*Supporting Information*

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

*Wataru Nomura, Haruo Aikawa, Nami Ohashi, Emiko Urano, Mathieu Métifiot, Masayuki Fujino, Kasthuraiah Maddali, Taro Ozaki, Ami Nozue, Tetsuo Narumi, Chie Hashimoto, Tomohiro Tanaka, Yves Pommier, Naoki Yamamoto, Jun A. Komano, Tsutomu Murakami, and Hirokazu Tamamura**

Department of Medicinal Chemistry, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, 2-3-10 Kandasurugadai, Chiyoda-ku, Tokyo 101-0062, JAPAN; AIDS Research Center, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, JAPAN; Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892-4255, USA, Department of Infectious Diseases, Osaka Prefectural Institute of Public Health, 1-3-69 Nakamichi, Higashinari-ku, Osaka 537-0025, JAPAN

*E-mail: tamamura.mr@tmd.ac.jp

## *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 $CH_3CN$ containing 0.1% (v/v) TFA at a flow rate of 1 $cm^3$ $min^{-1}$ 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 $CH_3CN$ solution containing 0.1% (v/v) TFA at a flow rate of 10 $cm^3$ $min^{-1}$ and a Cosmosil 5C18-AR II column (10 x 250 mm, Nacalai Tesque, Inc.) on a JASCO PU-2089 plus (JASCO Corporation, Ltd., Tokyo, Japan) in a suitable gradient mode of $CH_3CN$ solution containing 0.1% (v/v) TFA at a flow rate of 3 $cm^3$ $min^{-1}$.

ESI-TOFMS was recorded on a micrOTOF-2focus (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 $N^{\alpha}$-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, $OBu^{t}$ 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 $Ac_2O$ (100 μL) 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 $Ac_2O$ (100 μL) 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 $N^{\alpha}$-Fmoc and side-chain protected peptides was performed using 20 mol% of Grubbs second generation catalyst in degassed DCE for 2 h at 60 ºC. 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*

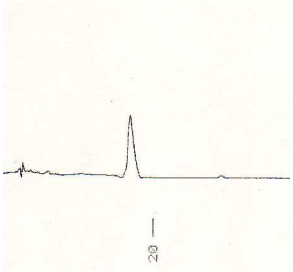
The synthetic peptides were cleaved from the resins with the deprotection of all the protecting groups by treatment with a mixture of TFA, water and triisopropylsilane (9.5/0.25/0.25, v/v) at room temperature for 90 min. The reaction mixtures were filtered, and the resins were washed with TFA (3 times). The filtrates and washed solutions were evaporated under vacuum, and the peptides were precipitated as solid powder by addition of cold $Et_2O$. After centrifugation, the supernatants were removed. The precipitations were washed with cold $Et_2O$ (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.

***Characterization Data of the Synthetic Peptides***
***Stapled Peptides 3S-9S and 11S and Linear Peptides 4L-6L, 8L, 9L and 11L***

| 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 |
| **6L** (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 |

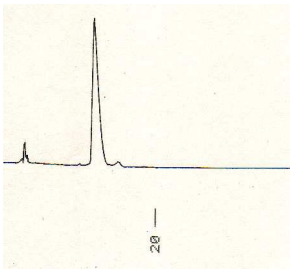
HPLC profiles

**3S**



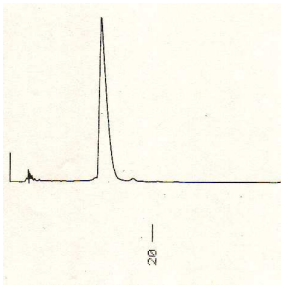
$CH_3CN$ (56%, isocratic)

**4S**



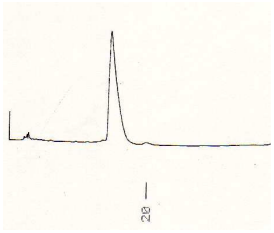
$CH_3CN$ (49%, isocratic)

