Supplementary Materials

[*The author's notes are added in the shaded areas on right-side-margin of the CompuSyn Report*]

CompuSyn Report

The analysis usually takes about 1 sec. Printing may take about 1 min.
The report contents depend on the selections at the "Generate Report" command in the menu.
Do not use "polygonogram" since it is for ⩾3 drug combos

Experiment Name: FD+PXT in MX-1 in Vitro

Date: 9. 15. 2015

Remove "*" in front of *. cse when give file name for saving

File Name: C:\Users\TingChaoChou\Desktop\FD.PXT.MX1. 9.15. 2015.cse

Description Combination of Fludelone (PD) and Panaxytriol (PXT) in Vitro against Mammary Cancer MX-1 Cell Growth, XTT assays

Drug: Fludelone (FD) [nM]

Can be different units. 1nM:1μM in this case.

Drug: Panaxytriol (PXT) [μM]

If both in μM, then the ratio is 1:1000

Drug Combo: Fludelone + Panaxytriol (FD+PXT) (FD+PXT [1:1])

Data for Drug: FD [nM]

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

Dose	Effect
0.05	0.0842
0.125	0.1208
0.25	0.2204
0.5	0.2222
1.25	0.3584
2.5	0.4531
5.0	0.6309
12.5	0.7308

Design Dose Range: Some doses above Dm, and some doses below the Dm value (e.g. here Dm=2.724nm);

The approximate Dm value can be from the preliminary data or from published literature

Do not enter fa <0.01 or Fa >0.99, unless the assay is very accurate
If enter fa=0 or fa=1, the computer will crash

8 data points entered.

X-int: 0.43522

Y-int: -0.2693 +/- 0.02517

m: 0.61871 +/- 0.03242

The "slope "of the median-effect (ME) plot, the dynamic order,
or the "shape" of dose-effect curve; m=1, >1 and <1 indicate

Dm: 2.72408

Dm: The median effect dose, in this case it is IC 50 value, which indicate "potency". The value can be obtained from the X-intercept of the ME-plot

r: 0.99186

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 or acceptable

Data for Drug: PXT [μM]

Dose	Effect
1.25	0.1305
2.5	0.2697
5.0	0.6349
12.5	0.9812
25.0	0.9949
50.0	0.9993

6 data points entered.

X-int: 0.50391

Y-int: -1.3101 +/- 0.16663

m: 2.59980 +/- 0.15768

Dm: 3.19086

r: 0.99272

D_1 has 8 doses (concentrations), and D_2 has 6 concentrations, not the same number is OK as long as they provide m1, $(Dm)_1$, m_2, and $(Dm)_2$ values from the dose-effect curves; Most cases, such as 5 vs 5, 6 vs 6 for D_1 and D_2 are OK.

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

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Data for Drug Combo: FD+PXT (FD+PXT [1:1])

In this case 1:1 means FD 0.5nM+PXT 0.5uM, etc

Dose A	Effect
0.5+	0.3218
1.25+	0.5136
2.5+	0.6332
5.0+	0.8777
12.5+	0.9786
25.0+	0.9943
50.0+	0.9995

7 data points entered.

X-int: 0.39023

Y-int: -0.6992 +/- 0.22052

m: 1.79184 +/- 0.18016

Dm: 2.45601

r: 0.97565

[NOTES]

Recommend to make a 1:1 mixture, and serial dilution them; Do not do more than 2-fold or 3-fold serial dilutions, otherwise the dose-range would be too large for the accurate measurements of effects.

The constant ratio combination allows computerized simulation of dose-effect curves, Fa-CI effect, Fa-DRI Plot, and isobologram based on the $m_{1,2}$ and $(Dm)_{1,2}$ 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.

[NOTES for manual calculation using a pocket calculator]

From the above Report, we obtain:
m_1=0.61871, $(Dm)_1$=2.72408nM ;
m_2=2.59980, $(Dm)_2$=3.19086μM;
$m_{1,2}$=1.79184, $(Dm)_{1,2}$=2.45601(1:1)=1.288nM+1.288μM

All parameters are calculated from the median-effect principle and equation of the mass-action law
$f_a/f_u = (D/D_m)^m$ or $D = D_m [f_a/(1-f_u)]^{1/m}$ (Chou equation)
$\log (f_a/f_u) = m \log (D) - m \log (D_m)$

Thus, the Median-effect Plot (MEP): $x = \log(D)$ $y = \log(f_a/f_u)$
gives the slope m, and the x-intercept logDm, 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…0.95 and 0.97

