SUPPORTING INFORMATION

Proteolytically Stable Cancer Targeting Peptides with High Affinity for Breast Cancer Cells

Rania Soudy[⊥], Avneet Gill[⊥], Tara Sprules[#], Afsaneh Lavasanifar[⊥], and Kamaljit Kaur[⊥]*

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Table S1. Peptide degradation in the presence of human serum (A) and liver homogenate (B).

(A)

Time (h)	% Intact Peptide[a]			
	18	**18-4**	**18-9**	**18-10**
0	100	100	100	100
0.5	5	100	100	100
1	0	100	100	100
5	0	100	100	100
24	0	100	100	100

[a]Percent intact peptide was calculated based on the area under the HPLC peak for the parent peptide.

(B)

Time (min/h)	% Intact Peptide[a]			
	18	**18-4**	**18-9**	**18-10**
0	100	100	100	100
5 min	35	100	100	100
15 min	3	100	100	100
30 min	0	100	100	100
1 h	0	100	100	100
6 h	0	100	100	100
24 h	0	100	100	100
48 h	0	100	100	100

[a]Percent intact peptide was calculated based on the area under the HPLC peak for the parent peptide.

Table S2. Chemical shift assignments for peptide p160 in water and TFE, and peptide 18 in TFE/water (4:1).

P160 Chemical Shifts, H_2O, 15ºC, referenced to H_2O at 4.868 ppm				
Residue	**NH**	**Hα**	**Hβ**	**Other**
Val 1	-	4.13	2.06	γCH_3 0.87, 1.02
Pro 2	-	4.48	1.90, 2.30	γCH_2 1.99, 2.00, δCH_2 3.72, 3.54
Trp 3	8.16	4.58	3.30, 3.21	2H 7.22, 4H 7.50, 5H 7.26, 6H 7.16, 7H 7.61, NH 10.25
Nle 4	7.58	4.17	1.44, 1.56	γCH_2 1.08, δCH_2 1.19, εCH_3 0.80
Glu 5	8.12	4.29	1.86, 1.99	γCH_2 2.29
Pro 6	-	4.28	1.78, 2.23	γCH_2 2.0, δCH_2 3.71, 3.79
Ala 7	8.49	4.21	1.30	
Tyr 8	7.99	4.51	3.01	2,6H 7.09, 3,5H 6.81
Gln 9	8.10	4.14	1.89, 1.84	γCH_2 2.15, δNH_2 7.50, 6.89
Arg 10	8.11	4.19	1.63	γCH_2 1.44, δCH_2 3.11, εNH 7.17
Phe 11	8.29	4.68	3.24, 2.95	2,6H 7.28, 3,5H 7.33
Leu 12	7.85	4.29	1.58	γCH 1.58, δCH_3 0.90, 0.86

P160 Chemical Shifts, TFE, 15ºC, referenced to TFE at 3.88 ppm				
Residue	**NH**	**Hα**	**Hβ**	**Other**
Val 1	-	3.83	1.23	γCH_3 0.46, 0.85
Pro 2	-	4.33	2.06, 2.34	γCH_2 2.06, 2.17, δCH_2 3.38, 3.56
Trp 3	6.71	4.68	3.28, 3.48	2H 7.09, 4H 7.50, 5H 7.32, 6H 7.22, 7H 7.59, NH 9.41
Nle 4	6.94	4.46	1.43	γCH_2 1.76, δCH_2 1.13, εCH_3 0.94
Glu 5	7.43	4.54	2.06, 2.14	γCH_2 2.52
Pro 6	-	4.31	1.81,2.30	γCH_2 2.03,2.09, δCH_2 3.65, 3.78
Ala 7	7.41	4.15	1.45	
Tyr 8	7.52	4.49	3.20	2,6H 7.10, 3,5H 6.84
Gln 9	7.98	4.14	2.08, 2.14	γCH_2 2.42, δNH_2 7.09, 6.14
Arg 10	7.52	4.15	1.70	γCH_2 1.35, 1.45, δCH_2 3.06, εNH 6.66
Phe 11	7.66	4.70	3.03, 3.30	2,6H 7.30, 3,5H 7.24
Leu 12	7.56	4.50	1.73	γCH 1.69, δCH_3 0.96

Peptide **18** Chemical Shifts, 80% TFE, 20% H_2O 15ºC, referenced to TFE at 3.88 ppm				
Residue	**NH**	**Hα**	**Hβ**	**Other**
Trp 1	-	4.32	3.41, 3.52	2H 7.28, 4H 7.47, 5H 7.24, 6H 7.15, 7H 7.63, NH 9.74
Nle 2	7.89	3.98	1.50, 1.71	γCH_2 0.96, δCH_2 1.22, εCH_3 0.82
Glu 3	8.97	4.22	2.03, 1.96	γCH_2 2.41
Ala 4	7.82	4.10	1.39	
Ala 5	7.76	4.22	1.38	
Tyr 6	7.55	4.44	3.07, 3.12	2,6H 7.09, 3,5H 6.81
Gln 7	7.77	4.10	2.04	γCH_2 2.23, 2.30, δNH_2 7.14, 6.38
Arg 8	7.66	4.18	1.666	γCH_2 1.35, 1.43, δCH_2 3.05, εNH 7.01
Phe 9	7.77	4.69	2.98, 3.28	2,6H 7.28, 3,5H 7.21
Leu 10	7.50	4.14	1.66	γCH 1.72, δCH_3 0.89, 0.91

Table S3. Structure calculation statistics for peptides p160 and **18**.

