

**MIR**  
**Discovery and Imaging Services**  
Formerly MIR Preclinical Services

Final Report to: Sanare S.A.

Study: SANA200805R1a (MIR1007)

Date: December 29, 2008

**EC<sub>50</sub> Determination for Peptides 13-24, Doxorubicin and Cisplatin as Single Agents  
and a Combination Peptide Mixture (13-24) against MCF-7 Human Pleural Effusion  
Breast Adenocarcinoma Cells**

## Executive Digest

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Sanare Peptides 13-24 were assayed for antiproliferative activity against MCF-7 human pleural effusion breast adenocarcinoma cells. The Sanare Peptides, as single agents and as a combination mixture, were serially diluted (1:5) starting at the individual peptide concentrations (based on solubility) listed in the table below and 1mM for both doxorubicin and cisplatin as the positive control single agents.

Peptide	Starting Treatment Concentration ( $\mu\text{M}$ )
13	250
14	250
15	125
16	31.25
17	3.91
18	250
19	250
20	125
21	125
22	31.25
23	250
24	62.5
Mixture (13-24)	Mixture of the Conc. above

Serially diluted peptides were added to the cells daily for four days for a total exposure of 96 hours. Cell proliferation was measured using MTT reduction at 96 hours post-treatment, reading the absorbance at 570nm and converting the values to Percent of Control to calculate the  $\text{EC}_{50}$  values.

MCF-7 cells treated with Peptides 17, 20, 21, 23 as single agents as well as with the Mixture (Peptides 13-24) produced the best antiproliferative activity based on the  $\text{EC}_{50}$  values from the MTT assay.

*Note: For the combination treatment with the peptide mixture, each day 100 $\mu\text{l}$  of culture supernatant was replaced with 100 $\mu\text{L}$  peptides in 48%PBS/52%media. This resulted in the nutrient rich media for the cells in the highest dosage level wells becoming more dilute with each consecutive daily treatment. This may have had a negative effect on cell proliferation in conjunction with any peptide treatment-related antiproliferative activity.*

A summary table of the  $\text{EC}_{50}$  results is displayed on the following page.

### EC<sub>50</sub> Results for SANA200805R1a (MIR1007)

Test Agent	EC <sub>50</sub> (μM)
Peptide 13-4061559	>250
Peptide 14-4061560	>250
Peptide 15-4061561	>125
Peptide 16-4061562	>31.25
Peptide 17-4061563	5.98
Peptide 18-4061564	>250
Peptide 19-4061565	>250
Peptide 20-4061566	124*
Peptide 21-4061567	122*
Peptide 22-4061568	>31.25
Peptide 23-4061569	250*
Peptide 24-4061570	>62.5
Peptide Mixture (13-24)	70*
Doxorubicin	0.05
Cisplatin	4.7

\* EC<sub>50</sub> values for peptides 20, 21, 23 and the mixture were obtained by constraining parameter "D" of the 4-parameter equation (i.e.- D = 0 for peptides 20, 21 and 23 and D = -30 for the mixture) since complete inhibition was not observed at the highest concentrations tested. This improves the accuracy of the EC<sub>50</sub> estimate. Similarly, the "A" parameter was constrained to 120 for Doxorubicin since its dose response curve was incomplete at the lowest concentrations tested.

All raw data and dose response curves are presented in Appendix III.

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## **Introduction**

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SANA200805R1a (MIR1007) was designed to determine the antiproliferative effects of exposure to Peptides 13-24 as single agents and the Peptide Mixture (13-24) as a combination therapy against MCF-7 cells (Human Pleural Effusion Breast Adenocarcinoma). Doxorubicin and Cisplatin served as the positive control agents.

Following exposure of the cells to test agents for 96 hours, proliferation was measured using an MTT assay. The MTT assay is based on the reduction of a yellow tetrazolium salt (MTT) by metabolically active cells to form purple formazan crystals which are subsequently solubilized in detergent and the absorbance at 570nm measured. The effect of the compounds on cell proliferation was compared to cells not exposed to compounds (No Drug Control) and expressed as Percent of Control treatment response. The data was plotted as Compound Concentration versus Percent of Control and analyzed by SoftMax® Pro software (Molecular Devices) to calculate EC<sub>50</sub> values.

## **Materials and Methods**

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### *Cell Culture*

The MCF-7 Human Pleural Effusion Breast Adenocarcinoma cell line was obtained from the National Cancer Institute (NCI). Cell cultures were established using standard *in vitro* culture methods and NCI recommended media (Appendix I) in 175cm<sup>2</sup> Greiner® tissue culture treated flasks. All cultures were incubated in humidified 37°C, 5% CO<sub>2</sub>, 95% air incubators. The cells were sub-cultured regularly to maintain log phase growth.

