

CompuSyn Report

Experiment Name: MCF-7 *in vitro*

Date: 14-08-21

File Name: C:\Users\User\Desktop\Esha-MDA-Compusyn (14-08-21)-report\MCF 14-8-21.cse

Description: Combination of Curcumin and Doxorubicin against breast cancer cell-line (MCF-7), *in vitro* study

Drug: Curcumin (Cur.) [μM]

Drug: Doxorubicin (Doxo.) [μM]

Drug Combo: Curcumin+ Doxorubicin (Combo) (Cur.+ Doxo. [100:1])

Data for Drug: Cur. [μM]

Data points usually from the average 'Fa' values of duplicate or triplicate assays

Dose	Effect
5.0	0.12
10.0	0.18
15.0	0.23
20.0	0.33
25.0	0.42
30.0	0.47
35.0	0.58
40.0	0.66
45.0	0.74
50.0	0.79
55.0	0.85
60.0	0.97

Design Dose Range: Some doses above 'Dm', and some doses below the 'Dm' value (e.g. here Dm= 23.401 μM)

The approximate 'Dm' value can be from the wet-lab experiments, preliminary data or from published literature

'Fa' values cannot be <0.01 or >0.99, unless the assay is very accurate. If, Fa=0 or 1 is entered, the software would crash

12 data points entered

X-int: 1.36923

Y-int: -2.5193 +/- 0.40900

The 'slope' of the median-effect (ME) plot, the dynamic order, or the 'shape' of

m: 1.83990 +/- 0.28078

Dose-Effect curve; m=1, >1 and <1

Dm: 23.4009

Dm: The median effect dose, in this case it is IC50 value, which indicate "potency"
The value can be obtained from the X-intercept of the ME-plot

r: 0.90061

r: The linear correlation coefficient of the ME-plot. It signifies the "conformity" of the data with the mass-action law, an indication of how good are the data, when r=1, it is perfect. For *in vitro* experiment, usually r>0.95 are considered good

Data for Drug: Doxo. [μM]

Dose Effect

0.25	0.2
0.5	0.34
1.0	0.45
1.25	0.53
1.5	0.61
1.75	0.68
2.0	0.74
2.25	0.79
2.75	0.85
3.0	0.94

D1 has 12 doses (concentrations), and D2 has 10 concentrations, not the same number is OK as long as they provide m_1 , Dm_1 and m_2 , Dm_2 values from the Dose-Effect curves

10 data points entered.

X-int: -0.0611

Y-int: 0.08773 +/- 0.06651

m: 1.43515 +/- 0.19298

Dm: 0.86871

r: 0.93468

The m_1 , Dm_1 as well as m_2 , Dm_2 are absolute requirements for determining synergism or antagonism or additive effect since they are required for the calculation of the CI value

Data for Drug Combo: Combo (Cur.+ Doxo. [100:1])

Dose A Effect

10.0+	0.43
15.0+	0.52
20.0+	0.61
25.0+	0.69
30.0+	0.77
35.0+	0.91
40.0+	0.99

In this case 100:1 means Curcumin 100 μM + Doxorubicin 1 μM , etc.

7 data points entered.