	P160 in H_2O	**P160 in TFE**	**P18 in TFE/H_2O (8:2)**
NOE upper distance limits	109	175	176
Intra-residue	41	53	55
Sequential	57	71	72
Medium range (i to i-2 or i+3)	11	51	49
Long range (i to i+4)	0	7	13
Final CYANA structures			
CYANA target function	$6.0 \times 10^{-2} \pm 1.74 \times 10^{-3}$ Å^2	0.13 ± 0 Å^2	$0.21 \pm 2.9 \times 10^{-2}$ Å^2
Average backbone RMSD to mean	1.81 ± 0.47 Å (1-12)	0.41 ± 0.10 Å (1-12)	0.61 ± 0.36 Å (1-10)
Average heavy atom RMSD to mean	3.10 ± 0.53 Å (1-12)	0.82 ± 0.10 Å (1-12)	1.32 ± 0.64 Å (1-10)
Distance restraint violations	1	0	0

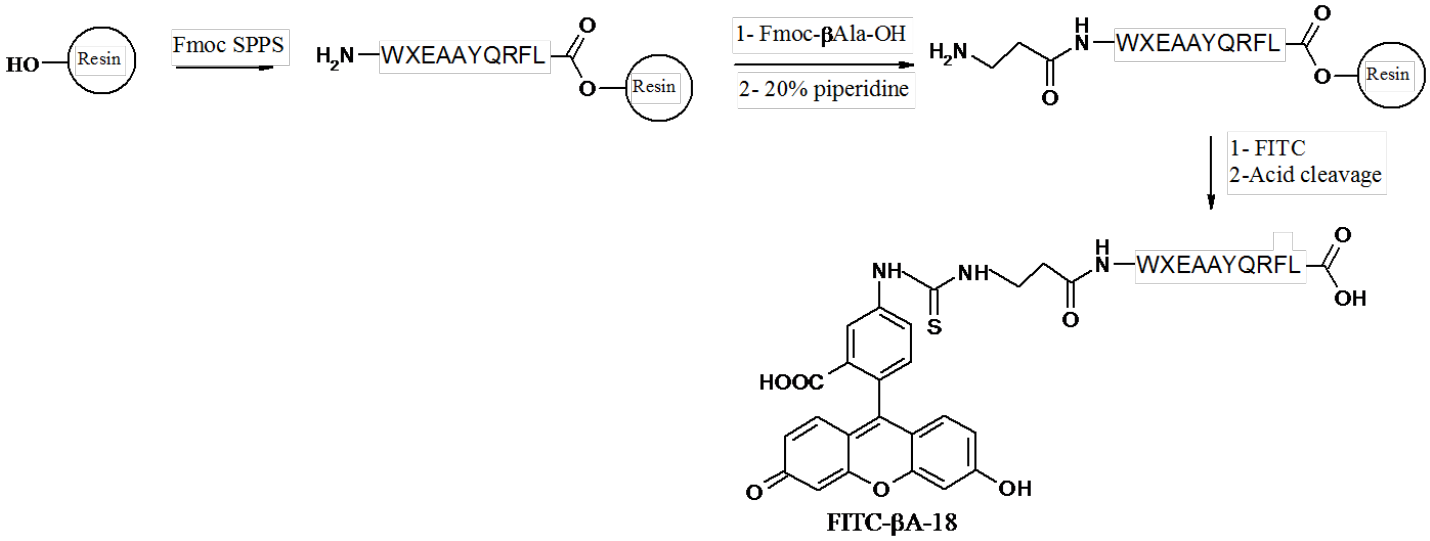
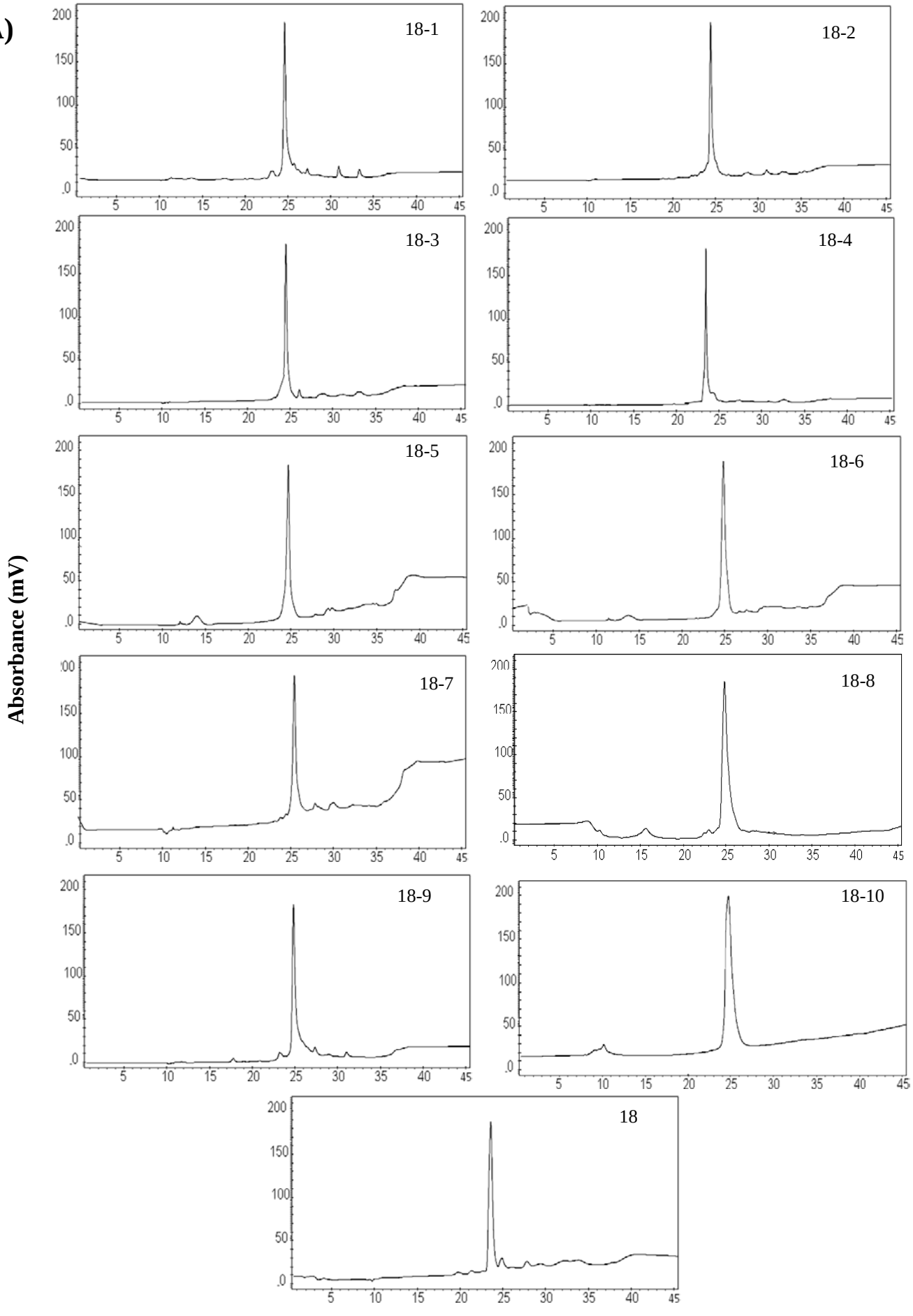