MCF-7 was brought up from cryopreservation using RPMI1640 media supplemented with 10%FBS and 1%PSG (Appendix I).

On the day of EC<sub>50</sub> plate seeding, the cells were removed from the culture flasks using 0.25% trypsin w/EDTA. The trypsin was deactivated using complete RPMI 1640 and the cells were aspirated by pipetting to form a single cell suspension and then pooled. The pooled cells were counted using trypan blue exclusion with a Neubauer Bright-Line® hemacytometer.

The MCF-7 cell suspension was centrifuged at 350xg for 5min at 4°C, the supernatant removed and the cell pellet diluted (based on live cell counts, using complete RPMI 1640) to yield a final 6.25x10<sup>4</sup> cells/ml suspension. The EC<sub>50</sub> plates were seeded according to previous growth curve data (i.e. 6.25x10<sup>3</sup> cells/100µl/well of the 96-well plate) and incubated overnight at 37°C in a 5% CO<sub>2</sub>, 95% air atmosphere.

### *Test Agent Preparation*

Peptides 13-14 (no lot information supplied; fine, white powders) were received from Sanare in Parafilm® sealed clear glass vials. They were stored at room temperature in a covered box to prevent exposure to light. Initially, 25mM stocks were made using Dulbecco's phosphate-buffered saline (DPBS), 1x. Seven out of the twelve peptides did not go into solution at the 25mM concentration. In an attempt to keep all of the peptides at the same concentration, another volume of DPBS was added to all peptide stocks (12.5mM). Some of the peptides would still not go into solution. Additional volumes of

DPBS were added, accompanied by intermittent vortexing and heating to 37°C in a water bath (5-10min), attempting to prepare solutions for the peptide stocks. As some of the stocks became uniform suspensions, it was determined that these stocks should not be diluted further (limiting further decreases in final dosage concentrations) but rather should be used as suspensions (assuming that they would probably go into solution with the final dilution as they were added to the wells of the treatment plates). The stocks were further diluted in complete media to yield 2x working solutions/suspensions. The table below displays the final stock concentrations for each peptide.

Doxorubicin (a translucent dark red solution, lot 07D610) was manufactured by Teva Parenteral Medicines. To prepare the treatment solution, the 2mg/ml stock solution was diluted using complete media to yield a 2mM (2x) working solution (see table below).

Cisplatin (a fine, dark yellow powder) was obtained from Sigma (M8407) and stored in a sealed amber vial at -20°C in a covered box to prevent exposure to light. A 4mM stock solution was made using 0.9% saline. The stock solution was aliquoted into microcentrifuge tubes (for one-time use) and frozen at -20°C. For this experiment, one frozen aliquot was quickly thawed in a 37°C water bath and placed on wet ice until ready to use. To prepare the treatment solution, the 4mM stock solution was diluted 1:2 using complete media to yield a 2mM (2x) working solution (see table below).

For all individual Peptides, the Peptide Mixture, doxorubicin and cisplatin, the working solutions were prepared in the first wells of the dilution reservoirs. Serial dilutions (1:5) were made in complete media across the remaining nine wells of each dilution reservoir (*i.e.* through well ten).

Test Agent Stock Preparation Table

Compound	M.W. or mM stock	Quantity (mg)	1x DPBS Vehicle (µl)	Stocks Final Conc. (mM)
Peptide 13-4061559	3125.5	5	128	12.5
Peptide 14-4061560	1732.9	5	128	12.5
Peptide 15-4061561	1910.2	5	313.5	6.25
Peptide 16-4061562	1909.3	5	525	1.56
Peptide 17-4061563	2399.0	5	668	0.195
Peptide 18-4061564	1326.5	5	128	12.5
Peptide 19-4061565	1129.3	5	128	12.5
Peptide 20-4061566	2223.7	5	270	6.25
Peptide 21-4061567	1746.2	5	343.5	6.25
Peptide 22-4061568	2070.3	5	482.5	1.56
Peptide 23-4061569	1570.8	5	128	12.5
Peptide 24-4061570	1886.2	5	424	3.125
Peptide Mixture (13-24)	N/A	N/A	N/A	12µl of each final stock above
Doxorubicin	580	173.9 µl	126.1(media)	2
Cisplatin	4mM	150µl	150 (media)	2

### *Cell Plating, Treatment and EC<sub>50</sub> Assay*

MCF-7 cells in the log phase of growth were seeded at the indicated density listed in Appendix I into 96-well culture plates in 0.1mL of complete media in all wells except column 12, which was reserved for the media only control (blank). The cells were allowed to attach overnight at 37°C.