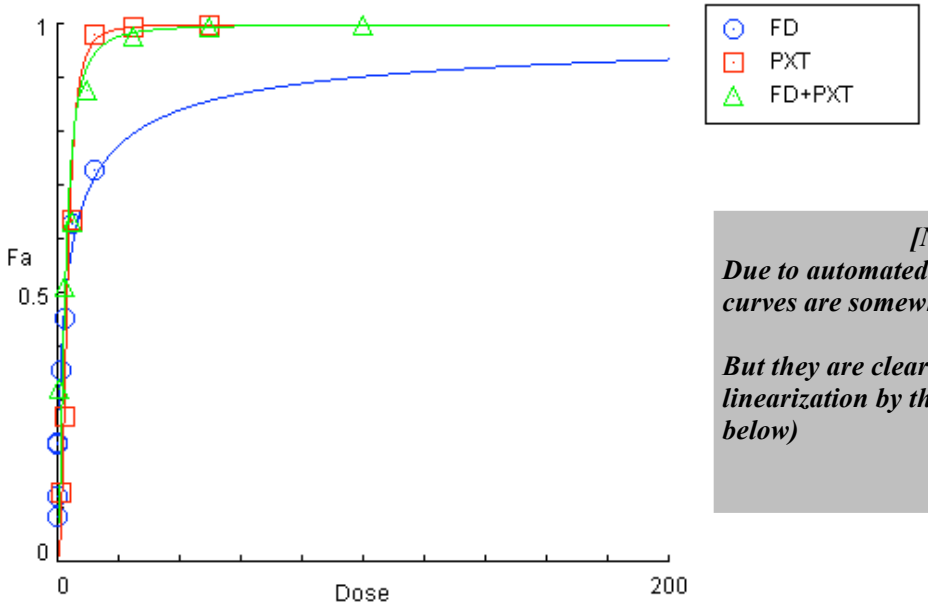
Based on the dose-reduction index (DRI) equations:

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

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

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

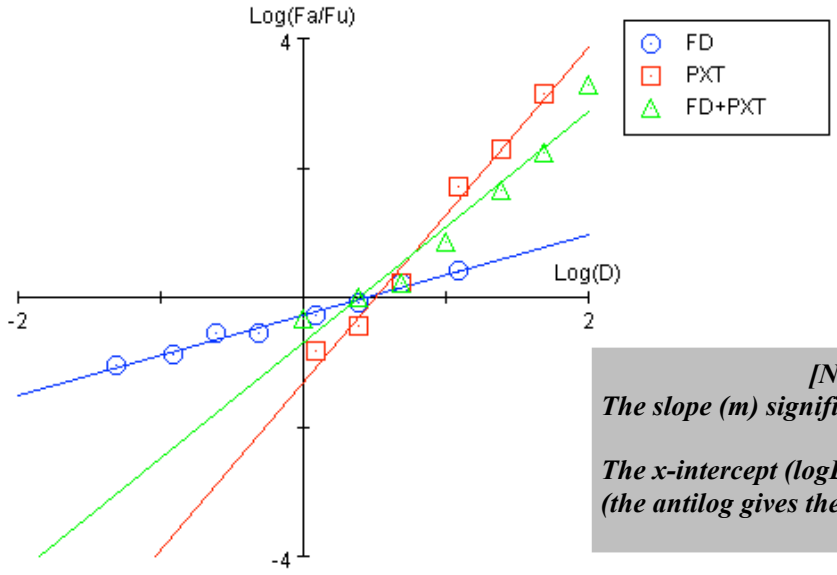
Dose-Effect Curve



[NOTES]
Due to automated scaling, the dose-effect curves are somewhat jammed.

But they are clearly separated after linearization by the media-effect plot (see 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: FD+PXT (FD+PXT [1:1])

Fa	CI Value	Total Dose
0.05	10.3965	0.47487
0.1	4.87333	0.72058
0.15	3.11063	0.93284
0.2	2.25725	1.13300
0.25	1.75974	1.33032
0.3	1.43726	1.53062
0.35	1.21357	1.73857
0.4	1.05105	1.95865
0.45	0.92913	2.19580
0.5	0.83565	2.45601
0.55	0.76303	2.74706
0.6	0.70641	3.07967
0.65	0.66262	3.46952
0.7	0.62968	3.94088
0.75	0.60660	4.53425
0.8	0.59342	5.32393
0.85	0.59185	6.46626
0.9	0.60745	8.37107
0.95	0.66132	12.7024
0.97	0.71467	17.0902

[NOTES]

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

This is Fa-CI table with Fa increment of 0.05.

At fa>0.45 showed synergistic effect (CI<1).

