



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

Final Report to: Sanare A.S.

Study: SANA200809R1a (MIR1036)

Date: January 8, 2009

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

The Charles River logo consists of a stylized blue wave graphic above the text 'charles river' in a lowercase, sans-serif font.

charles river

## Executive Digest

---

Twelve Sanare peptides previously assayed individually and as a single mixture of all twelve (SANA200805R1, MIR1007) were assayed again as a mixture of twelve peptides and as mixtures decreasing in quantity (12 – n; i.e. – mixtures ranging from 2 to 12 peptides). Serially diluted (1:5) peptide mixtures were added to MCF-7 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 (i.e.- No Drug Control) to calculate the EC<sub>50</sub> values.

The dose response curve for peptide combination mixture (“K”) produced the most favorable antiproliferative activity against MCF-7 of the eleven Sanare peptide mixtures tested. 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 antiproliferative activity at the highest concentration for the peptide mixtures appears to indicate some anti-growth activity. However, it should be considered that the results may be influenced by the increasing volume of DPBS + peptide exchanged daily for four days. 100µL of culture supernatant was replaced each day with 100µL peptide mixture, resulting in a daily decrease (8-48%) in growth nutrients for the cells/treatment day. Control wells of MCF-7 cells with DPBS + media ratios that corresponded to each Peptide Combination Mixture also showed a decrease in viable cells at the top DPBS + media “treatment” well.

A summary table of the EC<sub>50</sub> results is displayed on the following page.

### EC<sub>50</sub> Results for SANA200809R1a (MIR1036)

Mixture	Test Agents	EC <sub>50</sub> (μM)
A	Peptides 17, 20	87.4
B	Peptides 17, 20, 21	81.5
C	Peptides 17, 20, 21, 23	80.8
D	Peptides 17, 20, 21, 23, 24	65.1
E	Peptides 17, 20, 21, 23, 24, 13	75.4
F	Peptides 17, 20, 21, 23, 24, 13, 14	75.3
G	Peptides 17, 20, 21, 23, 24, 13, 14, 15	66.1
H	Peptides 17, 20, 21, 23, 24, 13, 14, 15, 16	73.6
I	Peptides 17, 20, 21, 23, 24, 13, 14, 15, 16, 18	75.1
J	Peptides 17, 20, 21, 23, 24, 13, 14, 15, 16, 18, 19	68.8
K	Peptides 17, 20, 21, 23, 24, 13, 14, 15, 16, 18, 19, 22	27.4
	Doxorubicin	<0.012
	Cisplatin	9.12

*\*EC<sub>50</sub> values for Peptide Combination Mixtures were obtained by constraining parameter "D" of the 4-parameter equation (i.e. - D = -20 to -35) since complete inhibition was not observed at the highest concentrations tested. This improves the accuracy of the EC<sub>50</sub> estimate.*

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

Molecular Imaging Research, Inc. (now known as Charles River Discovery and Imaging Services, Inc.) ("Charles River") has prepared this report for the sole use of the Client and for the intended purposes as stated in the written request of the Client under which this work was completed. This report may not be relied upon by any other party without the express written agreement of Charles River.

This report has been prepared at the request of the Client. The use of this report by unauthorized third parties shall be at their own risk, and Charles River accepts no duty of care to any such third party.

Any recommendations, opinions, findings or conclusions stated in this report are based on the information supplied by the Client and on the facts and circumstances as they existed at the time Charles River performed this work. Any changes in such facts and circumstances and information upon which this report is based may adversely affect any recommendations, opinions, findings or conclusions contained in this report.

This report is Confidential and no part of this report may be used, copied or duplicated by any third party without the express written permission of the Client and Charles River.

## Contents

---

<u>Section</u>	<u>Page</u>
A. Introduction	05
B. Materials and Methods	06
C. Results and Discussion	10
D. References	11
E. Appendices	
1. Cell Culture Information	12
2. Raw Data and Graphics	14

## Introduction

---

SANA200809R1a (MIR1036) was designed to determine the antiproliferative effects of 96 hour (daily treatment x four days) exposure to peptides 13-24 (a.k.a. 25-36) in various combination mixtures for therapy against MCF-7 cells (Human Pleural Effusion Breast Adenocarcinoma). Doxorubicin and Cisplatin served as single agent positive controls.

Following 96 hours of exposure of the cells to peptide combinations, the cell 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. 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.