The antiproliferative activity of Peptides 13-24 as single agents and the Peptide Mixture (13-24) as a combination therapy against MCF-7 were evaluated using the MTT Cell Proliferation Assay Kit (ATCC catalog # 30-1010K). The assay is based on the reduction of yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) by metabolically active cells forming purple formazan crystals. The purple formazan is solublized with detergent and quantified spectrophotometrically at 570nm (References 1-6). The MTT Cell Proliferation Assay Kit contains ready to use MTT and detergent solutions.

Following the serial dilutions described in "*Test Agent Preparation*", 0.1mL of diluted compound was transferred from each dilution reservoir well to the corresponding assay plate wells containing 0.1mL media + MCF-7 cells. This yielded a further 1:2 dilution and resulted in a 250-3.91µM final treatment concentration for the top dosage level of each Sanare Peptide test agent that was soluble at ≤25mM (concentration range varied depending on peptide solubility; see *Test Agent Preparation* above). This process was repeated daily for four consecutive days (96hrs). On Treatment Days 2-4, prior to the addition of the new peptide preparations, 100µl of culture supernatant was carefully removed from each well of each treatment plate for each single agent Peptide and the Peptide Mixture wells.

The positive control agents, doxorubicin and cisplatin, had final treatment starting concentrations of 1mM each. These agents were administered as a single treatment for this experiment.

The 1:5 serial dilutions across the dilution reservoirs and the test agent administration processes were carried out one compound at a time.

The test agents diluted in complete culture media were added to each well in a volume of 0.1mL for a total final well volume of 0.2mL/well. After the cells were exposed to test agents over four consecutive treatments (total 96 hours), 0.1mL of media was removed and 0.01mL of MTT reagent was added to each well. The plates were returned to the 37°C incubator for four hours. Detergent reagent (0.1mL) was then added and the plates incubated again at 37°C overnight in the dark. The absorbance at 570nm was measured 24 hours later with a SpectraMAX Plus plate reader (Molecular Devices).

Absorbance values were converted to Percent of Control and plotted against compound concentrations for EC<sub>50</sub> calculations using SoftMax® Pro (version 5.2, Molecular Devices). The plate blank average was subtracted from all wells prior to calculating Percent of Control. Percent of Control values were calculated by dividing the absorbance values for each test well by the No Drug control average (column 11 values) and multiplying by 100. Plots of Percent of Control vs. Compound Concentration were analyzed using the 4-parameter equation to obtain EC<sub>50</sub> values and other parameters that describe the sigmoidal dose response curve (see Appendix III).

### Data Retrieval

MIR Preclinical Services retains permanent “active” copies (on CD) of all experiments unless advised otherwise.

### Results and Discussion

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The experimentally determined EC<sub>50</sub> values for Peptides 13-24 as single agents, the Peptide Mixture (13-24) as a combination therapy, and Doxorubicin and Cisplatin (positive control single agents) against MCF-7 are summarized in the table below. The dose response curve for Peptides 17, 20, 21 and 23 as single agents as well as for the Mixture (Peptides 13-24) produced the most favorable anti-cancer activity against MCF-7 of the twelve Sanare Peptide test agents. Doxorubicin and cisplatin yielded dose response curves as expected, indicating inhibition of cell growth and/or cell death at the highest treatment concentrations (see raw data and graphs in Appendix II).

The dose response for anti-cancer activity for the Peptide Mixture treatment group may be influenced by the high volume of DPBS + peptide exchanged daily for four days. This resulted in a daily 48% decrease in growth nutrients for the cells.

Test Agent	EC <sub>50</sub> (μM) against MCF-7
Peptide 13-4061559	>250
Peptide 14-4061560	>250
Peptide 15-4061561	>125
Peptide 16-4061562	>31.25
Peptide 17-4061563	5.98
Peptide 18-4061564	>250
Peptide 19-4061565	>250
Peptide 20-4061566	124*
Peptide 21-4061567	122*
Peptide 22-4061568	>31.25
Peptide 23-4061569	250*
Peptide 24-4061570	>62.5
Peptide Mixture (13-24)	70*
Doxorubicin	0.05
Cisplatin	4.7

\* EC<sub>50</sub> values for Peptides 20, 21, 23 and the mixture were obtained by constraining parameter “D” of the 4-parameter equation (i.e.- D = 0 for peptides 20, 21 and 23 and D = -30 for the mixture) since complete inhibition was not observed at the highest concentrations tested. This improves the accuracy of the EC<sub>50</sub> estimate. Similarly, the “A” parameter was constrained to 120 for Doxorubicin since its dose response curve was incomplete at the lowest concentrations tested.