X-int: 1.16150

Y-int: -3.3060 +/- 1.14843

m: 2.84630 +/- 0.83489

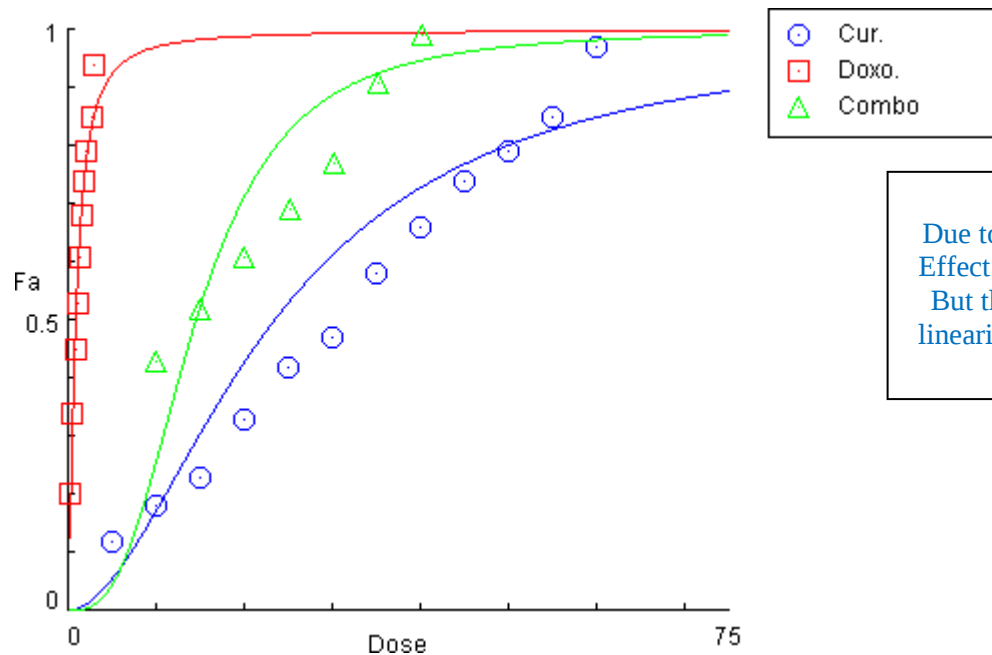
Dm: 14.5044

r: 0.83619

[NOTES]

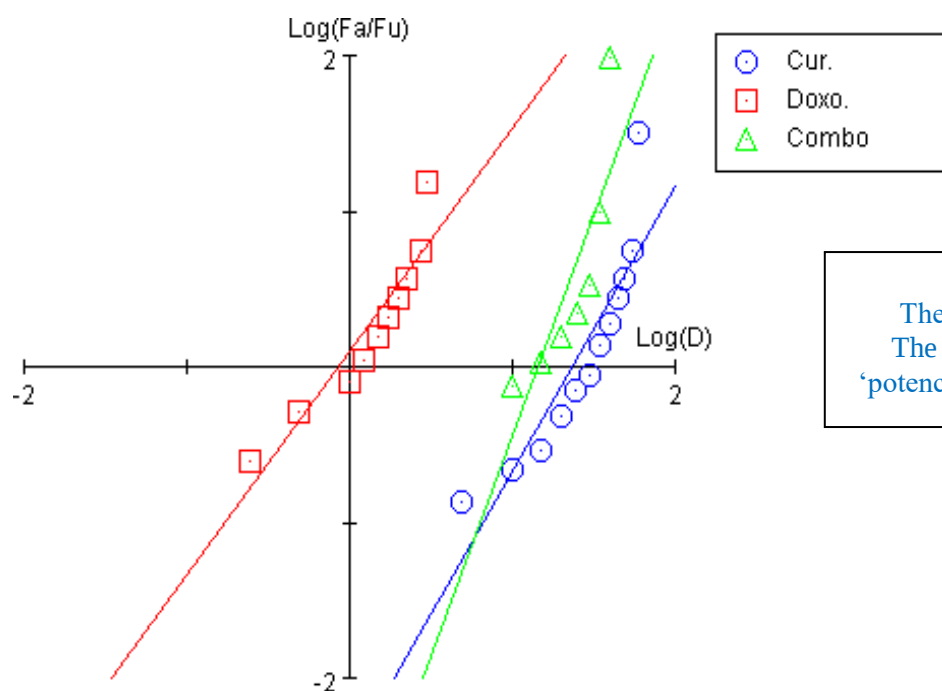
The constant ratio combination allows computerized simulation of dose-effect curves, Fa-CI effect, Fa-DRI Plot, and isobologram based on the m_1 , m_2 and Dm_1 , Dm_2 values. When combinations are at non-constant ratios, each "data point" has a ratio, the CI and DRI value can still be calculated, but automated computer simulation can't be carried out; therefore, the acquired conclusions are limited

Dose-Effect Curve

**[NOTES]**

Due to automated scaling, the Dose-Effect curves are somewhat jammed.
But they are clearly separated after linearization by the media-effect plot
(Find below)

Median-Effect Plot

**[NOTES]**

The 'slope' (m) signifies the shape.
The x-intercept (logDm) signifies the 'potency' (the antilog gives the Dm value)