**5S**



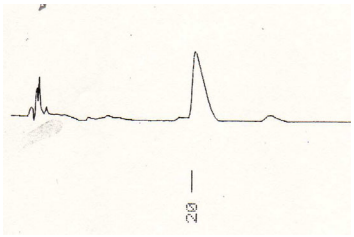
$CH_3CN$ (44%, isocratic)

**6S**



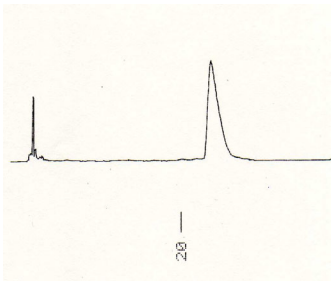
$CH_3CN$ (51%, isocratic)

**7S**



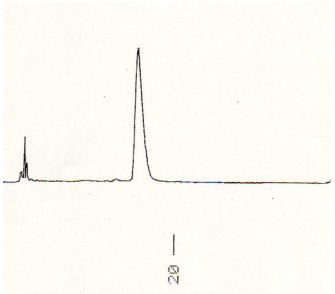
$CH_3CN$ (56%, isocratic)

**8S**



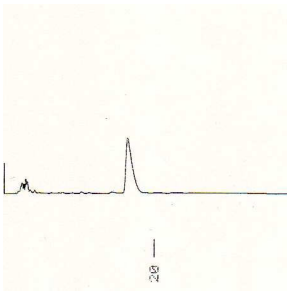

$CH_3CN$ (43%, isocratic)

**9S**



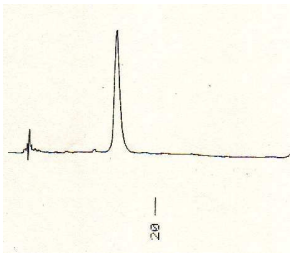

$CH_3CN$ (44%, isocratic)

**4L**



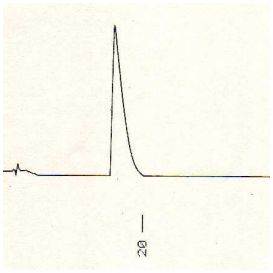

$CH_3CN$ (51%, isocratic)

**5L**



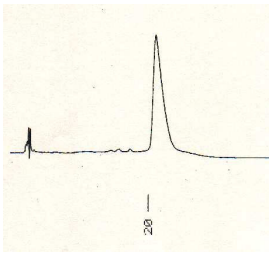

$CH_3CN$ (46%, isocratic)

**6L**



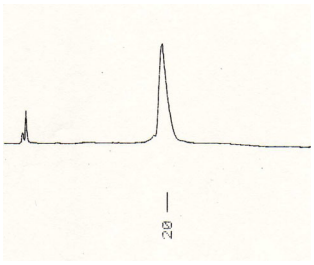
$CH_3CN$ (53%, isocratic)

**8L**



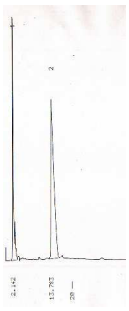
$CH_3CN$ (46%, isocratic)

**9L**



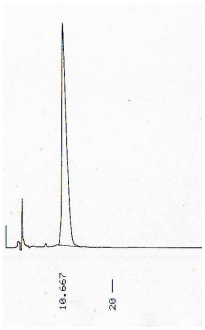
$CH_3CN$ (45%, isocratic)

**11S**



$CH_3CN$ (44%, isocratic)

**11L**



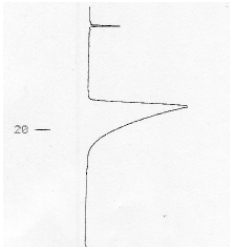

$CH_3CN$ (48%, isocratic)