## References

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1. MTT Cell Proliferation Assay (ATCC 30-1010K).
2. Van de Loosdrecht, A.A., et al. *J. Immunol. Methods* 174: 311-320, 1994.
3. Ferrari, M., et al. *J. Immunol. Methods* 131: 165-172, 1990.
4. Gerlier, D., and N. Thomasset. *J. Immunol. Methods* 94: 57-63, 1986.
5. Alley, M.C., et al. *Cancer Res.* 48: 589-601, 1988.
6. Mosmann, T. *J. Immunol. Methods* 65: 55-63, 1983.

## **Appendix I - Cell Culture**

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### **Cell Culture Protocol for Passaging Adherent Cells**

All manipulations were carried out in a Class II HEPA filtered biosafety hood using sterile technique.

1. Aspirated and discarded culture medium.
2. Added 2-3mL of 0.25% (w/v) Trypsin, 0.53mM EDTA solution (CellGro 25-053-CI) to each flask and ensured complete coverage of the cell monolayer by rocking gently in multiple directions. Flasks were returned to the 37°C incubator.
3. Observed cells periodically under an inverted microscope until cell layer was dispersed (usually within 1-3 minutes).
4. Once the cells were detached, an equal volume of fresh media was added to neutralize the trypsin. The cells were aspirated and gently pipetted to rinse and pool the monolayer of cells. Immediately following pooling of the single cell suspension, appropriate aliquots were added to new culture vessels (T175 flasks). Culture vessels contained 30mL fresh pre-warmed media (room temperature to 37°C).
5. Cultures were incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator. Cultures were subcultured and/or had media changed every 2 – 3 days.

### **Cell Line Propagation Conditions**

(Supplement percentages are volume/volume)

*Cell Line:* **MCF-7**

*Media:* RPMI1640 (CellGro 10-040-CV)

*Supplements:* 10% FBS, 1% PSG

*Atmosphere:* 5% CO<sub>2</sub>, 95% air

*Properties:* Adherent

\* FBS - Fetal Bovine Serum (Gibco 10082-147; lot #1354986)

\* PSG – Penicillin, Streptomycin, L-Glutamine Solution (CellGro 30-009-CI)

### **Seeding Density for EC<sub>50</sub> Assay**

<b>Cell Line</b>	<b>cells/well (x10<sup>3</sup>)</b>
MCF-7	6.25

## **Appendix II – Protocol**

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Client Study No.: **SANA200805R1a** Study Title: **IC50 Assays of 12 Sanare Peptides Against MCF-7 Cells**  
 MIR No.: **1007**

Study Type : **IC<sub>50</sub>**  
 Cell Type : **Breast**  
 Media : **ATCC recommended**  
 Cells : **MCF-7**

Study Leader: **R. Lister**  
 Number of Compounds : **12 peptides + mixture + doxorubicin**  
 Estimated Start Date :  
 Number of plates : **3**

		Compound Concentration (µM)											
		compound 1		compound 2		compound 3		compound 4		compound 5			
		1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>		500	500	500	500	500	500	500	500	500	500	Cells Only	No Cells
<b>B</b>		100	100	100	100	100	100	100	100	100	100	Cells Only	No Cells
<b>C</b>		20	20	20	20	20	20	20	20	20	20	Cells Only	No Cells
<b>D</b>		4	4	4	4	4	4	4	4	4	4	Cells Only	No Cells
<b>E</b>		1	1	1	1	1	1	1	1	1	1	Cells Only	No Cells
<b>F</b>		0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	Cells Only	No Cells
<b>G</b>		0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	Cells Only	No Cells
<b>H</b>		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	Cells Only	No Cells

		Compound Concentration (µM)											
		compound 6		compound 7		compound 8		compound 9		compound 10			
		1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>		500	500	500	500	500	500	500	500	500	500	Cells Only	No Cells
<b>B</b>		100	100	100	100	100	100	100	100	100	100	Cells Only	No Cells
<b>C</b>		20	20	20	20	20	20	20	20	20	20	Cells Only	No Cells
<b>D</b>		4	4	4	4	4	4	4	4	4	4	Cells Only	No Cells
<b>E</b>		1	1	1	1	1	1	1	1	1	1	Cells Only	No Cells
<b>F</b>		0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	Cells Only	No Cells
<b>G</b>		0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	Cells Only	No Cells
<b>H</b>		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	Cells Only	No Cells