Figure S1. Solid phase peptide synthesis of FITC-βA-**18** or FITC-**18**.

(A)


18-1
18-2
18-3
18-4
18-5
18-6
18-7
18-8
18-9
18-10
18
Absorbance (mV)
Time (min)

(B)

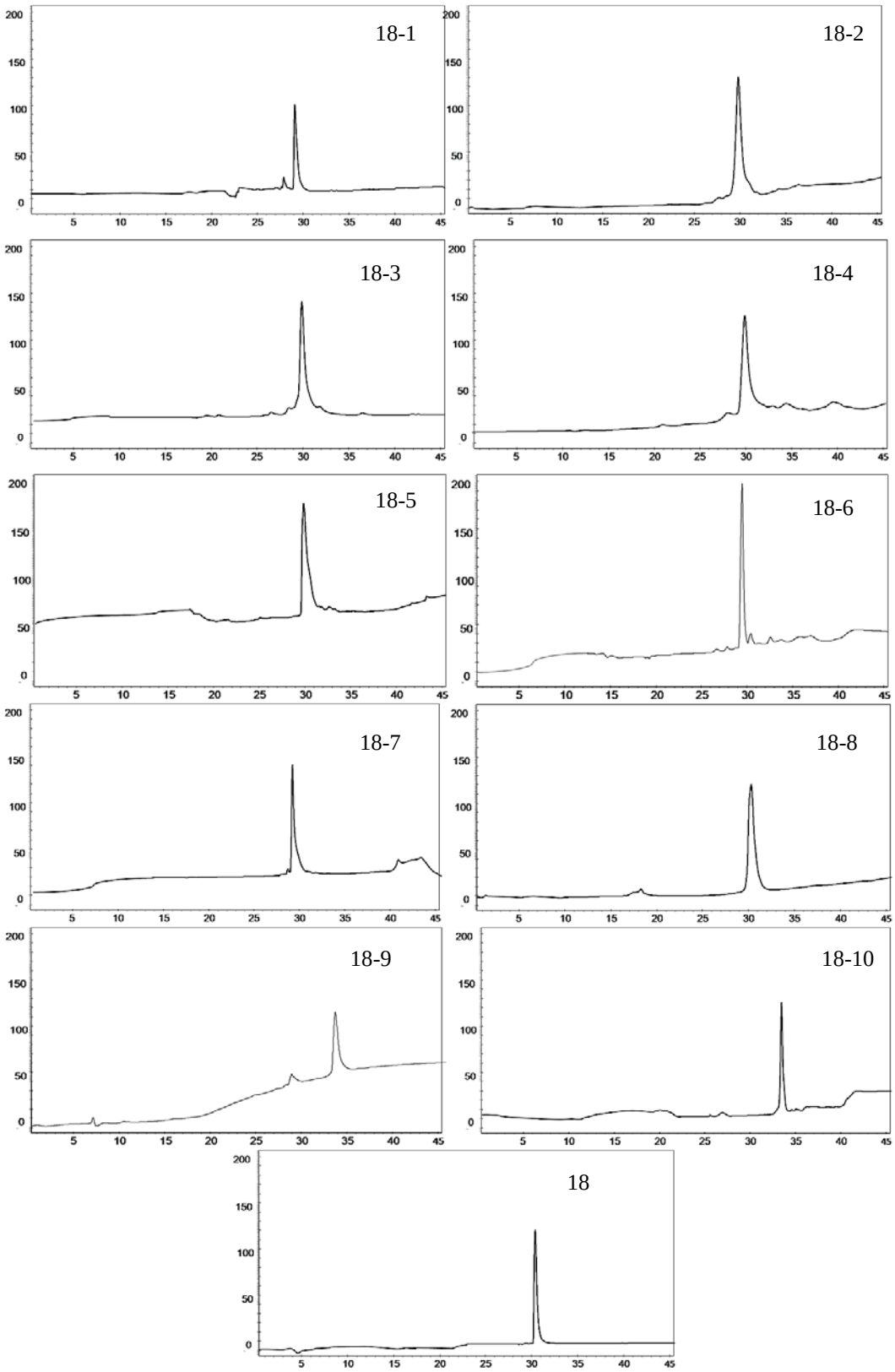
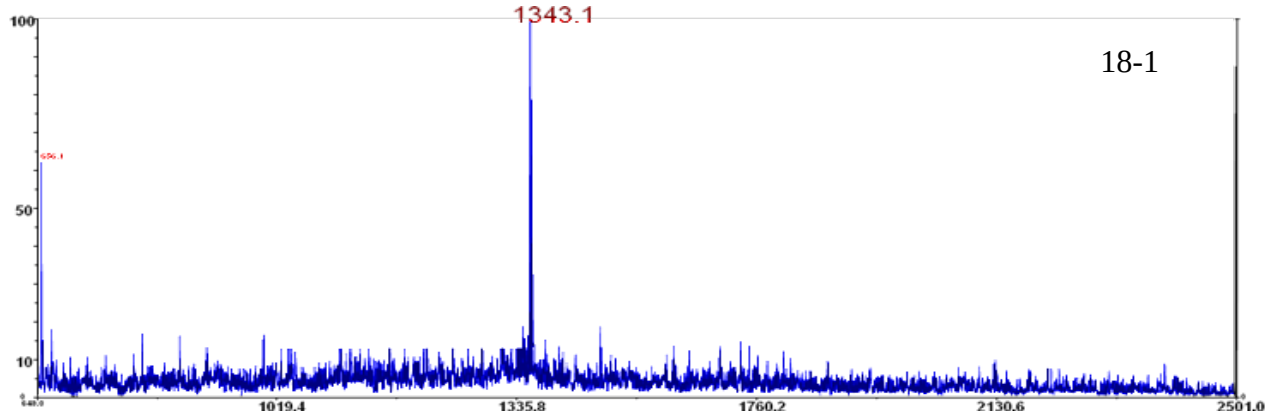
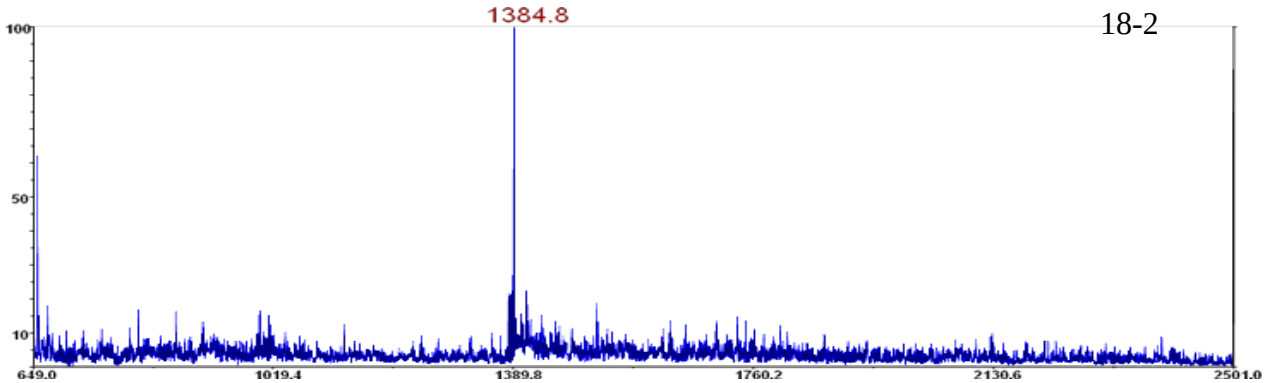


Figure S2. Analytical RP-HPLC chromatograms of the pure FITC-β-ala-peptides (**18-1** - **18-10**) and parent peptide **18** (FITC-β-ala-**18**) in two solvent systems, namely, (A) IPA/water or solvent 1 and (B) acetonitrile/water or solvent 2. The HPLC method used for solvent 1 was 15-50% IPA/water in 35 min with a flow rate of 1 mL/min and for solvent 2 was 15-55% acetonitrile/water in 35 min with a flow rate of 1 mL/min (Vydac C18 analytical column).