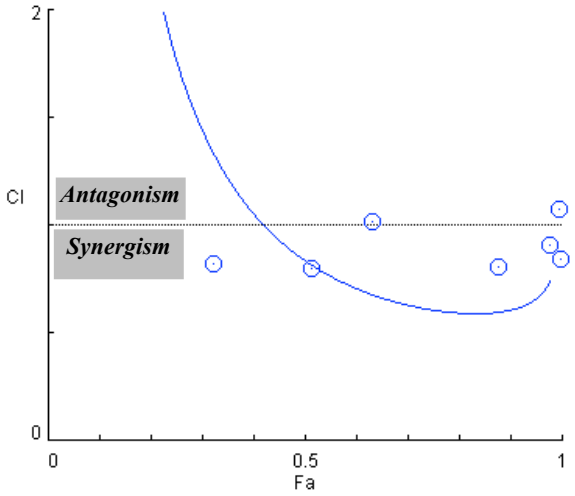
For anti-cancer agents, synergism (CI<1) at high dose (high effect) is more relevant to the therapy than the CI values at low dose (low effect).

CI values for actual experimental points:

Total Dose	Fa	CI Value
1.0	0.3218	0.82116
2.5	0.5136	0.80387
5.0	0.6332	1.01482
10.0	0.8777	0.81016
25.0	0.9786	0.90988
50.0	0.9943	1.07814
100.0	0.9995	0.84229

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

Combination Index Plot



FD+PXT

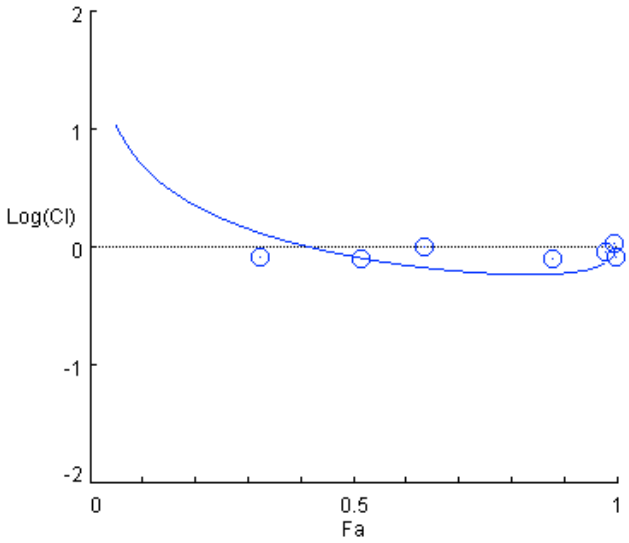
1:1 (nM vs μM)

[NOTES]

Among 7 combination data points 5 of them are on the synergy side (CI<1), the other 2 points are nearly additive

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



FD+PXT

Antagonism

Additive effect

Synergism

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: FD+PXT (FD+PXT [1:1])

Fa	Dose FD	Dose PXT	DRI FD	DRI PXT
0.05	0.02336	1.02811	0.09837	4.33008
0.1	0.07815	1.37045	0.21690	3.80375
0.15	0.16506	1.63736	0.35389	3.51047
0.2	0.28982	1.87210	0.51160	3.30468
0.25	0.46139	2.09115	0.69365	3.14384
0.3	0.69258	2.30339	0.90497	3.00974
0.35	1.00160	2.51477	1.15221	2.89292
0.4	1.41452	2.73008	1.44439	2.78772
0.45	1.96953	2.95383	1.79390	2.69044
0.5	2.72408	3.19086	2.21830	2.59841
0.55	3.76772	3.44691	2.74309	2.50953
0.6	5.24604	3.72941	3.40688	2.42195
0.65	7.40878	4.04872	4.27078	2.33388
0.7	10.7144	4.42027	5.43759	2.24329
0.75	16.0833	4.86889	7.09416	2.14761
0.8	25.6042	5.43860	9.61853	2.04308
0.85	44.9573	6.21830	13.9052	1.92331
0.9	94.9582	7.42939	22.6872	1.77501
0.95	317.709	9.90318	50.0234	1.55926
0.97	750.275	12.1503	87.8018	1.42191

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 2.72408 nm of FD, and requires 3.19086μM of PXT