DPBS + media controls specific to each Peptide Mixture Combination therapy were used for comparison to the corresponding treatment wells on each EC<sub>50</sub> test plate. These controls were used to account for the effects promoted by the dilution of nutrient rich complete media with daily therapy.

## Materials and Methods

---

### *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.

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

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 25°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 13-24, also known as peptides 25-36, respectively, (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.

In the previous experiment (SANA200805R1a/MIR1007), initial 25mM stocks were made using Dulbecco's phosphate-buffered saline (DPBS). 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 Peptide stock solutions and suspensions remaining from SANA200805R1a/MIR1007 were used for this experiment. Additional amounts of Peptides that were necessary for four treatments in multiple combinations were weighed out, prepared identically to the Peptides used in SANA200805R1a/MIR1007 and added to the residual Peptide stocks remaining from SANA200805R1a/MIR1007. Once each individual Peptide stock was made, the Peptide Combination Mixture stocks were made by combining 60µl of each Peptide in the combination to each mixture test agent vial.

These vials were labeled “A” through “K” and were stored in a covered box at 4°C when not in use. The Peptide Combination stock vials were used once daily for treatments of the MCF-7 cells throughout the experiment. For therapy, these final stock Peptide Mixtures 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 Peptide Combinations, doxorubicin and cisplatin, the 2x 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
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 (a.k.a. Peptides 25-36, respectively) as Peptide 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 solubilized 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 under “*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 Combination test agent that was soluble at ≤25mM conc. (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 Peptide Combination Mixture.

The positive control agents, doxorubicin and cisplatin, had final treatment starting concentrations of 1mM each. These agents were each 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 final starting concentration for the individual Peptide test agents in Combination Mixtures is listed in the Table below followed by the Peptide Combination Mixture Table.

<b>Peptide</b>	<b>Treatment Starting Concentration (µM) in Mixtures “A-K”</b>
<b>17 (a.k.a. 29)</b>	<b>3.91</b>
<b>20 (a.k.a. 32)</b>	<b>125</b>
<b>21 (a.k.a. 33)</b>	<b>125</b>
<b>23 (a.k.a. 35)</b>	<b>250</b>
<b>24 (a.k.a. 36)</b>	<b>62.5</b>
<b>13 (a.k.a. 25)</b>	<b>250</b>
<b>14 (a.k.a. 26)</b>	<b>250</b>
<b>15 (a.k.a. 27)</b>	<b>125</b>
<b>16 (a.k.a. 28)</b>	<b>31.25</b>
<b>18 (a.k.a. 30)</b>	<b>250</b>
<b>19 (a.k.a. 31)</b>	<b>250</b>
<b>22 (a.k.a. 34)</b>	<b>31.25</b>

Mixture	Peptide Mixture Combinations	Starting Treatment Concentration (µM)
A	17, 20	≤250 for each Sanare Peptide
B	17, 20, 21	≤250
C	17, 20, 21, 23	≤250
D	17, 20, 21, 23, 24	≤250
E	17, 20, 21, 23, 24, 13	≤250
F	17, 20, 21, 23, 24, 13, 14	≤250
G	17, 20, 21, 23, 24, 13, 14, 15	≤250
H	17, 20, 21, 23, 24, 13, 14, 15, 16	≤250
I	17, 20, 21, 23, 24, 13, 14, 15, 16, 18	≤250
J	17, 20, 21, 23, 24, 13, 14, 15, 16, 18, 19	≤250
K	17, 20, 21, 23, 24, 13, 14, 15, 16, 18, 19, 22	≤250

The Peptide Mixture test agents, diluted in complete culture media, were added to each well in a volume of 0.1mL for a total final daily 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. The EC<sub>50</sub> values for Peptides Combination Mixtures were obtained by constraining parameter “D” of the 4-parameter equation (i.e. - D = -20 to -35) since complete inhibition was not observed at the highest concentrations tested. This improves the accuracy of the EC<sub>50</sub> estimate (see Appendix II).