## *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 |

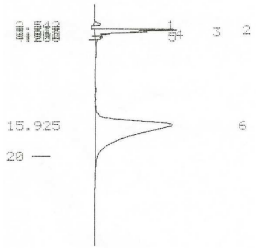
HPLC profiles

**17**



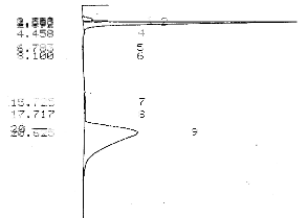

$CH_3CN$ (40%, isocratic)

**18**




$CH_3CN$ (44%, isocratic)

**19**



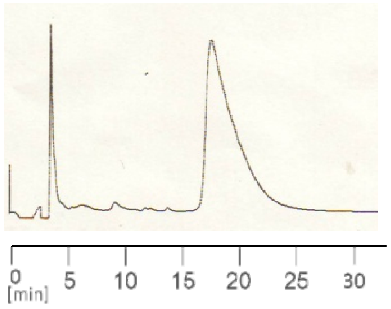
$CH_3CN$ (43%, isocratic)

## *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 |

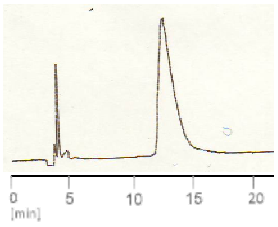
HPLC profiles

**20**



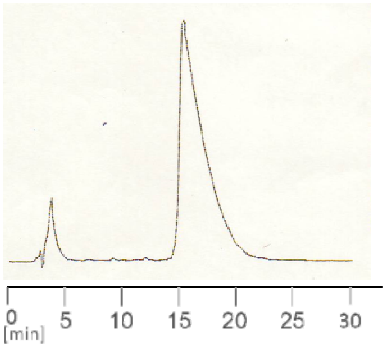

$CH_3CN$ (43%, isocratic)

**21**



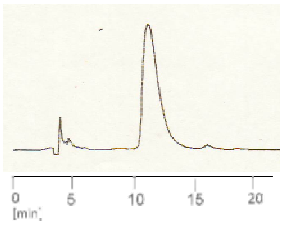

$CH_3CN$ (44%, isocratic)

**22**




$CH_3CN$ (43%, isocratic)

**23**




$CH_3CN$ (43%, isocratic)

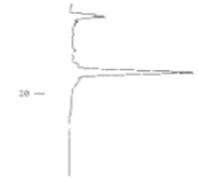
## *Fluorescein-labeled Peptides 3S-F, 6S-F, 8S-F, 6L-F, 1-F and 2-F*

| peptide | sequence |
|---|---|
| **3S-F** (stapled) | Fluorescein-GABA-**c**AII**c**ILQQLLFIHFRIG-$NH_2$ |
| **6S-F** (stapled) | Fluorescein-GABA-EAI**c**RIL**c**QLLFIHFRIG-$NH_2$ |
| **8S-F** (stapled) | Fluorescein-GABA-EAIIR**c**LQQ**c**LFIHFRIG-$NH_2$ |
| **6L-F** (linear) | Fluorescein-GABA-EAI**c**RIL**c**QLLFIHFRIG-$NH_2$ |
| **1-F** | Fluorescein-GABA-EAIIRILQQLLFIHFRIG-$NH_2$ |
| **2-F** | Fluorescein-GABA-EAIIRILQQLLFIHFRIG-$R_8$-$NH_2$ |

| 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**



$CH_3CN$ (56%, isocratic)

**6S-F**

$CH_3CN$ (51%, isocratic)

**8S-F**

$CH_3CN$ (46%, isocratic)

**6L-F**

$CH_3CN$ (53%, isocratic)

**1-F**

$CH_3CN$ (48%, isocratic)

**2-F**

$CH_3CN$ (40%, isocratic)