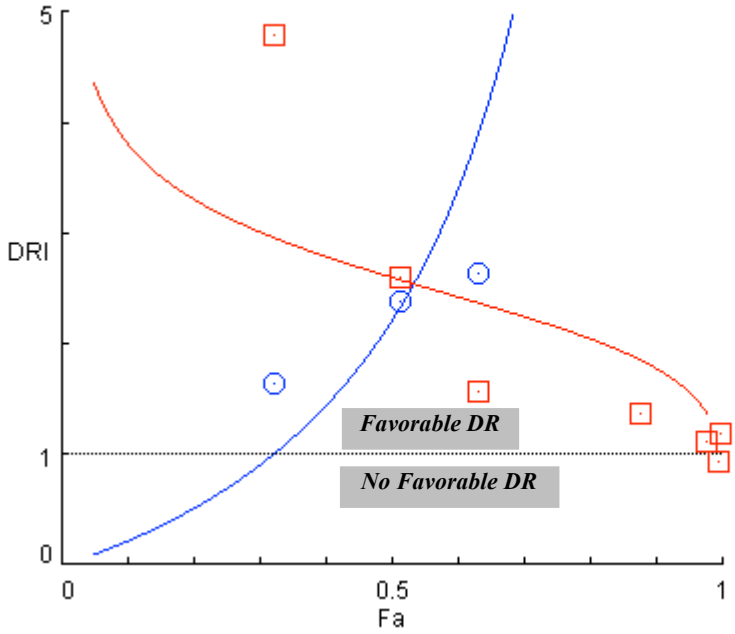
However, it requires 2.2183-fold less FD plus 2.5984-fold less PXT to achieve the same 50% inhibition (i.e., 1.2280nM FD+1.2280μM PXT)(1:1 combination)

DRI values calculated at experimental points

Fa	Dose FD	Dose PXT	DRI FD	DRI PXT
0.3218	0.81643	2.39536	1.63286	4.79071
0.5136	2.97451	3.25835	2.37961	2.60668
0.6332	6.58349	3.93651	2.63340	1.57460
0.8777	65.8592	6.80979	13.1718	1.36196
0.9786	1313.82	13.8833	105.105	1.11066
0.9943	11437.1	23.2352	457.484	0.92941
0.9995	589141.	59.3678	11782.8	1.18736

DRI values of each drug at each combination data point

DRI Plot for Combo: FD+PXT (FD+PXT [1:1])



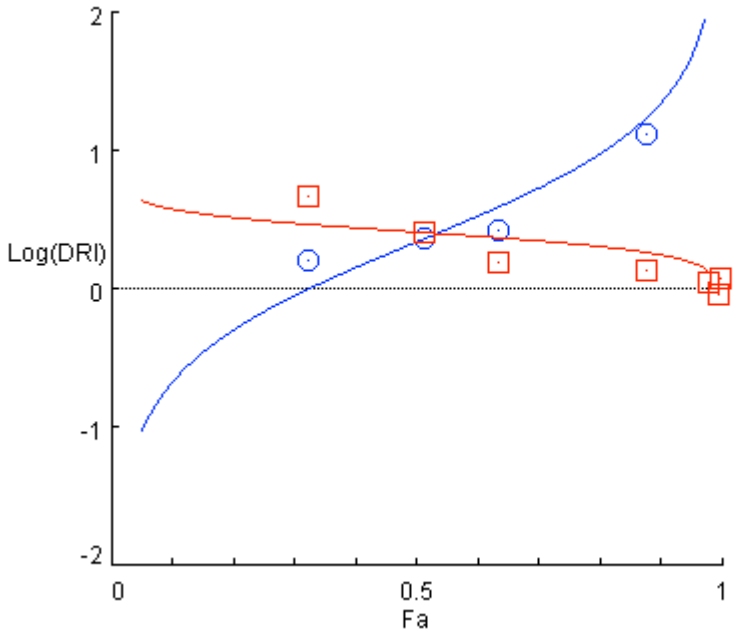
DRI values for FD (○) and PXT(□) are shown.

Most combinations show favorable DRI (>1).

The simulation just to show the trends.

Log(DRI) Plot for Combo: FD+PXT (FD+PXT [1:1])