#### *Data Retrieval*

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

## Results and Discussion

The experimentally determined EC<sub>50</sub> values for Peptides 13-24 (a.k.a. Peptides 25-36, respectively) in mixtures as combination therapy, and Doxorubicin and Cisplatin (positive control single agents) against MCF-7 are summarized in the table below. The dose response curve for Peptide Combination Mixture (“K”) produced the most efficacious antiproliferation activity against MCF-7 of the eleven Sanare Peptide Mixtures tested. 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 antiproliferative activity at the highest concentration for the Peptide Mixtures appears to indicate some anti-growth activity. However, it should be considered that the results may be influenced by the increasing volume of DPBS + Peptide Combinations exchanged daily for four days. This exchange resulted in a 8-48% decrease in growth nutrients for the cells/treatment day. Controls of DPBS + media that corresponded to each Peptide Combination Mixture also evidenced a decrease of viable cells in the top DPBS + media dilution wells for all Combination therapies.

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

Mixture	Test Agents	EC <sub>50</sub> (μM)
A	Peptides 17, 20	87.4
B	Peptides 17, 20, 21	81.5
C	Peptides 17, 20, 21, 23	80.8
D	Peptides 17, 20, 21, 23, 24	65.1
E	Peptides 17, 20, 21, 23, 24, 13	75.4
F	Peptides 17, 20, 21, 23, 24, 13, 14	75.3
G	Peptides 17, 20, 21, 23, 24, 13, 14, 15	66.1
H	Peptides 17, 20, 21, 23, 24, 13, 14, 15, 16	73.6
I	Peptides 17, 20, 21, 23, 24, 13, 14, 15, 16, 18	75.1
J	Peptides 17, 20, 21, 23, 24, 13, 14, 15, 16, 18, 19	68.8
K	Peptides 17, 20, 21, 23, 24, 13, 14, 15, 16, 18, 19, 22	27.4
	Doxorubicin	<0.012
	Cisplatin	9.12

*\*EC<sub>50</sub> values for Peptides Combination Mixtures were obtained by constraining parameter “D” of the 4-parameter equation (i.e.- D = -20 to -35) since complete inhibition was not observed at the highest concentrations tested. This improves the accuracy of the EC<sub>50</sub> estimate.*

## References

---

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**

---

### **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 – Raw Data and Graphics**

---

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

	Absorbance 570nm			Absorbance 570nm			Absorbance 570nm			Absorbance 570nm			Absorbance 570nm	
Conc. (μM)	Mixture "A"		Conc. (μM)	Mixture "B"		Conc. (μM)	Mixture "C"		Conc. (μM)	Mixture "D"		Conc. (μM)	Mixture "E"	
≤250	0.050	0.153	≤250	0.064	0.047	≤250	0.052	0.044	≤250	-0.009	0.066	≤250	0.048	0.052
≤50	0.795	1.021	≤50	1.020	1.049	≤50	1.078	1.007	≤50	0.606	1.017	≤50	0.960	0.962
≤10	0.945	1.112	≤10	1.116	1.164	≤10	1.197	1.085	≤10	0.909	1.176	≤10	1.116	1.125
≤2	1.114	1.230	≤2	1.224	1.269	≤2	1.283	1.184	≤2	0.952	1.297	≤2	1.195	1.224
≤0.4	1.113	1.280	≤0.4	1.299	1.311	≤0.4	1.343	1.234	≤0.4	0.937	1.370	≤0.4	1.274	1.286
≤0.08	1.062	1.263	≤0.08	1.296	1.349	≤0.08	1.333	1.278	≤0.08	1.354	1.414	≤0.08	1.324	1.296
≤0.016	1.114	1.203	≤0.016	1.277	1.321	≤0.016	1.373	1.236	≤0.016	1.261	1.390	≤0.016	1.262	1.294
≤3.20E-03	0.900	1.270	≤3.20E-03	1.234	1.238	≤3.20E-03	1.305	1.190	≤3.20E-03	1.125	1.334	≤3.20E-03	1.225	1.215
0.000	See DPBS+Media Treatment Control Table													