## *Representative Primary Data for Values of $IC_{50}$, $EC_{50}$ and $CC_{50}$*

**$IC_{50}$ (μM) strand transfer**

**Table 4**

| peptide conc. (μM) | peptide **20** | | | | | | peptide **21** | | | | | | peptide **22** | | | | | | peptide **23** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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) | peptide **17** | | | | | | peptide **6S** | | | | | | peptide **1** | | | | peptide **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 | | | | | | | | | |

**$EC_{50}$ (μM) p24 ELISA**

**Table 3**

| | peptide **17** | | | | peptide **18** | | | | peptide **19** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| peptide conc. (μM) | 10 | 5 | 2.5 | 1.3 | 10 | 5 | 2.5 | 1.3 | 20 | 10 | 5 | 2.5 |
| 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 |
| % inhibition | 116 | 75 | -37 | -45 | 58 | -68 | -35 | -23 | -7 | 2 | 24 | 30 |
| | peptide **6S** | | | | peptide **6L** | | | | peptide **8S** | | | |
| peptide conc. (μM) | 10 | 5 | 2.5 | 1.3 | 10 | 5 | 2.5 | 1.3 | 10 | 5 | 2.5 | 1.3 |
| 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 |
| % inhibition | 116 | 11 | -6 | 1 | -6 | 0 | 7 | 4 | -15 | -8 | 2 | 2 |
| | peptide **8L** | | | | peptide **5S** | | | | peptide **1** | | | |
| 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 |
| % inhibition | -6 | 0 | 5 | 9 | -1 | 3 | 11 | -4 | -31 | 6 | 8 | -2 |
| | peptide **2** | | | | **AZT** | | | | | | | |
| peptide conc. (μM) | 10 | 5 | 2.5 | 1.3 | 0.8 | 0.16 | 0.032 | 0.0064 | | | | |
| OD | -300035 | -104264 | 1531826 | 1550471 | -307027 | -297705 | -66974.1 | 1860442 | | | | |
| % of control | -21 | -7 | 105 | 106 | -21 | -20 | -5 | 127 | | | | |
| % inhibition | 121 | 107 | -5 | -6 | 121 | 120 | 105 | -27 | | | | |

**Table 4**

| | peptide **20** | | | | peptide **21** | | | | peptide **22** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| peptide conc. (μM) | 10 | 5 | 2.5 | 1.25 | 10 | 5 | 2.5 | 1.25 | 10 | 5 | 2.5 | 1.25 |
| 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 |
| % 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 |
| % 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 **23** | | | | peptide **17** | | | | peptide **6S** | | | |
| peptide conc. (μM) | 10 | 5 | 2.5 | 1.25 | 5 | 2.5 | 1.25 | 0.625 | 20 | 10 | 5 | 2.5 |
| 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 | -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 |
| | peptide **1** | | | | peptide **2** | | | | **AZT** | | | |
| peptide conc. (μM) | 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 |
| % 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 | 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.