18-1



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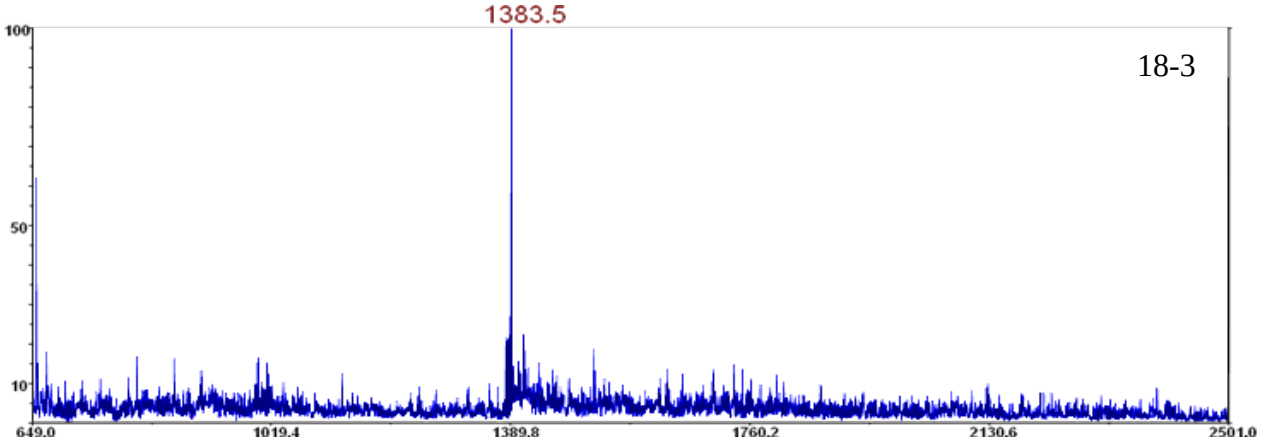

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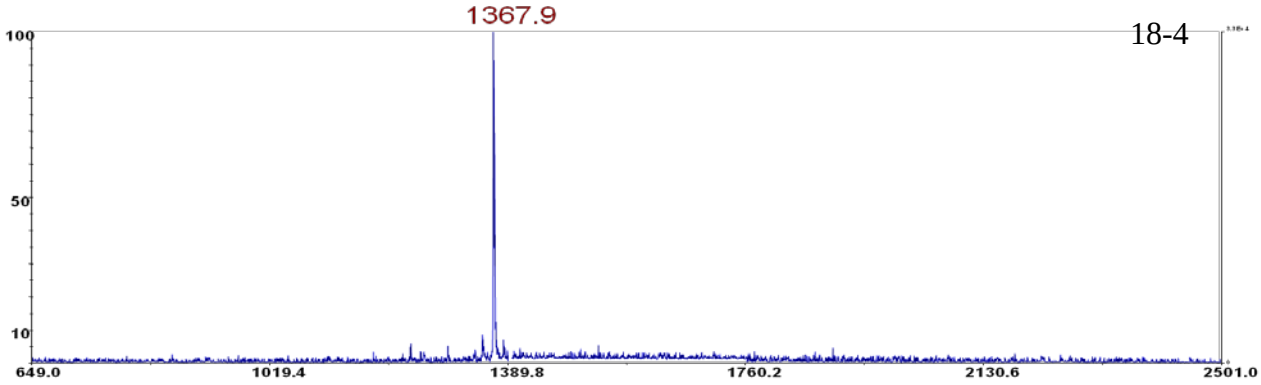
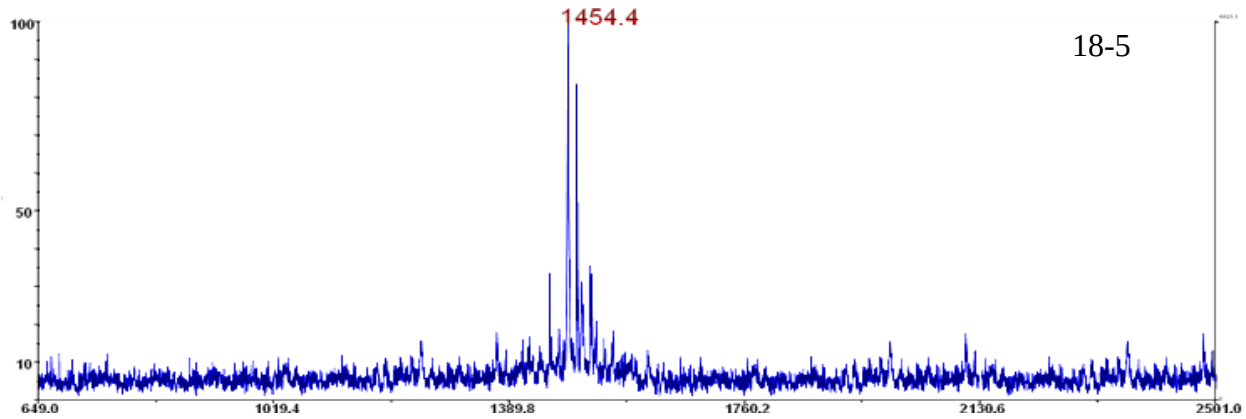