The condensed log(DRI) scale



Favorable DR

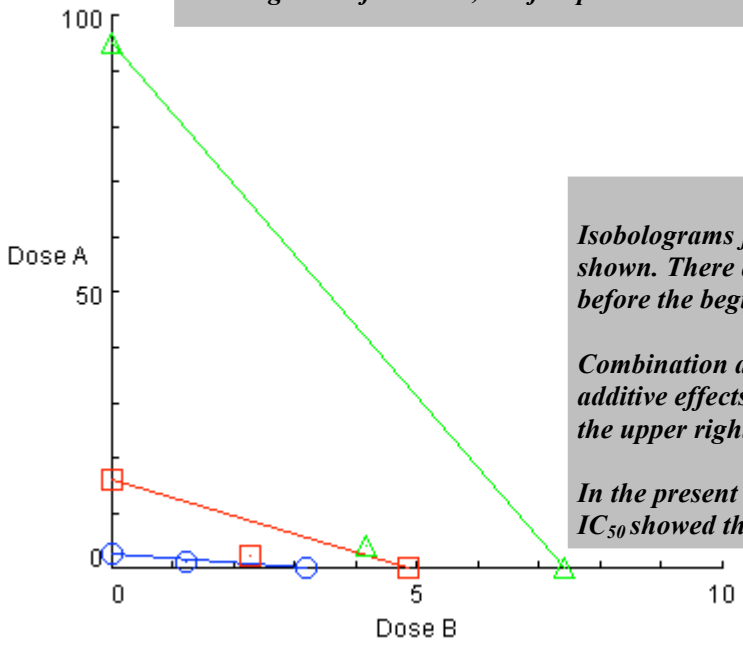
No Dose-Reduction

Not favorable DR

In this case, FD (blue circles) is very potent and toxic; Favorable dose-reduction is very beneficial to FD

Isobologram for Combo: FD+PXT (FD+PXT [1:1])

Isobologram is for visual, not for quantitative determination of synergism or antagonism



[NOTES]
Isobolograms for 50%, 75% and 90% inhibition are shown. There are options in menu to select other fa levels before the beginning of data entries

Combination data point on the diagonal line indicates additive effects, on the lower left indicates synergism, on the upper right indicates antagonism.

In the present case, IC_{90} and IC_{75} showed synergism, at IC_{50} showed the nearly additive effect.

Summary Table

Most of the contents are used for constructing Table 1.

Experiment Name: FD+PXT in MX-1 in Vitro

Date: 9. 15. 2015

File Name: C:\Users\TingChaoChou\Desktop\FD.PXT.MX1. 9.15. 2015.cse

Description Combination of Fludelone (PD) and Panaxytriol (PXT) in Vitro against Mammary Cancer MX-1 Cell Growth, XTT assays

Drug: Fludelone (FD) [nM]

Drug: Panaxytriol (PXT) [uM]

Drug Combo: FLudelone + Panaxytriol (FD+PXT) (FD+PXT [1:1])

Drug/Combo	Dm	m	r
FD	2.72408	0.61871	0.99186
PXT	3.19086	2.59980	0.99272
FD+PXT	2.45601	1.79184	0.97565

For "Parameters" in Table 1

CI values at:

Combo	ED50	ED75	ED90	ED95
FD+PXT	0.83565	0.60660	0.60745	0.66132

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 FD	Dose PXT
FD		2.72408	
PXT			3.19086
FD+PXT	0.83565	1.22801	1.22801

These data are illustrated for the ED_{50}-Isobologram at Fa=0.5 (in Fig. 1d)

For DRI at fa=0.5 (bottom of Table 1)
for FD=2.72408/1.22801=2.2183
for PXT=3.19086/1.22801=2.5984

Data for Fa = 0.75

Drug/Combo	CI value	Dose FD	Dose PXT
FD		16.0833	
PXT			4.86889
FD+PXT	0.60660	2.26713	2.26713

For ED_{75}-isobologram in Fig. 1d

Data for Fa = 0.9

Drug/Combo	CI value	Dose FD	Dose PXT
FD		94.9582	
PXT			7.42939
FD+PXT	0.60745	4.18553	4.18553

For ED_{90}-isobologram in Fig. 1d

Data for Fa = 0.95

Drug/Combo	CI value	Dose FD	Dose PXT
FD		317.709	
PXT			9.90318
FD+PXT	0.66132	6.35121	6.35121

Synergy (CI<1) at high effect levels (e.g., at fa>0.90) is more relevant to 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 FD	Dose PXT
FD		750.275	
PXT			12.1503
FD+PXT	0.71467	8.54510	8.54510

Supplementary Table1. Citation of Combination Index Method for Synergy Determination

Highly Cited Published Paper By T.C. Chou	Thomson Reuters Web of Science Citation Data Base*					
	Trends				Total	Average Citations per
	2011	2012	2013	2014	Citations	Year Since Publication
A. Chou, TC & Talalay, P Introduction of Combination Index (CI) / isobologram algorithms Adv. Eng. Regul. 22:27-55, 1984 [2]	289	257	283	324	3,524	110.1
B. Chou, TC Combination index /isobologram (Review) Pharmacol. Rev. 58: 621-681, 2006 [1]	119	146	158	203	968	96.8
C. Chou, TC Combination Index Method, (FAQ) Cancer Res. 70: 440-446, 2010 [3]	41	75	123	173	536	89.3

* Based on Thomson Reuters Web of Science All Database Collection, as of September 28, 2015.