| **$EC_{50}$ (μM) MTT assay** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |
| **Table 3** | | | | | | | | | |
| | peptide **17** | | | | | | | | |
| peptide conc. (μM) | 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 |
| | peptide **18** | | | | | | | | |
| peptide conc. (μM) | 10 | 5 | 2.5 | 1.3 | 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 | 8 | 83 | 95 | 97 | 97 | 97 | 96 | 97 | 97 |
| | peptide **19** | | | | | | | | |
| peptide conc. (μM) | 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 |
| % of control | 10 | 8 | 4 | 3 | 3 | 3 | 4 | 4 | 4 |
| % inhibition | 90 | 92 | 96 | 97 | 97 | 97 | 96 | 96 | 96 |
| | peptide **6S** | | | | | | | | |
| peptide conc. (μM) | 10 | 5 | 2.5 | 1.3 | 0.6 | 0.3 | 0.2 | 0.1 | 0 |
| OD | 1 | 1.225 | 0.084 | 0.04 | 0.041 | 0.04 | 0.038 | 0.047 | 0.045 |
| % of control | 75 | 92 | 6 | 3 | 3 | 3 | 3 | 4 | 3 |
| % inhibition | 25 | 8 | 94 | 97 | 97 | 97 | 97 | 96 | 97 |
| | 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 **5S** | | | | | | | | |
| 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** | | | | | | | | |
| peptide conc. (μM) | 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 |
| | peptide **2** | | | | | | | | |
| peptide conc. (μM) | 10 | 5 | 2.5 | 1.3 | 0.6 | 0.3 | 0.2 | 0.1 | 0 |
| OD | 0.009 | 0.714 | 0.348 | 0.101 | 0.05 | 0.043 | 0.043 | 0.047 | 0.048 |
| % of control | 1 | 54 | 26 | 8 | 4 | 3 | 3 | 4 | 4 |
| % inhibition | 99 | 46 | 74 | 92 | 96 | 97 | 97 | 96 | 96 |
| | **AZT** | | | | | | | | |
| peptide conc. (μM) | 100 | 20 | 4 | 0.8 | 0.16 | 0.032 | 0.006 | 0.001 | 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) MIT assay** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |
| **Table 4** | | | | | | | | | |
| | peptide **20** | | | | | | | | |
| peptide conc. (μM) | 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 | 0.0195 | 0.0205 |
| % 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 | 99.76280835 |
| | peptide **21** | | | | | | | | |
| peptide conc. (μM) | 20 | 10 | 5 | 2.5 | 1.25 | 0.625 | 0.3125 | 0.15625 | 0.078125 |
| OD | 0.0025 | 0.4495 | 0.9015 | 0.1135 | 0.0385 | 0.0275 | 0.0235 | 0.0225 | 0.0175 |
| % of control | -2.32447818 | 61.29032258 | 125.6166983 | 13.47248577 | 2.79886148 | 1.233396584 | 0.664136622 | 0.521821632 | -0.18975332 |
| % inhibition | 102.3244782 | 38.70967742 | -25.6166983 | 86.52751423 | 97.20113852 | 98.76660342 | 99.33586338 | 99.47817837 | 100.1897533 |