	Absorbance 570nm			Absorbance 570nm			Absorbance 570nm			Absorbance 570nm			Absorbance 570nm	
Conc. (μM)	Mixture "F"		Conc. (μM)	Mixture "G"		Conc. (μM)	Mixture "H"		Conc. (μM)	Mixture "I"		Conc. (μM)	Mixture "J"	
≤250	0.022	0.056	≤250	-0.023	-0.004	≤250	-0.030	-0.029	≤250	-0.028	-0.010	≤250	-0.034	-0.037
≤50	0.970	0.961	≤50	0.617	0.917	≤50	0.961	0.946	≤50	0.949	0.968	≤50	0.559	0.926
≤10	1.063	1.054	≤10	0.792	1.082	≤10	1.179	1.121	≤10	1.151	1.154	≤10	0.900	1.180
≤2	1.185	1.171	≤2	1.198	1.193	≤2	1.262	1.249	≤2	1.222	1.280	≤2	1.141	1.316
≤0.4	1.293	1.266	≤0.4	1.134	1.297	≤0.4	1.337	1.299	≤0.4	1.301	1.346	≤0.4	1.087	1.370
≤0.08	1.324	1.288	≤0.08	1.287	1.277	≤0.08	1.332	1.289	≤0.08	1.304	1.324	≤0.08	1.202	1.418
≤0.016	1.287	1.281	≤0.016	1.176	1.264	≤0.016	1.302	1.261	≤0.016	1.281	1.350	≤0.016	1.053	1.310
≤3.20E-03	1.167	1.221	≤3.20E-03	0.955	1.299	≤3.20E-03	1.300	1.251	≤3.20E-03	1.275	1.330	≤3.20E-03	1.056	1.278
0.000	See DPBS+Media Treatment Control Table													

	Absorbance 570nm			Absorbance 570nm			Absorbance 570nm	
Conc. (µM)	Mixture "K"		Conc. (µM)	Doxorubicin		Conc. (µM)	Cisplatin	
≤250	-0.038	-0.035	1,000	0.206	0.158	1,000	-0.038	-0.038
≤50	0.258	0.275	200	0.069	0.066	200	0.051	0.057
≤10	0.863	0.908	40	0.157	0.146	40	0.340	0.349
≤2	1.008	1.033	8	0.193	0.182	8	0.559	0.598
≤0.4	1.107	1.073	1.6	0.204	0.218	1.6	0.906	0.937
≤0.08	1.145	1.159	0.32	0.143	0.140	0.32	1.158	1.263
≤0.016	1.215	1.220	0.064	0.395	0.387	0.064	1.192	1.255
≤3.20E-03	1.166	1.148	1.28E-02	0.555	0.544	1.28E-02	1.165	1.209
0.000	See DPBS+Media Treatment Control Table							

### Individual DPBS+Media Peptide Combinations Control

	MCF-7 (Absorbance 570nm)												
	96hrs. DPBS+Media Treatment Control												
"Conc"	Mixture A	Mixture B	Mixture C	Mixture D	Mixture E	Mixture F	Mixture G	Mixture H	Mixture I	Mixture J	Mixture K	Doxorubicin	Cisplatin
≤250	0.753	0.942	0.821	0.517	0.769	0.594	0.363	0.452	0.333	0.246	0.247	1.005	1.005
≤50	1.334	1.263	1.297	1.273	1.261	1.243	1.250	1.278	1.309	1.203	1.267	1.104	1.104
≤10	1.297	1.275	1.276	1.306	1.292	1.259	1.304	1.335	1.299	1.238	1.309	1.111	1.111
≤2	1.328	1.239	1.303	1.312	1.303	1.278	1.318	1.341	1.350	1.250	1.318	1.125	1.125
≤0.4	1.315	1.278	1.308	1.320	1.302	1.284	1.325	1.345	1.343	1.231	1.318	1.109	1.109
≤0.08	1.330	1.281	1.352	1.335	1.296	1.289	1.334	1.336	1.421	1.309	1.379	1.095	1.095
≤0.016	1.308	1.289	1.297	1.313	1.303	1.259	1.360	1.367	1.346	1.276	1.343	1.153	1.153
≤3.20E-03	1.295	1.268	1.286	1.246	1.268	1.214	1.348	1.351	1.333	1.197	1.325	1.106	1.106
No Cells>>> (Growth plate)	-0.014	0.000	0.002	0.004	0.005	0.003	0.005	-0.004					
	<b>AVE</b>	<b>STDDV</b>	<b>%CV</b>										
	1.2203	0.1947	16.0										