18-4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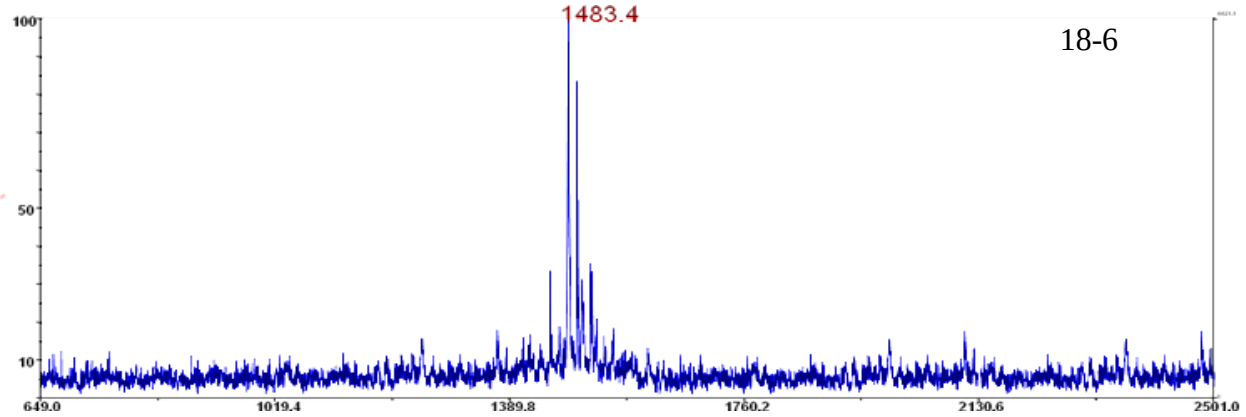
18-5
100
50
10
649.0
1019.4
1389.8
1760.2
2130.6
2501.0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50
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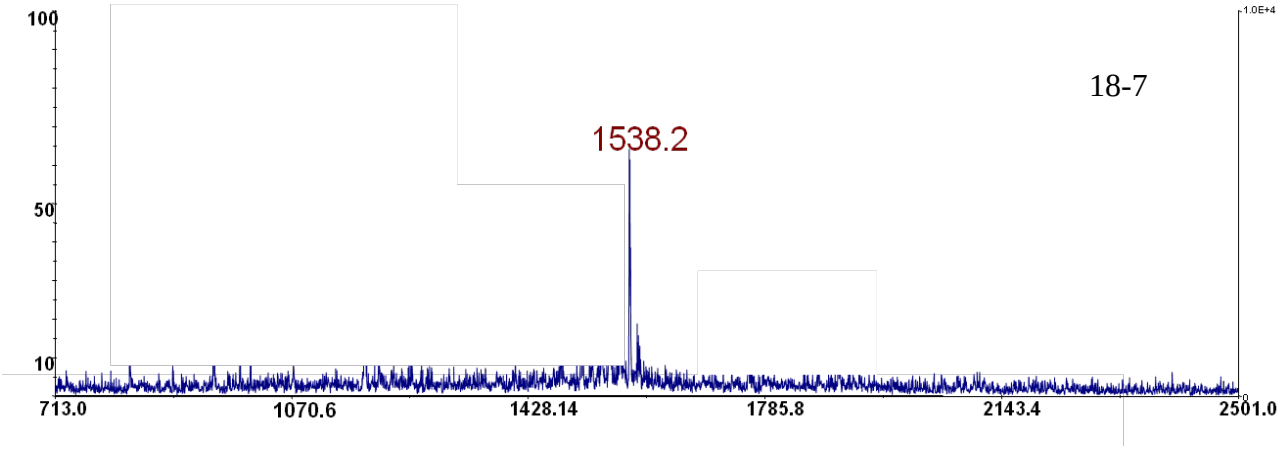
2501.0


1538.2
18-7
100
50
10
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713.0
1070.6
1428.14
1785.8
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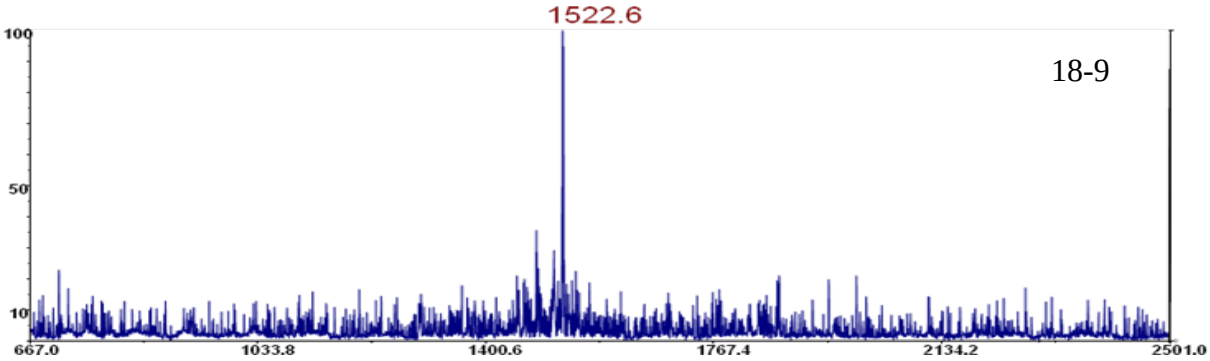
2501.0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667.0
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2134.2
2501.0