| | peptide **22** | | | | | | | | |
| peptide conc. (μM) | 20 | 10 | 5 | 2.5 | 1.25 | 0.625 | 0.3125 | 0.15625 | 0.078125 |
| OD | 0.0005 | 0.0995 | 0.4255 | 0.0655 | 0.0355 | 0.0275 | 0.0145 | 0.0145 | 0.0185 |
| % of control | -0.94623656 | 11.82795699 | 53.89247312 | 7.440860215 | 3.569892473 | 2.537634409 | 0.860215054 | 0.860215054 | 1.376344086 |
| % inhibition | 100.9462366 | 88.17204301 | 46.10752688 | 92.55913978 | 96.43010753 | 97.46236559 | 99.13978495 | 99.13978495 | 98.62365591 |
| | peptide **23** | | | | | | | | |
| peptide conc. (μM) | 20 | 10 | 5 | 2.5 | 1.25 | 0.625 | 0.3125 | 0.15625 | 0.078125 |
| OD | 0.0005 | 0.2595 | 0.2145 | 0.0245 | 0.0205 | 0.0215 | 0.0105 | 0.0115 | 0.0145 |
| % 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 | 73.33333333 | 97.84946237 | 98.3655914 | 98.23655914 | 99.65591398 | 99.52688172 | 99.13978495 |
| | peptide **17** | | | | | | | | |
| peptide conc. (μM) | 20 | 10 | 5 | 2.5 | 1.25 | 0.625 | 0.3125 | 0.15625 | 0.078125 |
| OD | 0.0005 | 0.0045 | 0.2285 | 0.0335 | 0.0225 | 0.0195 | 0.0085 | 0.0115 | 0.0135 |
| % of control | -0.94623656 | -0.43010753 | 28.47311828 | 3.311827957 | 1.892473118 | 1.505376344 | 0.086021505 | 0.47311828 | 0.731182796 |
| % inhibition | 100.9462366 | 100.4301075 | 71.52688172 | 96.68817204 | 98.10752688 | 98.49462366 | 99.91397849 | 99.52688172 | 99.2688172 |
| | peptide **6S** | | | | | | | | |
| peptide conc. (μM) | 20 | 10 | 5 | 2.5 | 1.25 | 0.625 | 0.3125 | 0.15625 | 0.078125 |
| OD | 1.0155 | 0.9175 | 0.0725 | 0.0265 | 0.0175 | 0.0165 | 0.0175 | 0.0175 | 0.0165 |
| % of control | 141.8406072 | 127.8937381 | 7.637571157 | 1.091081594 | -0.18975332 | -0.33206831 | -0.18975332 | -0.18975332 | -0.33206831 |
| % inhibition | -41.8406072 | -27.8937381 | 92.36242884 | 98.90891841 | 100.1897533 | 100.3320683 | 100.1897533 | 100.1897533 | 100.3320683 |
| | **AZT** | | | | | | | | |
| peptide conc. (μM) | 100 | 20 | 4 | 0.8 | 0.16 | 0.032 | 0.0064 | 0.00128 | 0.000256 |
| OD | 0.6065 | 0.9595 | 0.9005 | 0.9285 | 0.7885 | 0.0515 | 0.0185 | 0.0075 | 0.0145 |
| % of control | 70.24661893 | 112.3707239 | 105.3301512 | 108.6714399 | 91.96499602 | 4.017501989 | 0.079554495 | -1.23309467 | -0.39777247 |
| % inhibition | 29.75338107 | -12.3707239 | -5.33015115 | -8.67143994 | 8.035003978 | 95.98249801 | 99.92044551 | 101.2330947 | 100.3977725 |