CI Data for Drug Combo: Combination (Cur.+ Doxo.) [100:1]

Fa	CI Value	Total Dose
0.05	1.53782	5.15504
0.1	1.28926	6.70260
0.15	1.15747	7.88553
0.2	1.06789	8.91203
0.25	0.99956	9.85988
0.3	0.94373	10.7700
0.35	0.89594	11.6693
0.4	0.85359	12.5786
0.45	0.81499	13.5170
0.5	0.77900	14.5044
0.55	0.74471	15.5639
0.6	0.71139	16.7250
0.65	0.67834	18.0283
0.7	0.64483	19.5336
0.75	0.60999	21.3368
0.8	0.57256	23.6061
0.85	0.53051	26.6789
0.9	0.47970	31.3875
0.95	0.40828	40.8101
0.97	0.36440	49.1915

[NOTES]

CI<1 indicates synergism,
CI=1 indicates additive effect and CI>1 indicates
antagonism.

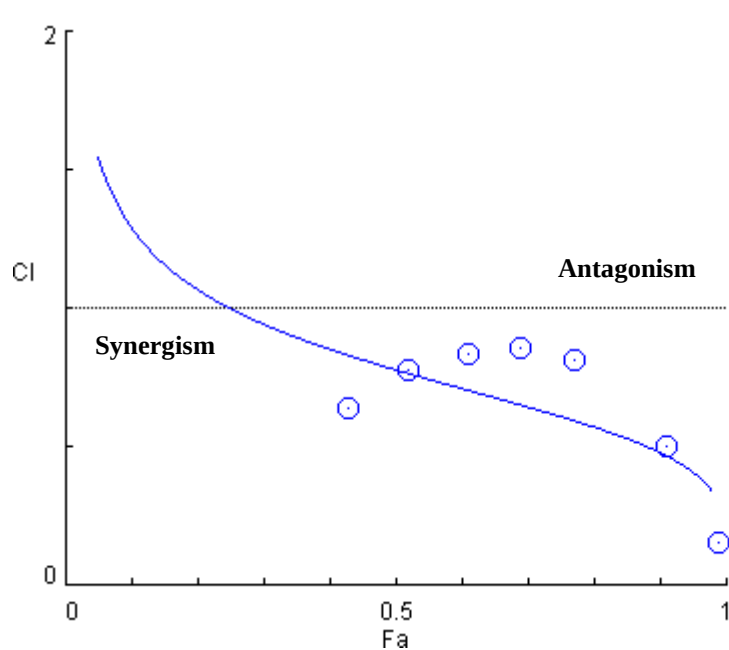
This is Fa-CI table with Fa increment of 0.05
At Fa>0.5 showed synergistic effect (CI<1)
For anti-cancer agents, synergism (CI<1) at high
dose (high effect) is more relevant to the therapy
than the CI values at low dose (low effect)

CI values for actual experimental points:

Total Dose	Fa	CI Value
10.1	0.43	0.63817
15.15	0.52	0.77702
20.2	0.61	0.83879
25.25	0.69	0.85638
30.3	0.77	0.81358
35.35	0.91	0.50569
40.4	0.99	0.15940