Compound Concentration ( $\mu\text{M}$ )												
	compound 11		compound 12		mixture		doxorubicin		cisplatin		11	12
	1	2	3	4	5	6	7	8	9	10		
A	500	500	500	500	500	500	1,000	1,000	1,000	1,000	Cells Only	No Cells
B	100	100	100	100	100	100	200	200	200	200	Cells Only	No Cells
C	20	20	20	20	20	20	40	40	40	40	Cells Only	No Cells
D	4	4	4	4	4	4	8	8	8	8	Cells Only	No Cells
E	1	1	1	1	1	1	2	2	2	2	Cells Only	No Cells
F	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.3	Cells Only	No Cells
G	0.03	0.03	0.03	0.03	0.03	0.03	0.1	0.1	0.1	0.1	Cells Only	No Cells
H	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	Cells Only	No Cells

Drug	Required Amount (mg)	Vehicle	Stability	Comments
peptides 1 - 12	10 - 20 mg each	PBS	Unknown	

Procedures
<p>Cell lines will be grown using media, serum, and culture conditions recommended by the A.T.C.C. Stock cultures will be allowed to grow to 70-80% confluence for this study. The antiproliferative activity of test compounds against the indicated cell lines will be evaluated <i>in vitro</i> using the the ATCC's MTT Cell Proliferation Assay Kit (Catalog No. 30-1010K). Cells are seeded into 96-well culture plates at a predetermined density on day 0 in a volume of 0.1 mL complete medium. Plates are placed in a humidified incubator at 37°C with 5% CO<sub>2</sub> and 95% HEPA filtered room air for 24hrs. 0.1mL of 2x drug are added to indicated wells and the plates are returned to the incubator. Peptides/media will be replaced every 24 hrs (i.e.- at time 0, 24, 48 &amp; 72 hrs.) for a total of 96 hrs. exposure. Cells will be dosed every 24 hrs. by first removing 100uL of media and then adding 100uL fresh 2x conc. peptide in media for a total of 96 hrs. exposure. Cisplatin &amp; doxorubicin will be a single dose. Cell proliferation is measured by addition of MTT reagent to each well and incubated for an additional 4 hrs. followed by addition of cell lysis/MTT solubilization reagent and incubating overnight. Absorbance (570nm) of the plate wells will be measured and quantitated relative to no drug control wells. Results will be expressed as percent inhibition versus compound concentration graph. Growth at 24h to be verified by incubation of an additional plate to be harvested at 24 h (same time as drug addition).</p>
Comments
<p>Additional cost associated with multiple drug additions (3x after the initial dose) and doxorubicin control.</p>

**Appendix III – Raw Data and Graphs**

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**MCF-7 EC<sub>50</sub> Raw Data**

	Absorbance 570nm			Absorbance 570nm			Absorbance 570nm			Absorbance 570nm			Absorbance 570nm	
Conc. (μM)	Peptide 13		Conc. (μM)	Peptide 14		Conc. (μM)	Peptide 15		Conc. (μM)	Peptide 16		Conc. (μM)	Peptide 17	
250	0.377	1.765	250	1.226	1.590	125	1.224	1.506	31.25	0.766	1.433	3.91	0.291	0.304
50	0.967	1.829	50	1.843	1.387	25	1.736	1.462	6.25	1.409	1.582	0.782	1.813	1.529
10	1.085	1.456	10	1.765	1.364	5	1.871	1.391	1.25	1.408	1.575	0.156	1.586	1.568
2	1.164	1.707	2	1.915	1.516	1	1.601	1.286	0.25	1.724	1.518	0.031	1.466	1.688
0.4	1.163	1.717	0.4	1.756	1.562	0.2	1.555	1.254	0.05	1.543	1.656	6.26E-03	1.232	1.486
0.08	1.309	1.953	0.08	1.761	1.662	0.04	1.756	1.306	0.01	1.658	1.427	1.25E-03	1.515	1.486
0.016	1.092	1.694	0.016	1.765	1.348	0.008	1.446	1.329	0.002	1.568	1.564	2.50E-04	1.221	1.406
3.20E-03	0.328	1.722	3.20E-03	1.088	1.601	1.60E-03	1.056	1.624	4.00E-04	0.973	1.659	5.005E-05	1.062	1.290
0.000	1.001	1.519	1.298	1.492	1.419	1.693	1.500	1.450						