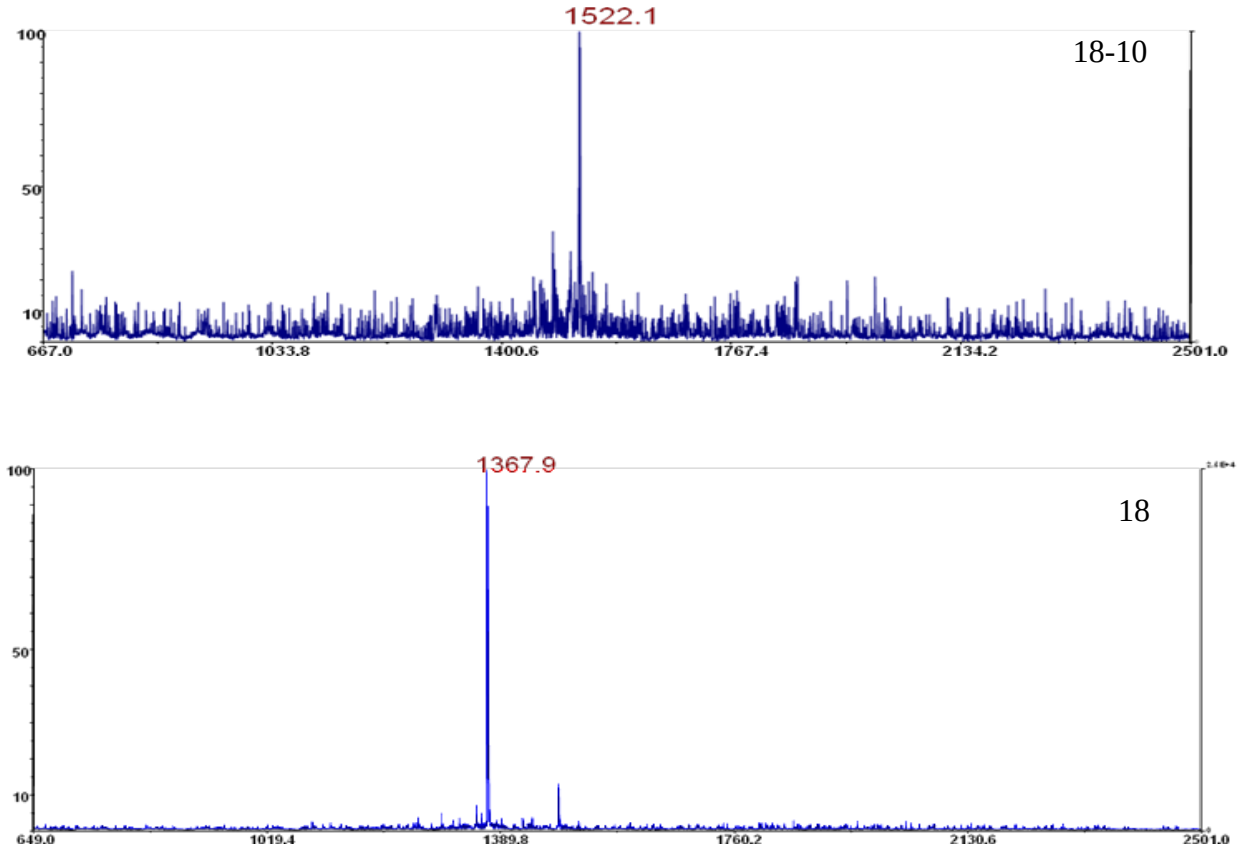

Figure S3. MALDI-TOF of β-ala-peptides showing $[M+H]^+$ peaks.

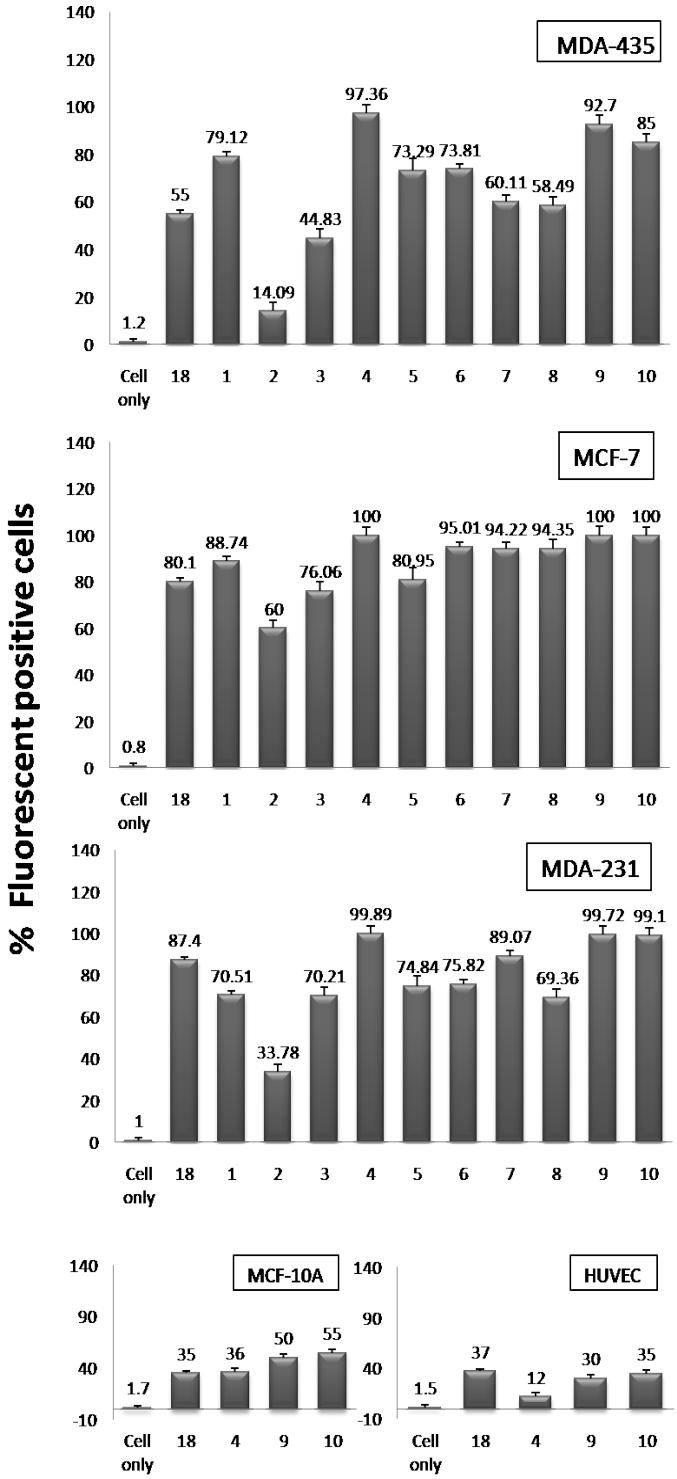

Figure S4. Fluorescence of the cells observed after uptake of the FITC-labeled peptides using flow cytometry. The FITC-labeled peptides (10^{-5} mol/L) were incubated with breast cancer cell lines MDA-MB-435, MCF-7, and MDA-MB-231, or control cell lines MCF-10A and HUVEC for 30 min prior to FACS analysis. Bars represent the percent number of fluorescent cells bound with the peptide as mean (SD of data obtained from three separate experiments).