The CI values for each individual
combination data point without a simulation

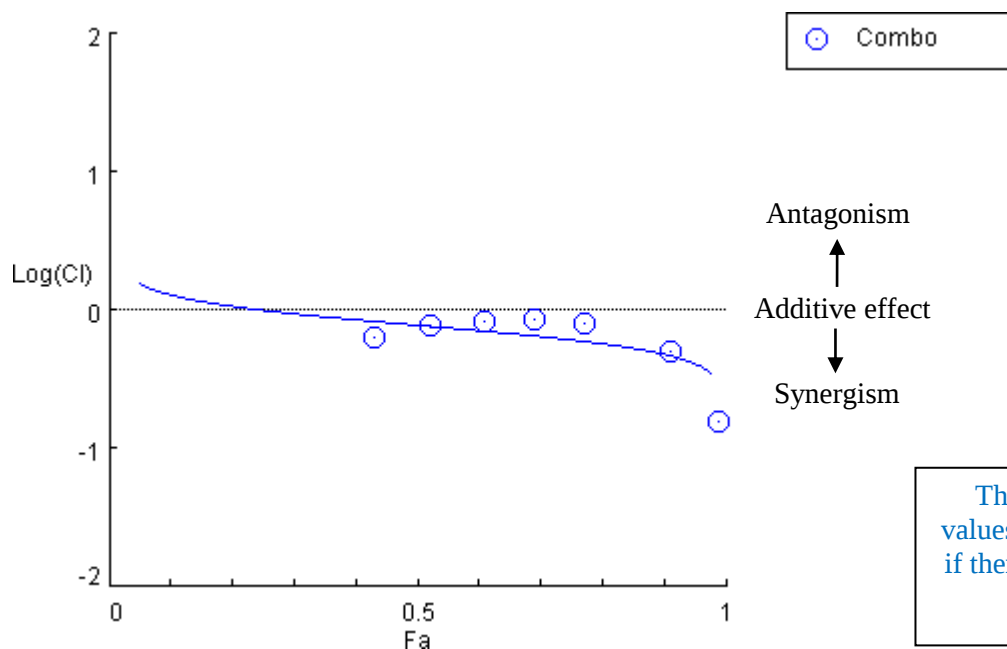
Combination Index Plot

**[NOTES]**

Among 7 combination data points all are on the synergy side (CI<1), among them, 3 points are slightly synergistic and 4 are highly synergistic

The simulation at low Fa showed substantial antagonism. This is of less concern since CI values for synergism is 0-1, and for antagonism is 1- ∞ low Fa is less relevant to therapy than high Fa (i.e. Killing cancer cells in small fraction is not useful in cancer therapy)

Logarithmic Combination Index Plot



The logarithmic scale for CI values is to condense the graph so if there are out of scale data point can be shown

DRI Data for Drug Combo: Combo (Cur.+Doxo.) [100:1]

Fa	Dose Cur.	Dose Doxo.	DRI Cur.	DRI Doxo.
0.05	4.72302	0.11165	0.92536	2.18745
0.1	7.08915	0.18792	1.06825	2.83168
0.15	9.11576	0.25939	1.16757	3.32240
0.2	11.0156	0.33065	1.24840	3.74721
0.25	12.8799	0.40404	1.31936	4.13875

Fa	Dose Cur.	Dose Doxo.	DRI Cur.	DRI Doxo.
0.3	14.7650	0.48136	1.38464	4.51413
0.35	16.7152	0.56434	1.44673	4.88450
0.4	18.7726	0.65490	1.50735	5.25851
0.45	20.9829	0.75535	1.56785	5.64401
0.5	23.4009	0.86871	1.62950	6.04915
0.55	26.0975	0.99908	1.69356	6.48338
0.6	29.1702	1.15232	1.76155	6.95868
0.65	32.7606	1.33722	1.83535	7.49151
0.7	37.0878	1.56775	1.91765	8.10616
0.75	42.5159	1.86778	2.01254	8.84137
0.8	49.7115	2.28235	2.12694	9.76519
0.85	60.0720	2.90928	2.27418	11.0138
0.9	77.2450	4.01588	2.48562	12.9225
0.95	115.943	6.75922	2.86945	16.7282
0.97	154.789	9.79002	3.17812	20.1009

[NOTES]

DRI>1 and <1 indicate favorable and not favorable Dose-reduction,
DRI=1 indicates no Dose-reduction
This is Fa-DRI table with Fa increment of 0.05

