SUPPLEMENTARY METHODS

Methods for assessing drug interaction

Chou-Talalay Combination Index for Loewe Additivity

Loewe additivity is a dose-effect model which states that additivity occurs in a two-drug combination if the sum of the ratios of the dose vs the median-effect for each individual drug is 1:

$$a/D_m(A) + b/D_m(B) = 1$$

where a, b are the doses for the two drugs A and B, and $D_m(A)$ and $D_m(B)$ represent the IC_{50} doses (median-effect) for the two drugs, respectively.

The *Chow-Talalay Combination Index* for Loewe Additivity model (1) assigns a quantitative measure to any given effect x produced by the combination of dose a of drug A and dose b of drug B:

$$CI = a/D_x(A) + b/D_x(B)$$

where $D_x(A)$ is the dose of drug A that alone produces the effect x and $D_x(B)$ is the dose of drug B that alone produces the effect x. For any given endpoint of the effect measurement CI estimates additive effect ($CI = 1$), synergism ($CI < 1$), or antagonism ($CI > 1$).

The combination index (CI) coefficients were computed based on the Chou-Talalay method implemented in *CalcuSyn* v2.11 (http://www.biosoft.com/w/calcusyn.htm). The degree of interaction between drugs was estimated according to the classification presented by Chou-Talalay (1):

CI range	Log10(CI) range	Classification
< 0.1	< -1	very strong synergism
0.1 to 0.3*	-1 to -0.52*	strong synergism
0.3 to 0.7	-0.52 to -0.15	Synergism
0.7 to 0.85	-0.15 to -0.07	moderate synergism
0.85 to 0.9	-0.07 to -0.05	slight synergism
0.9 to 1.1	-0.05 to 0.04	nearly additive
1.1 to 1.2	0.04 to 0.08	slight antagonism
1.2 to 1.45	0.08 to 0.16	moderate antagonism
1.45 to 3.3	0.16 to 0.52	Antagonism
3.3 to 10	0.52 to 1	strong antagonism
> 10	> 1	very strong antagonism

*any range in the table is left-side inclusive and right-side exclusive, i.e., "*a* to *b*" means " ≥ *a* and < *b* ".

The log *CI* - f_a plots showing log10(*CI*) on the *y* axis and the inhibitory fraction f_a on the *x* axis were restricted to *CI* scores computed for the range of concentrations of drug combinations where the effect of an individual drug was less than a fractional inhibition of 0.9. If the fractional inhibition score in paired combination with DMSO and with the other drug is > 0.9, this dose was eliminated from the analysis since the additional effect of the second drug could not be reliably calculated.

The Bliss Independence method is an effect-based strategy that compare the effect resulting from the combination of two drugs directly to the effects of its individual components (2,3). The model predicts that if the individual drugs have the inhibitory effects f_1 and f_2 then the *expected* combined effect of the two drugs is

$$E(f_{12}) = 1 - (1 - f_1)(1 - f_2) = f_1 + f_2 - f_1 f_2$$

The difference between the *observed* combined effect f_{12} and the *expected* combined effect of the two drugs is called the Excess over Bliss (*eob*):

$$eob = f_{12} - E(f_{12})$$

Positive *eob* values are indicative of synergistic interaction whereas negative *eob* values are indicative of antagonistic behavior. Null *eob* values indicate no drug interaction. For each drug pair the *eob* scores were depicted as 3D surface plots (Excel MSOffice v10) across all of the drug dose combinations and highlighted with gradient red for synergy and gradient blue for antagonism.

REFERENCES FOR SUPPLEMENTARY METHODS

1. Chou T-C. Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. Pharmacol Rev. 2006;58:621–81.
2. Bliss CI. The calculation of microbial assays. Bacteriol Rev. 1956;20:243–58.
3. Greco WR, Bravo G, Parsons JC. The search for synergy: a critical review from a response surface perspective. Pharmacol Rev. 1995;47:331–85.