	Absorbance 570nm			Absorbance 570nm			Absorbance 570nm			Absorbance 570nm			Absorbance 570nm	
Conc. (μM)	Peptide 18		Conc. (μM)	Peptide 19		Conc. (μM)	Peptide 20		Conc. (μM)	Peptide 21		Conc. (μM)	Peptide 22	
250	0.208	1.363	250	0.963	1.509	125	0.714	0.931	125	0.497	0.883	31.25	0.791	1.025
50	0.870	1.542	50	1.706	1.464	25	1.428	1.317	25	1.473	1.562	6.25	1.349	1.552
10	0.996	1.494	10	1.859	1.508	5	1.519	1.236	5	1.670	1.364	1.25	1.513	1.419
2	1.192	1.597	2	1.730	1.296	1	1.740	1.204	1	1.606	1.368	0.25	1.534	1.356
0.4	1.055	1.640	0.4	1.564	1.362	0.2	1.443	1.377	0.2	1.404	1.437	0.05	1.575	1.373
0.08	1.159	1.627	0.08	1.648	1.310	0.04	1.660	1.134	0.04	1.688	1.336	0.01	1.500	1.486
0.016	0.987	1.525	0.016	1.889	1.383	0.008	1.560	1.316	0.008	1.413	1.269	0.002	1.422	1.545
3.20E-03	0.413	1.605	3.20E-03	1.528	1.474	1.60E-03	1.488	1.317	1.60E-03	1.202	1.452	4.00E-04	1.203	1.296
0.000	0.855	1.575	1.362	1.385	1.399	1.453	1.411	1.520						

	Absorbance 570nm			Absorbance 570nm			Absorbance 570nm			Absorbance 570nm			Absorbance 570nm	
Conc. (µM)	Peptide 23		Conc. (µM)	Peptide 24		Conc. (µM)	Peptide Mixture (13-24)		Conc. (µM)	Doxorubicin		Conc. (µM)	Cisplatin	
250	0.303	1.331	250	0.974	1.278	≤250	-0.070	-0.064	1000	0.277	0.327	1000	-0.052	-0.055
50	1.016	1.749	50	1.889	1.700	≤50	1.138	1.032	200	0.091	0.099	200	0.066	0.055
10	1.155	1.753	10	1.743	1.653	≤10	1.339	1.435	40	0.154	0.173	40	0.371	0.341
2	1.333	1.751	2	1.852	1.356	≤2	1.492	1.597	8	0.264	0.277	8	0.570	0.566
0.4	1.179	1.536	0.4	1.868	1.278	≤0.4	1.741	1.407	1.6	0.285	0.296	1.6	1.069	1.016
0.08	1.208	1.580	0.08	2.047	1.492	≤0.08	1.468	1.633	0.32	0.424	0.450	0.32	1.345	1.591
0.016	1.058	1.562	0.016	1.731	1.424	≤0.016	1.720	1.321	0.064	0.708	0.723	0.064	1.653	1.537
3.20E-03	1.002	1.684	3.20E-03	1.462	1.465	≤3.20E-03	1.611	1.333	0.013	1.394	1.514	0.013	1.525	1.496
0.000	0.334	0.713	0.387	0.822	1.418	1.399	1.257	1.138						

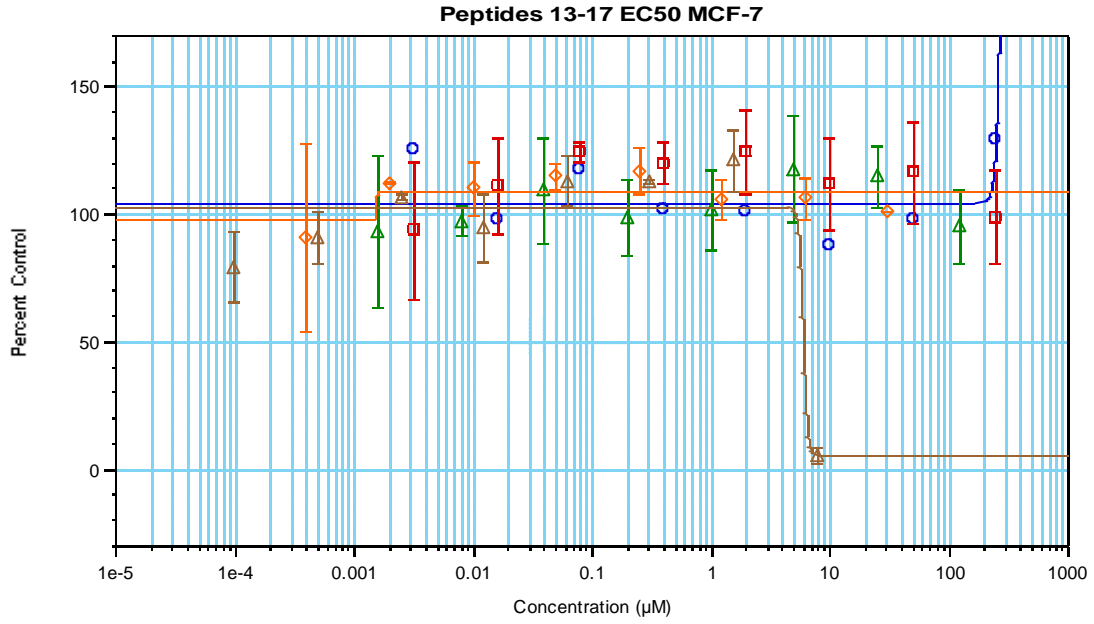
24hour Growth  $A_{570nm} = 0.235$  (CV = 6.7%) i.e. - MTT signal at beginning of drug exposure. This value is subtracted from the 96 hours exposure data shown in the plate data above before Percent of Control is calculated.