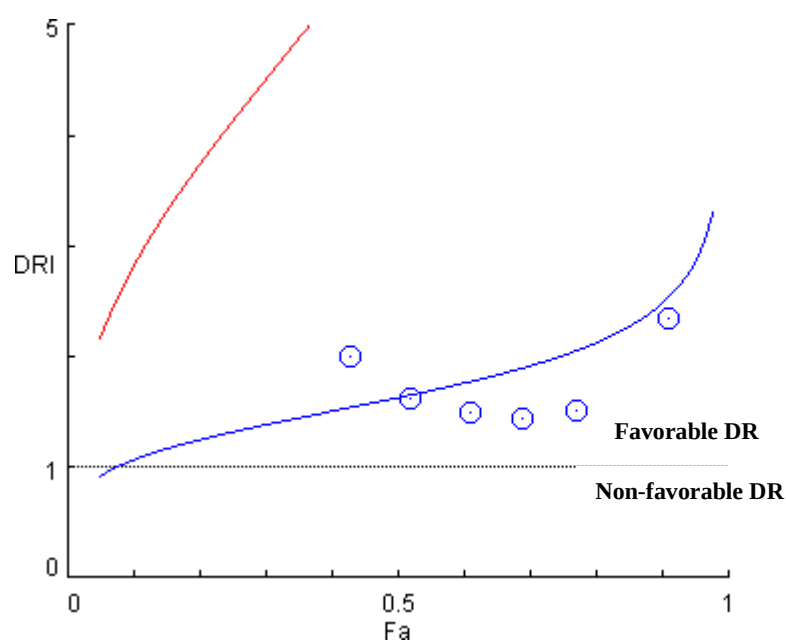
At 50% inhibition, it requires 23.4009µM of Curcumin, and requires 0.8687µM of Doxorubicin.
However, it requires 1.62950-fold less Curcumin plus 6.04915-fold less Doxorubicin to achieve the same 50% inhibition (i.e., **14.361µM Cur. + 0.14361µM Doxo.**) (100:1 combination)

DRI values calculated at experimental points

Fa	Dose Cur.	Dose Doxo.	DRI Cur.	DRI Doxo.
0.43	20.0772	0.71381	2.00772	7.13808
0.52	24.4414	0.91853	1.62943	6.12355
0.61	29.8412	1.18641	1.49206	5.93206
0.69	36.1488	1.51705	1.44595	6.06819
0.77	45.1279	2.01615	1.50426	6.72050
0.91	82.2902	4.35520	2.35115	12.4434
0.99	284.365	21.3508	7.10912	53.3769

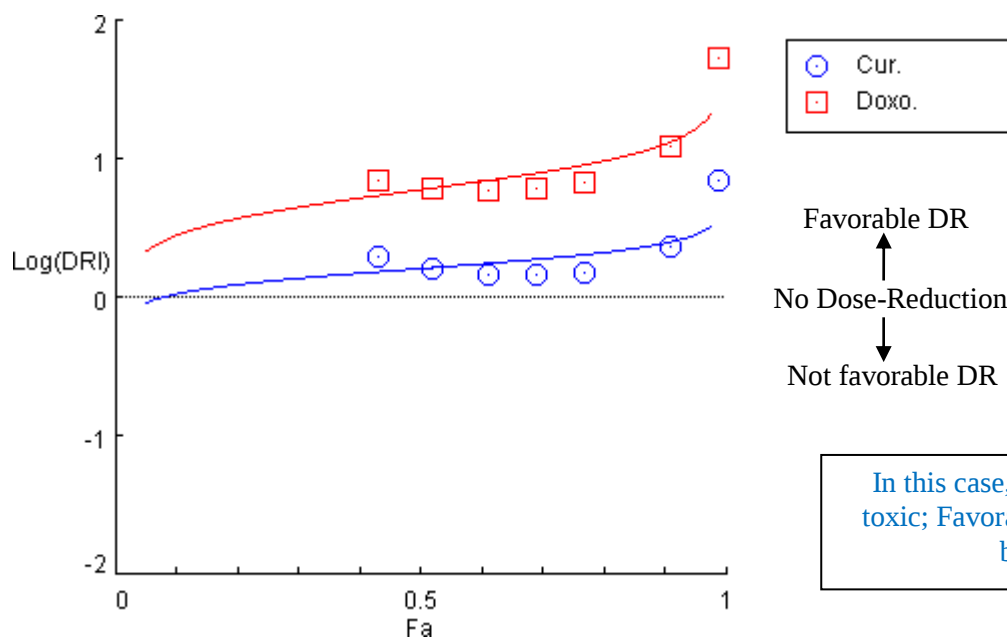
DRI values of each drug at each combination data point

DRI Plot for Combo: Combo (Cur.+Doxo.) [100:1]