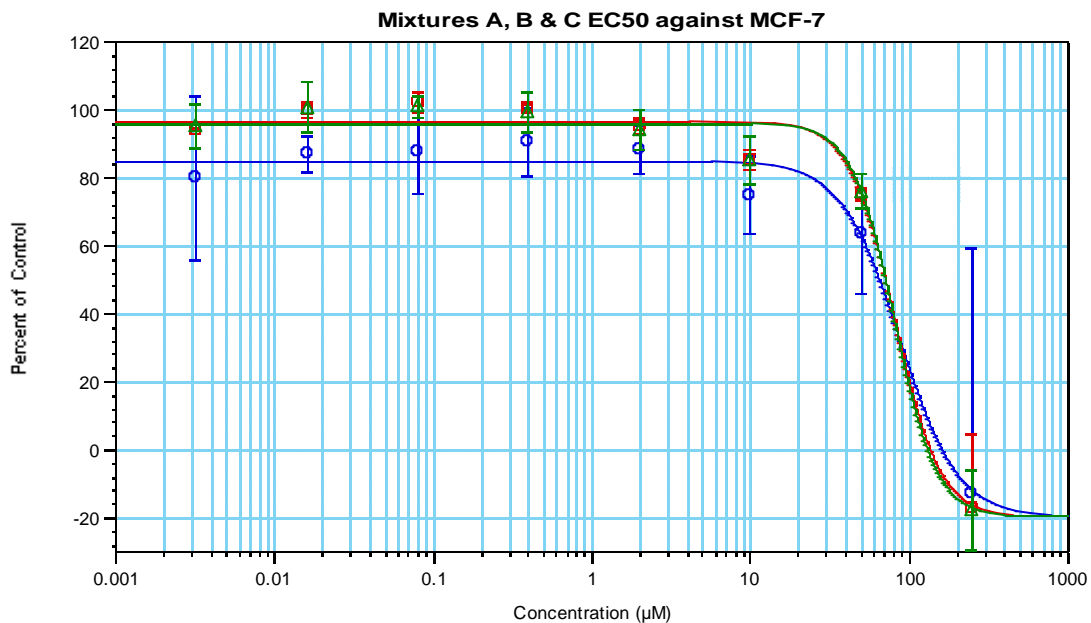
24hour Growth  $A_{570nm} = 0.235$  (CV = 4.7%) i.e. - MTT signal at beginning of drug exposure. This value is subtracted from the 96 hours exposure data shown in the plate data above before Percent of Control is calculated.

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

	A2780 (Absorbance 570nm)										
	24 hrs. growth						96hrs. growth				
	6250 cells/well	0.240	0.231	0.233	0.245	0.242	0.235	1.493	1.312	1.296	1.224
	0.234	0.236	0.241	0.239	0.246	0.215	1.733	1.726	1.646	1.271	1.245
	0.235	0.241	0.231	0.248	0.258	0.230	1.745	1.558	1.785	1.301	1.249
	0.239	0.238	0.240	0.245	0.260	0.219	1.610	1.554	1.545	1.353	1.234
	0.233	0.235	0.233	0.247	0.250	0.219	1.574	1.644	1.516	1.270	1.230
	0.234	0.236	0.229	0.246	0.249	0.216	1.586	1.637	1.491	1.235	1.275
	0.244	0.227	0.221	0.249	0.235	0.214	1.626	1.322	1.314	1.240	1.243
	0.231	0.226	0.220	0.245	0.228	0.215	1.332	1.165	1.287	1.292	1.308
No Cells>>	-0.014	0.000	0.002	0.004	0.005	0.003	0.005	-0.004			
	<b>AVE</b>	<b>STDDV</b>	<b>%CV</b>								
	0.2354	0.0111	4.7								

24hour Growth  $A_{570nm} = 0.235$  (CV = 4.7%)

## EC<sub>50</sub> Assay of Peptide Combination Mixtures “A”, “B” & “C” 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>
○ Peptides 17, 20 (Mixture A: Conc vs Pcnt Control)	84.9	2.36	87.4	-20	0.98
■ Peptides 17, 20, 21 (Mixture B: Conc vs Pcnt Control)	96.5	3.06	81.5	-20	0.982
△ Peptides 17, 20, 21, 23 (Mixture C: Conc vs Pcnt Control)	96	3.26	80.8	-20	0.985

Curve Fit Option - Fixed Weight Value

Fixed Parameter(s):