| **$CC_{50}$ (μM) MTT assay** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |
| **Table 3** | | | | | | | | | |
| | peptide **17** | | | | | | | | |
| peptide conc. (μM) | 20 | 10 | 5 | 2.5 | 1.3 | 0.6 | 0.3 | 0.2 | 0.1 |
| OD | 0.006 | 0.061 | 0.968 | 1.072 | 1.036 | 0.981 | 1.093 | 1.336 | 0.808 |
| % of control | 1 | 6 | 93 | 103 | 99 | 94 | 105 | 128 | 78 |
| % inhibition | 99 | 94 | 7 | -3 | 1 | 6 | -5 | -28 | 22 |
| | peptide **18** | | | | | | | | |
| peptide conc. (μM) | 10 | 5 | 2.5 | 1.3 | 0.6 | 0.3 | 0.2 | 0.1 | 0 |
| OD | 1.0915 | 1.2785 | 1.3395 | 1.3935 | 1.3155 | 1.1325 | 1.1285 | 1.2435 | 0.8595 |
| % of control | 79 | 92 | 97 | 100 | 95 | 82 | 81 | 90 | 62 |
| % inhibition | 21 | 8 | 3 | 0 | 5 | 18 | 19 | 10 | 38 |
| | peptide **19** | | | | | | | | |
| peptide conc. (μM) | 10 | 5 | 2.5 | 1.3 | 0.6 | 0.3 | 0.2 | 0.1 | 0 |
| OD | 1.0705 | 1.1125 | 1.6235 | 1.3395 | 1.2045 | 1.1935 | 1.4045 | 1.4195 | 1.1035 |
| % of control | 77 | 80 | 117 | 97 | 87 | 86 | 101 | 102 | 80 |
| % inhibition | 23 | 20 | -17 | 3 | 13 | 14 | -1 | -2 | 20 |
| | peptide **6S** | | | | | | | | |
| peptide conc. (μM) | 10 | 5 | 2.5 | 1.3 | 0.6 | 0.3 | 0.2 | 0.1 | 0 |
| OD | 1.2 | 1.335 | 1.505 | 1.28 | 1.435 | 1.169 | 0.95 | 1.097 | 1.134 |
| % of control | 90 | 101 | 113 | 96 | 108 | 88 | 72 | 83 | 85 |
| % inhibition | 10 | -1 | -13 | 4 | -8 | 12 | 28 | 17 | 15 |
| | peptide **6L** | | | | | | | | |
| peptide conc. (μM) | 10 | 5 | 2.5 | 1.3 | 0.6 | 0.3 | 0.2 | 0.1 | 0 |
| OD | 1.0745 | 1.2185 | 1.2955 | 1.5585 | 1.0075 | 1.2775 | 1.3365 | 1.4385 | 0.7205 |
| % of control | 98 | 111 | 118 | 142 | 92 | 117 | 122 | 131 | 66 |
| % inhibition | 2 | -11 | -18 | -42 | 8 | -17 | -22 | -31 | 34 |
| | peptide **8S** | | | | | | | | |
| peptide conc. (μM) | 10 | 5 | 2.5 | 1.3 | 0.6 | 0.3 | 0.2 | 0.1 | 0 |
| OD | 1.3385 | 1.2735 | 1.3795 | 1.0535 | 1.5005 | 0.9835 | 1.4335 | 1.4645 | 0.4955 |
| % of control | 122 | 116 | 126 | 96 | 137 | 90 | 131 | 134 | 45 |
| % inhibition | -22 | -16 | -26 | 4 | -37 | 10 | -31 | -34 | 55 |
| | peptide **8L** | | | | | | | | |
| peptide conc. (μM) | 10 | 5 | 2.5 | 1.3 | 0.6 | 0.3 | 0.2 | 0.1 | 0 |
| OD | 1.0575 | 0.9885 | 1.1385 | 1.0675 | 1.2825 | 1.2255 | 1.1865 | 1.4475 | 1.3565 |
| % of control | 97 | 90 | 104 | 97 | 117 | 112 | 108 | 132 | 124 |
| % inhibition | 3 | 10 | -4 | 3 | -17 | -12 | -8 | -32 | -24 |
| | peptide **5S** | | | | | | | | |
| peptide conc. (μM) | 10 | 5 | 2.5 | 1.3 | 0.6 | 0.3 | 0.2 | 0.1 | 0 |
| OD | 1.2575 | 1.2075 | 1.3615 | 1.3785 | 1.3795 | 1.5485 | 1.5665 | 1.3165 | 1.3495 |
| % of control | 79 | 76 | 85 | 86 | 87 | 97 | 98 | 83 | 85 |
| % inhibition | 21 | 24 | 15 | 14 | 13 | 3 | 2 | 17 | 15 |
| | peptide **1** | | | | | | | | |
| peptide conc. (μM) | 10 | 5 | 2.5 | 1.3 | 0.6 | 0.3 | 0.2 | 0.1 | 0 |
| OD | 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** | | | | | | | | |
| peptide conc. (μM) | 10 | 5 | 2.5 | 1.3 | 0.6 | 0.3 | 0.2 | 0.1 | 0 |
| OD | 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 | 97 |
| % 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 |
| OD | 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 |
| % inhibition | 37 | 40 | 23 | 20 | 5 | 17 | 29 | 25 | -12 |

| **$CC_{50}$ (μM) MTT assay** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |
| **Table 4** | | | | | | | | | |
| | peptide **20** | | | | | | | | |
| peptide conc. (μM) | 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. (μM) | 20 | 10 | 5 | 2.5 | 1.25 | 0.625 | 0.3125 | 0.15625 | 0.078125 |
| OD | 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. (μM) | 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 | 97.01937407 |
| % 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 | 8.177408177 |
| | **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 | 100.3115871 |
| % inhibition | -2.53164557 | -1.01265823 | -1.01265823 | -15.6183057 | -13.164557 | -3.81694255 | -11.2950341 | -1.01265823 | -0.31158715 |