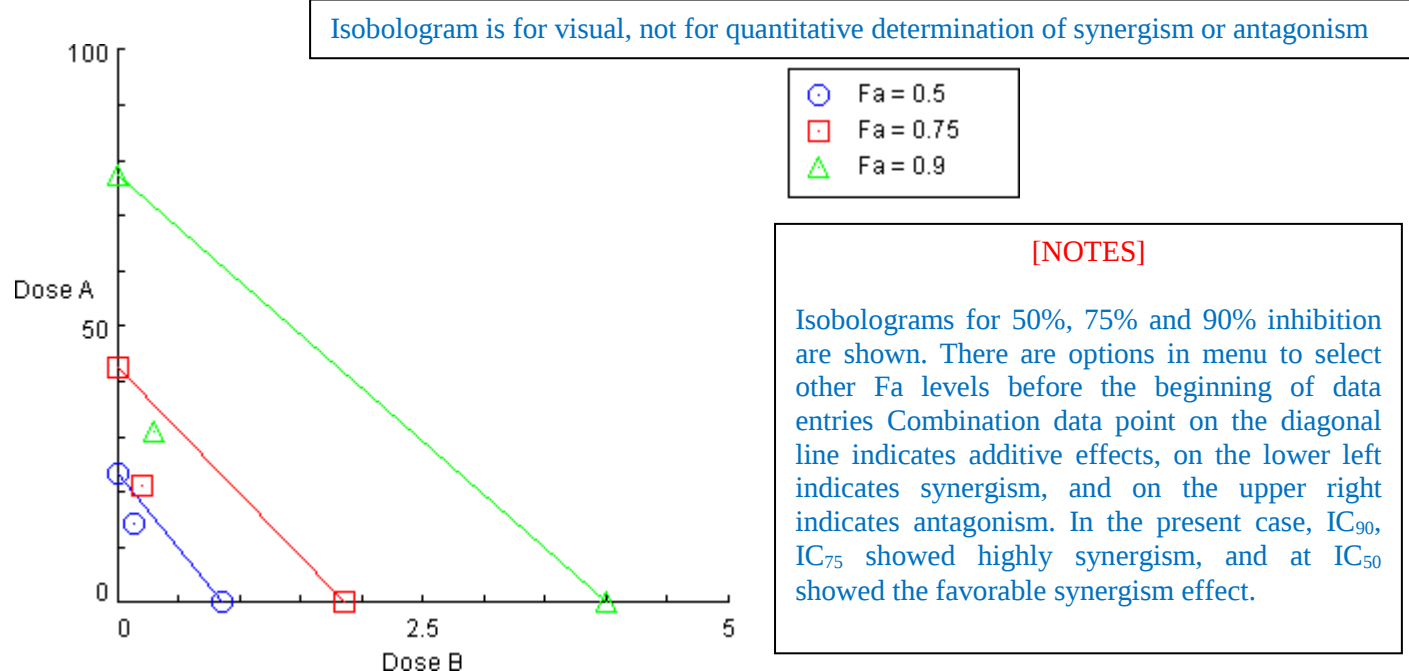


DRI values for Cur. (○) and Doxo. (□) are shown.
All combinations show favorable DRI (>1).
The simulation just to show the trends

Log(DRI) Plot for Combo: Combo (Cur.+Doxo.) [100:1]



Isobologram for Combo: Combo (Cur.+ Doxo.) [100:1]



Summary Table

Experiment Name:	MCF-7 in vitro	Most of the contents are used for constructing Table: 1
Date:	14-08-21	
File Name:	C:\Users\User\Desktop\Esha-MDA-Compusyn (14-08-21)-report\MCF 14-8-21.cse	
Description	Combination of Curcumin and Doxorubicin against breast cancer cell-line (MCF-7), in vitro study	
Drug:	Curcumin (Cur.) [μM]	
Drug:	Doxorubicin (Doxo.) [μM]	
Drug Combo:	Curcumin+Doxorubicin (Combo) (Cur.+ Doxo.) [100:1]	

Drug/Combo	Dm	m	r
Cur.	23.4009	1.83990	0.90061
Doxo.	0.86871	1.43515	0.93468
Combo	14.5044	2.84630	0.83619

From 'Parameters' in Table: 1

CI values at:

Combo	ED50	ED75	ED90	ED95
Combo	0.77900	0.60999	0.47970	0.40828

From the 'Fa-CI Table' of simulation of CI values
For numbers given in CI column of Table 1 (at bottom)