D

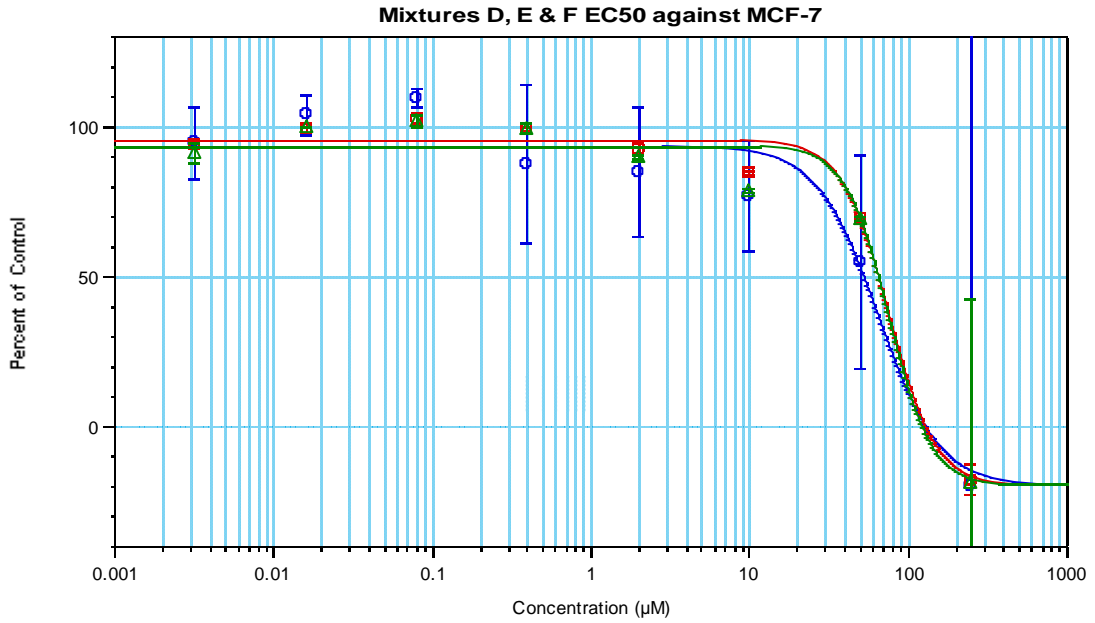
A = Top

B = Slope

C = EC<sub>50</sub>

D = Bottom

**EC<sub>50</sub> Assay of Peptide Combination Mixtures “D”, “E” & “F” 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>
○ Peptides (17, 20, 21, 23, 24) (Mixture D: Conc vs Pcnt Co...	93.4	2.25	65.1	-20	0.939
■ Peptides 17, 20, 21, 23, 24, 13 (Mixture E: Conc vs Pcnt ...	95.6	2.93	75.4	-20	0.982
△ Peptides (17, 20, 21, 23, 24, 13, 14) (Mixture F: Conc vs ...	93.7	3.18	75.3	-20	0.965

Curve Fit Option - Fixed Weight Value

Fixed Parameter(s):