### **MCF-7 EC<sub>50</sub> Raw Data (24hr & 96hr Growth Control)**

	A2780 (Absorbance 570nm)										
	24 hrs Growth						96hrs Growth				
	0.242	0.218	0.260	0.261	0.260	0.218	1.261	1.196	1.336	1.295	1.381
0.241	0.213	0.251	0.247	0.231	0.209	1.788	1.519	1.481	1.479	1.389	
0.235	0.213	0.235	0.225	0.240	0.199	1.647	1.356	1.350	1.498	1.371	
0.242	0.200	0.233	0.231	0.244	0.199	1.788	1.406	1.603	1.368	1.336	
0.240	0.236	0.237	0.225	0.237	0.247	1.751	1.337	1.395	1.387	1.346	
0.244	0.243	0.233	0.212	0.234	0.244	1.668	1.379	1.402	1.472	1.379	
0.241	0.247	0.250	0.222	0.259	0.251	1.708	1.459	1.491	1.424	1.439	
0.242	0.240	0.238	0.227	0.227	0.253	1.387	1.286	1.424	1.472	1.454	
No Cells>>	-0.003	-0.008	-0.010	-0.002	-0.005	0.018	-0.005	0.015			
	<b>AVE</b>	<b>STDDV</b>	<b>%CV</b>								
	0.2348	0.0157	6.7								

24hour Growth  $A_{570nm} = 0.235$  (CV = 6.7%)

## EC<sub>50</sub> Assay of Peptides 13-17, against MCF-7 Human Pleural Effusion Adenocarcinoma



4-P Fit:  $y = (A - D) / (1 + (x/C)^B) + D$ :

	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>	<u>R<sup>2</sup></u>
● 13-4061559 (Peptide 13: Conc vs PcntControl)	104	16.9	327	2.46e+03	0.349
■ 14-4061560 (14: Conc vs PcntControl)	122	8.46	4.16e+08	114	-0.823
▲ 15-4061561 (15: Conc vs PcntControl)	111	47.6	2.75e+03	99.8	-0.77
◆ 16-4061562 (16: Conc vs PcntControl)	97.4	168	0.00155	109	0.529
▲ 17-4061563 (17: Conc vs PcntControl)	103	23.8	5.98	5.12	0.863

Curve Fit Option - Fixed Weight Value

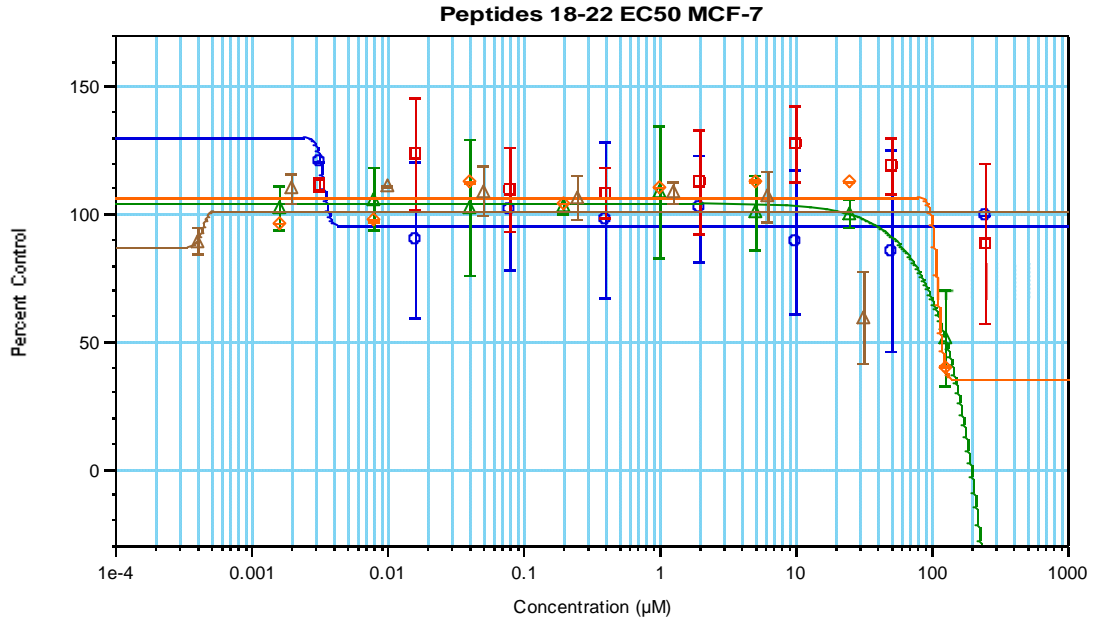
A = Top

B = Slope

C = EC<sub>50</sub>

D = Bottom

## EC<sub>50</sub> Assay of Peptides 18-22 against MCF-7 Human Pleural Effusion Adenocarcinoma



4-P Fit:  $y = (A - D) / (1 + (x/C)^B) + D$ :

