0.3	0.2	0.1	(
% of control % inhibition peptide conc. (μM)	77 23 peptide 68	80	110400	1 1 1 2 1	1 2045	1 1935	1 4045	1 4195	1 1034
weptide conc. (μM)	23 peptide 68		117	97	87	86	101	102	80
eptide conc. (µM)	peptide 6S	20	-17	3	13	14	-1	-2	20
eptide conc. (µM)	Pepude 05		17		15	17	1	*	20
reprise cone. (µm)	10	5	2.5	13	0.6	0.3	0.2	0.1	(
ac	12	1 335	1 505	1.28	1 435	1 169	0.95	1.097	1134
% of control	90	101	113	06	108	88	72	83	84
% inhibition	30	101	113	30	108	12	28	17	0.
/6 111110101011	nantida 61	-1	-15	4	-0	12	20	17	1.
antida anna (cMO	10	6	2.6	1.2	0.6	0.2	0.2	0.1	(
optide conc. (µM)	1.0745	1 2195	1.2055	1.5	1.0075	1.2775	1.2265	1.4296	0 7206
JD // of control	1.0743	1.2165	1.2933	1.5565	1.0075	1.2773	1.5505	1.4585	0.7203
/6 OI COILLOI	96	111	110	142	92	117	122	21	2/
/6 IIIIIDILIOII	nantida 96	-11	-10	-42	0	-17	-22	-51	34
antida cono. (uM)	10	5	2.5	1.2	0.6	0.3	0.2	0.1	0
	1 2295	1 2725	1 2705	1.0525	1 5005	0.0825	1 4225	1 4645	0.4055
JD K of control	1.5565	1.2/55	1.5795	1.0555	1.3003	0.9855	1.4555	1.404.5	0.4933
/6 OI COILIOI	122	116	126	90	27	90	21	24	43
/6 1111101001	-22 nontido 91	-10	-20	4	-57	10	-51	-34	32
antida non a 6.00	peptide 8L	E	2.6	1.2	0.6	0.2	0.2	0.1	0
peptide conc. (µM)	10	0.0005	2.5	1.3	0.6	0.3	0.2	0.1	1200
	1.0575	0.9885	1.1385	1.0675	1.2825	1.2255	1.1865	1.4475	1.3562
% of control	97	90	104	97	117	112	108	132	124
% inhibition	3	10	-4	3	-17	-12	-8	-32	-24
	peptide 58								
beptide conc. (µM)	10	5	2.5	1.3	0.6	0.3	0.2	0.1	(
JD	1.2575	1.2075	1.3615	1.3785	1.3795	1.5485	1.5665	1.3165	1.3495
% of control	79	76	85	86	8/	97	98	83	85
% inhibition	21	24	15	14	13	3	2	17	15
	peptide 1								
beptide conc. (µM)	10	5	2.5	1.3	0.6	0.3	0.2	0.1	(
DD	1.368	0.998	0.942	1.394	1.536	1.638	1.436	1.178	1.363
% of control	103	75	71	105	116	123	108	89	103
% inhibition	-3	25	29	-5	-16	-23	-8	11	-3
	peptide 2								
eptide conc. (µM)	10	5	2.5	1.3	0.6	0.3	0.2	0.1	
DD	0.007	0.871	1.225	1.418	1.066	1.041	1.543	1.265	1.286
% of control	1	66	92	107	80	78	116	95	93
% inhibition	99	34	8	-7	20	22	-16	5	3
	AZT								
peptide conc. (µM)	100	20	4	0.8	0.16	0.03	0.006	0.001	0.0003
DD	0.9965	0.9635	1.2215	1.2745	1.5105	1.3175	1.1375	1.2005	1.7855
% of control	63	60	77	80	95	83	71	75	112

CC50 (µM) MTT assay									
Table 4									
	peptide 20								
nentide conc. (uM)	20	10	5	2.5	1.25	0.625	0.3125	0.15625	0.078125
OD	0.0325	1.1065	0.9665	1.0035	0.9805	0.9305	0.8865	0.9475	0.7005
% of control	4.504504505	153.3610534	133.957034	139.0852391	135,8974359	128.967429	122.8690229	131.3236313	97.08939709
% inhibition	95,4954955	-53.3610534	-33,957034	-39.0852391	-35,8974359	-28,967429	-22.8690229	-31.3236313	2.910602911
	peptide 21								
peptide conc. (uM)	20	10	5	2.5	1.25	0.625	0.3125	0.15625	0.078125
OD U	0.0035	0.9245	1.0065	0.9465	0.9035	0.8555	0.8335	0.9155	0.7785
% of control	0.485100485	128.1358281	139,5010395	131,1850312	125.2252252	118.5724186	115.5232155	126.8884269	107,9002079
% inhibition	99,51489951	-28,1358281	-39,5010395	-31,1850312	-25,2252252	-18.5724186	-15.5232155	-26.8884269	-7.9002079
	peptide 22								
peptide conc. (uM)	20	10	5	2.5	1.25	0.625	0.3125	0.15625	0.078125
OD	0.0005	0.6615	0.9375	0.6135	0.8285	0.8465	0.8235	0.8475	0.7595
% of control	0.063870556	84.50074516	119.7572919	78.36917181	105.8335108	108.1328508	105.1948052	108.2605919	97.01937403
% inhibition	99.93612944	15.49925484	-19.7572919	21.63082819	-5.83351075	-8.13285076	-5.19480519	-8.26059187	2.980625931
	peptide 23								
peptide conc. (µM)	20	10	5	2.5	1.25	0.625	0.3125	0.15625	0.078125
OD	-0.0005	0.4925	0.9065	0.7095	0.9215	0.8425	0.8035	0.7755	0.7355
% of control	-0.06387056	62.91249734	115.7973174	90.6323185	117.7134341	107.6218863	102.639983	99.06323185	93.9535874
% inhibition	100.0638706	37.08750266	-15.7973174	9.367681499	-17.7134341	-7.62188631	-2.63998297	0.93676815	6.046412604
	peptide 17								
peptide conc. (µM)	20	10	5	2.5	1.25	0.625	0.3125	0.15625	0.078125
OD	0.0005	0.0235	0.7205	0.6885	0.6515	0.8235	0.8695	0.8905	0.9035
% of control	0.063870556	3.001916117	92.03747073	87.94975516	83.22333404	105.1948052	111.0708963	113.7534597	115.4140941
% inhibition	99.93612944	96.99808388	7.962529274	12.05024484	16.77666596	-5.19480519	-11.0708963	-13.7534597	-15.4140941
	peptide 6S								
peptide conc. (µM)	20	10	5	2.5	1.25	0.625	0.3125	0.15625	0.078125
OD	1.0615	1.0075	0.9675	0.9635	0.9375	0.9705	0.7485	0.7485	0.6625
% of control	147.1240471	139.6396396	134.0956341	133.5412335	129.9376299	134.5114345	103.7422037	103.7422037	91.82259182
% inhibition	-47.1240471	-39.6396396	-34.0956341	-33.5412335	-29.9376299	-34.5114345	-3.74220374	-3.74220374	8.177408173
	AZT								
peptide conc. (µM)	100	20	4	0.8	0.16	0.032	0.0064	0.00128	0.000256
OD	0.8775	0.8645	0.8645	0.9895	0.9685	0.8885	0.9525	0.8645	0.8585
% of control	102.5316456	101.0126582	101.0126582	115.6183057	113.164557	103.8169426	111.2950341	101.0126582	100.311587
% inhibition	-2.53164557	-1.01265823	-1.01265823	-15.6183057	-13 164557	-3.81694255	-11 2950341	-1.01265823	-0.31158715