D

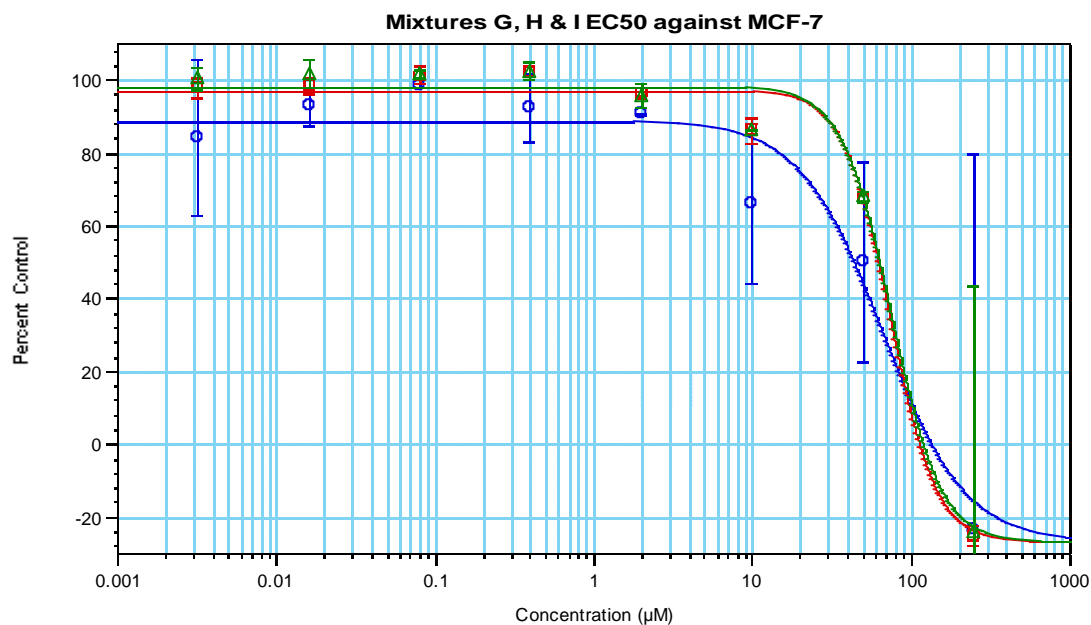
A = Top

B = Slope

C = EC<sub>50</sub>

D = Bottom

## EC<sub>50</sub> Assay of Peptide Combination Mixtures "G", "H" & "I" 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>
○ Peptides 17,20,21,23,24,13,14,15 (Mixture G: Conc vs Pc...	88.9	1.67	66.1	-27	0.951
■ Peptides 17,20,21,23,24,13,14,15,16 (Mixture H: Conc v s ...	97.2	2.98	73.6	-27	0.988
△ Peptides 17,20,21,23,24,13,14,15,16,18 (Mixture I: Conc v ...	98.3	2.78	75.1	-27	0.986

Curve Fit Option - Fixed Weight Value

Fixed Parameter(s):

D

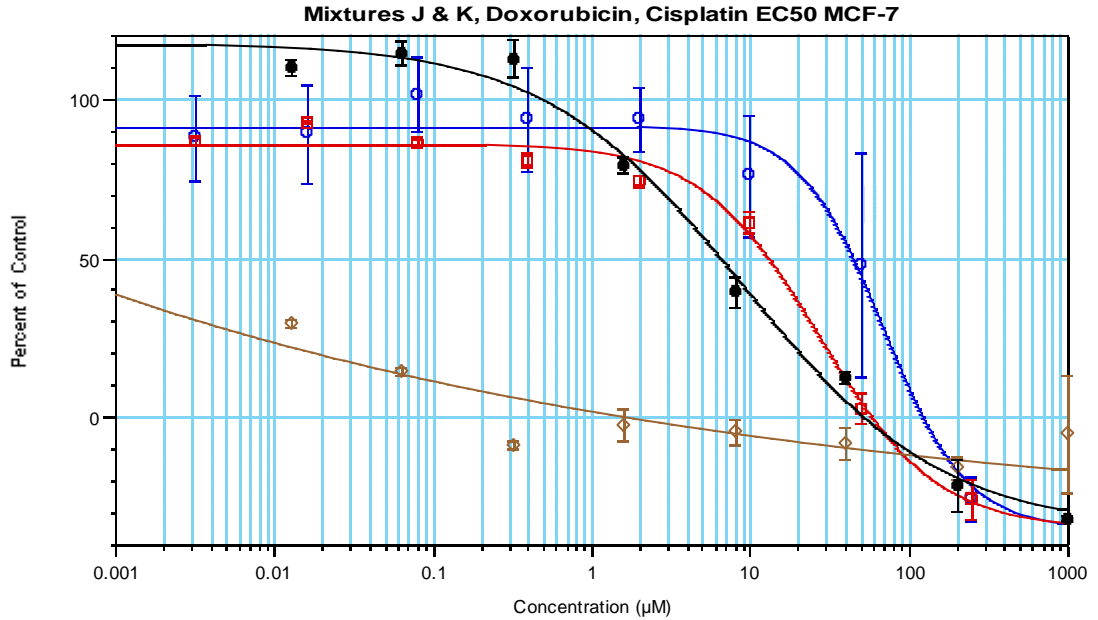
A = Top

B = Slope

C = EC<sub>50</sub>

D = Bottom

**EC<sub>50</sub> Assay of Peptide Combination Mixtures “J” & “K” and Doxorubicin and Cisplatin as Single Agents 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>
○ Peptides 17,20,21,23,24,13,14,15,16,18,19 (Mixture J: Con...	91.5	1.64	68.8	-35	0.979
◻ Peptides 17,20,21,23,24,13,14,15,16,18,19,22 (Mixture K: ...	85.9	1.17	27.4	-35	0.991
◇ Doxorubicin Positive Control (Doxorubicin: Conc vs Pcnt ...	4.16e+09	0.1	5.14e-81	-35	0.716
● Cisplatin Positive Control (Cisplatin: Conc vs Pcnt Control)	118	0.692	9.12	-35	0.992