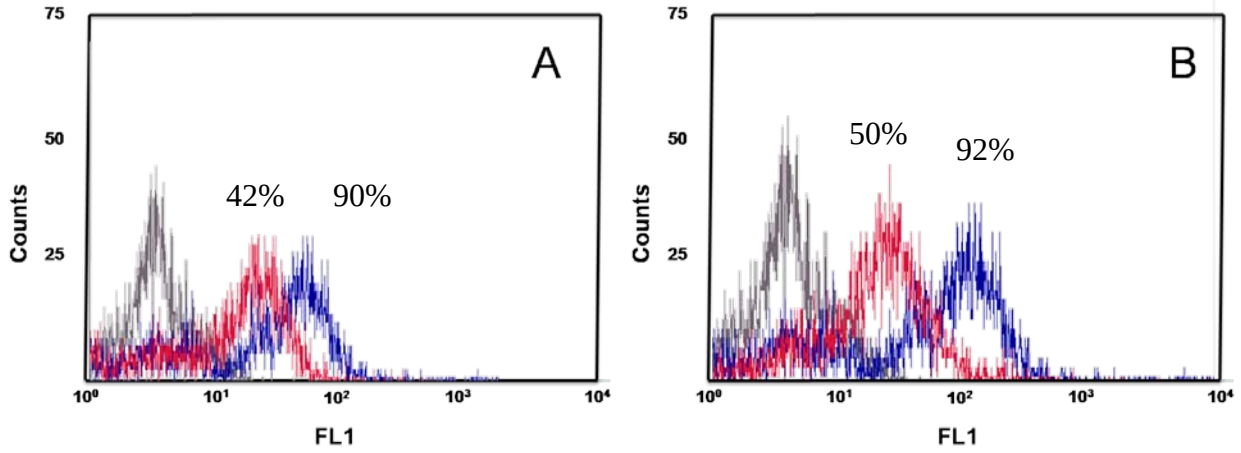

Figure S5. FACS analysis for the competitive binding of the peptides **18-4** (A) and **18-9** (B) to MDA-MB-435 cells, showing autofluorescence of MDA-MB-435 cells (grey), fluorescence (blue) of the cells after incubation with 10^{-5} mol/L FITC-**18-4** (A) or FITC-**18-9** (B), and fluorescence (red) of cells after incubation with the FITC-peptides in the presence of 50-fold excess unlabeled **18-4** (A) or **18-9** (B). Experiments were done using Beckman Coulter QUANTA SC flow cytometer.

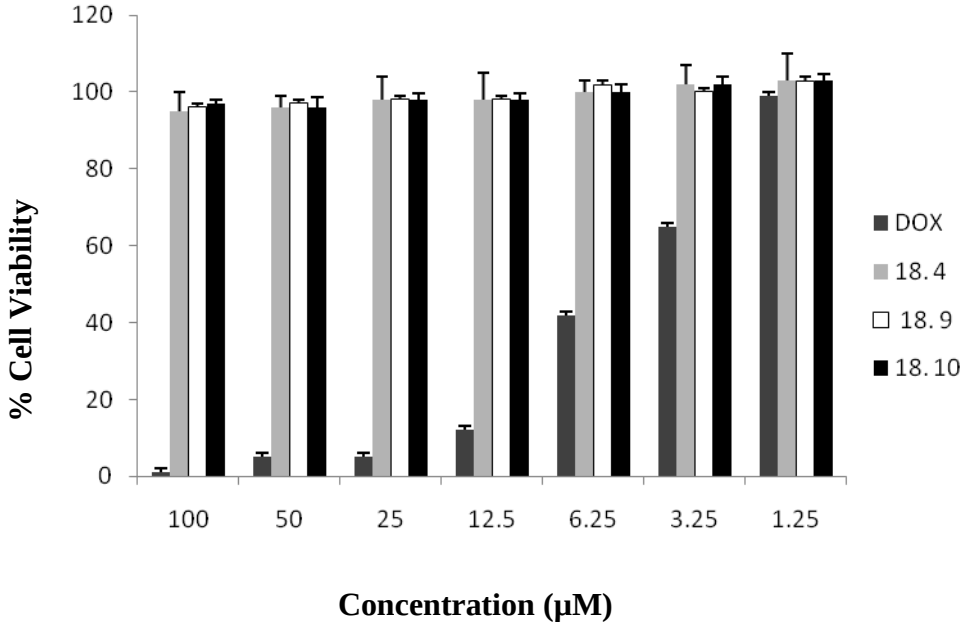

Figure S6. Cytotoxicity of peptides **18-4** (grey), **18-9** (white) and **18-10** (black) compared to doxorubicin (dark grey) monitored in MDA-MB-435 cancer cell line using the MTT assay. Cells were incubated with different concentration of peptides for 48 h. The data represent the mean ± S.D of two independent experiments, and each concentration was done in triplicate.