	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>	<u>R<sup>2</sup></u>
○ 18-4061564 (18: Conc vs PcntControl)	130	19.1	0.00337	95.2	0.672
□ 19-4061565 (19: Conc vs PcntControl)					
△ 20-4061566 (20: Conc vs PcntControl)	104	1.54	2.34e+05	-5.59e+06	0.982
◇ 21-4061567 (21: Conc vs PcntControl)	107	19.8	110	34.4	0.922
△ 22-4061568 (22: Conc vs PcntControl)	86.7	18.4	0.000435	102	0.0587

Curve Fit Option - Fixed Weight Value

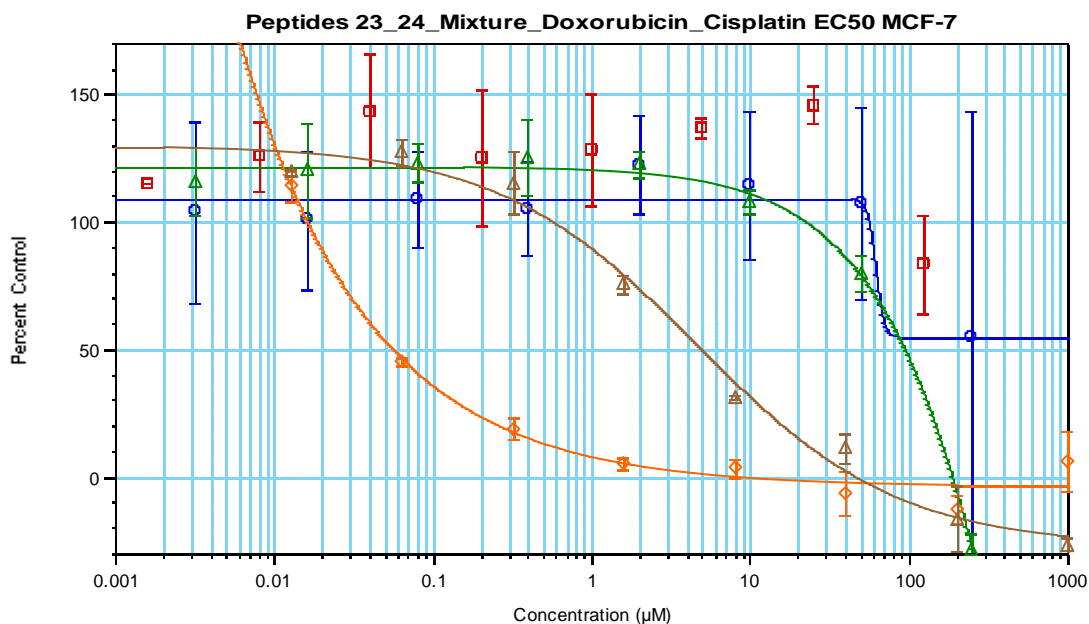
A = Top

B = Slope

C = EC<sub>50</sub>

D = Bottom

**EC<sub>50</sub> Assay of Peptides 23, 24, Doxorubicin and Cisplatin as Single Agents and Combination Mixture (Peptides 13-24) against MCF-7 Human Pleural Effusion adenocarcinoma**



4-P Fit:  $y = (A - D) / (1 + (x/C)^B) + D$ :

	A	B	C	D	R <sup>2</sup>
● 23-4061569 (23: Conc vs PcntControl)	109	16.5	61.6	54.5	0.89
■ 24-4061570 (24: Conc vs PcntControl)					
▲ Peptide Mixture (#s 13-24) (Mixture (13-24): Conc vs Pcnt...	122	0.879	1.27e+03	-654	0.996
◆ Doxorubicin (Doxorubicin: Conc vs PcntControl)	1.01e+10	0.529	1.3e-17	-4.04	0.981
▲ Cisplatin (Cisplatin: Conc vs Pcnt Control)	130	0.685	4.7	-27.2	0.99

Curve Fit Option - Fixed Weight Value

A = Top

B = Slope

C = EC<sub>50</sub>

D = Bottom