Curve Fit Option - Fixed Weight Value

Fixed Parameter(s):

D

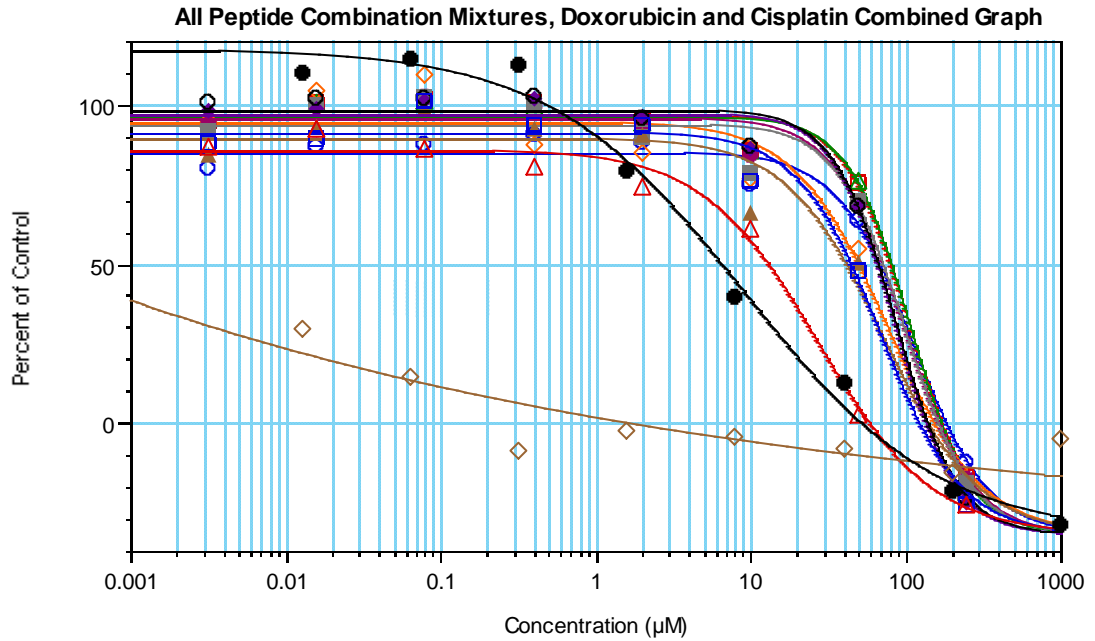
A = Top

B = Slope

C = EC<sub>50</sub>

D = Bottom

**EC<sub>50</sub> Assay of All Peptide Combination Mixtures and Doxorubicin and Cisplatin as Single Agents 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>
○ Mixture A (Mixture A@Experiment#1: Conc vs Pc...	85.2	1.78	112	-35	0.982
□ Mixture B (Mixture B@Experiment#1: Conc vs Pc...	96.7	2.1	106	-35	0.984
△ Mixture C (Mixture C@Experiment#1: Conc vs Pc...	96.2	2.18	107	-35	0.986
◇ Mixture D (Mixture D@Experiment#2: Conc vs Pc...	94.7	1.44	77.3	-35	0.948
● Mixture E (Mixture E@Experiment#2: Conc vs Pc...	95.9	1.93	97.7	-35	0.984
■ Mixture F (Mixture F@Experiment#2: Conc vs Pc...	94.1	2	99.1	-35	0.968
▲ Mixture G (Mixture G@Experiment#4: Conc vs Pc...	89.6	1.4	71.6	-35	0.957
◆ Mixture H (Mixture H@Experiment#4: Conc vs Pc...	97.4	2.23	85.6	-35	0.989
◊ Mixture I (Mixture I@Experiment#4: Conc vs Pc...	98.5	2.16	86.3	-35	0.987
◻ Mixture J (Mixture J: Conc vs Pc...	91.5	1.64	68.8	-35	0.979
△ Mixture K (Mixture K: Conc vs Pc...	85.9	1.17	27.4	-35	0.991
◇ Doxorubicin (Doxorubicin: Conc vs Pc...	4.16e+09	0.1	5.14e-81	-35	0.716
● Cisplatin (Cisplatin: Conc vs Pc...	118	0.692	9.12	-35	0.992

Curve Fit Option - Fixed Weight Value  
Fixed Parameter(s):

A = Top

B = Slope

C = EC<sub>50</sub>

D = Bottom