Data for Fa = 0.5

Drug/Combo	CI value	Dose Cur.	Dose Doxo.
Cur.		23.4009	
Doxo.			0.86871
Combo	0.77900	14.3608	0.14361

These data are illustrated for the ED₅₀- Isobologram at
Fa=0.5 (in Fig.1d)
For DRI at Fa=0.5 (bottom of Table: 1)
For Curcumin=23.4009/1.62950 =14.3608
For Doxorubicin=0.8687/6.04915=0.14361

Data for Fa = 0.75

Drug/Combo	CI value	Dose Cur.	Dose Doxo.
Cur.		42.5159	
Doxo.			1.86778
Combo	0.60999	21.1255	0.21125

For ED₇₅-isobologram in Fig. 1d

Data for Fa = 0.9

Drug/Combo	CI value	Dose Cur.	Dose Doxo.
Cur.		77.2450	
Doxo.			4.01588
Combo	0.47970	31.0767	0.31077

For ED₉₀-isobologram in Fig. 1d

Data for Fa = 0.95

Drug/Combo	CI value	Dose Cur.	Dose Doxo.
Cur.		115.943	
Doxo.			6.75922
Combo	0.40828	40.4061	0.40406

Synergy (CI<1) at high effect levels, (e.g., at
Fa>0.90) is more relevant for anticancer
(therapeutic) effect than the CI at low effect
levels (e.g., at Fa<0.3)

Data for Fa = 0.97

Drug/Combo	CI value	Dose Cur.	Dose Doxo.
Cur.		154.789	
Doxo.			9.79002
Combo	0.36440	48.7044	0.48704

[NOTES for manual calculation]

From the above Report, we obtain:

$$m_1=1.83990, (Dm)_1=23.4009\mu M;$$

$$m_2=1.43515, (Dm)_2=0.86871\mu M;$$

$$m_{1,2}=2.84630, (Dm)_{1,2}=14.5044\mu M; \text{Cur.+ Doxo. [100:1]} = \mathbf{14.361\mu M \text{ Curcumin} + 0.14361\mu M \text{ Doxorubicin}}$$

All parameters are calculated from the median-effect principle and equation of the mass-action law

$$fa/fu = (D/Dm)^m \text{ or } D = Dm [fa/(1-fu)]^{1/m} \quad (\text{Chou equation})$$

$$\log(fa/fu) = m \log(D) - m \log(Dm)$$

Thus, the Median-effect Plot (MEP): $x = \log(D)$ $y = \log(fa/fu)$

Gives the slope m , and the x -intercept $\log(Dm)$, then the antilog of the X -intercept gives the Dm value.

Based on the Combination index Theorem (CIT) and the Median-Effect Equation and Plot, when the combination $D_{1,2}$ for D_1 and D_2 is P/Q , we got:

$$CI = \frac{(D)_1}{(D_x)_1} + \frac{(D)_2}{(D_x)_2} = \frac{(D)_{1,2} [P/(P+Q)]}{(D_m)_1 [f_a/(1-f_a)]^{1/m_1}} + \frac{(D)_{1,2} [Q/(P+Q)]}{(D_m)_2 [f_a/(1-f_a)]^{1/m_2}}$$

Therefore, substituting, the m and Dm parameters, combination ratio P/Q into the corresponding equations given above, and setting $fa=0.01-0.99$, the CI values at all effect levels can be simulated as $Fa-CI$ table or $Fa-CI$ Plot. The default setting for the CompuSyn is $fa=0.05, 0.1, 0.15 \dots 0.95$ and 0.97

Based on the dose-reduction index (DRI) equations:

$$(DRI)_1 = \frac{(D_x)_1}{(D)_1}, \quad (DRI)_2 = \frac{(D_x)_2}{(D)_2}$$

$$(DRI)_1 = \frac{(D_m)_1 [f_a/(1-f_a)]^{1/m_1}}{(D)_1}, \quad (DRI)_2 = \frac{(D_m)_2 [f_a/(1-f_a)]^{1/m_2}}{(D)_2}$$

Similarly, $(DRI)_1$ and $(DRI)_2$ values at a particular combination data point can be determined or at different fa value can be simulated.