

Supplemental Information

Data for the initial 45 mixtures tested are listed in Table S.1. The column *Observed Responses* lists synergism scores.

Table S.1 . Data for the initial 45 mixtures

Drug/ Mixture	Log IC50 (μL)	SE Log IC50 (μL)	IC50 (μL)	Observed Responses	Cross- Validation Predictions	Doxorubicin Dose Reduction	Number of Drugs
Baicalein	3.33	0.11	28.04 (0.75 μg/ml)	-	-	-	1
Curcumin	4.44	0.03	85.18 (1.71 μg/ml)	-	-	-	1
Doxorubicin	1.65	0.15	5.22 (0.65 μg/ml)	-	-	-	1
EGCG	3.55	0.05	34.67 (86.68 μg/ml)	-	-	-	1
Juglone	4.40	0.03	81.35 (1.59 μg/ml)	-	-	-	1
Luteolin	4.00	0.06	54.35 (4.10 μg/ml)	-	-	-	1
Plumbagin	3.83	0.03	45.90 (1.49 μg/ml)	-	-	-	1
Quercetin	3.83	0.08	46.17 (4.16 μg/ml)	-	-	-	1
Rhein	3.92	0.05	50.55 (7.79 μg/ml)	-	-	-	1
Vitamin K3	4.35	0.02	77.48 (10.38 μg/ml)	-	-	-	1
M1	4.58	0.04	97.43	0.07	0.31	4.01	10
M2	4.39	0.03	80.53	0.20	0.15	-	6
M3	4.16	0.04	63.83	-0.07	-0.06	-	2
M4	1.42	0.11	4.12	-0.20	-0.24	7.40	2
M5	4.05	0.07	57.48	0.00	0.01	-	2
M6	3.77	0.05	43.25	0.04	-0.02	-	3
M7	4.48	0.06	88.53	0.06	0.31	-	2
M8	3.55	0.07	34.96	0.00	0.00	6.71	7
M9	4.81	0.04	122.95	0.07	0.39	-	4
M10	4.13	0.14	61.90	0.07	0.05	3.98	7

M11	3.82	0.04	45.56	-0.01	-0.07	–	2
M12	4.13	0.03	61.94	0.04	0.09	–	2
M13	3.95	0.06	51.92	-0.05	0.02	–	2
M14	4.41	0.04	81.95	0.01	0.11	–	5
M15	3.04	0.10	20.93	-0.09	-0.07	4.16	3
M16	3.89	0.02	49.13	0.12	0.00	–	2
M17	4.31	0.05	74.70	0.14	0.03	–	5
M18	4.23	0.09	68.88	0.04	0.18	–	2
M19	3.69	0.07	40.22	0.13	-0.02	8.08	8
M20	2.99	0.10	19.92	-0.25	-0.18	6.62	3
M21	2.98	0.10	19.62	-0.11	-0.16	9.36	5
M22	3.59	0.05	36.11	0.01	0.00	–	2
M23	4.06	0.05	58.24	0.06	0.04	5.09	8
M24	4.06	0.05	58.24	-0.05	0.02	4.29	6
M25	1.38	0.14	3.97	-0.12	-0.23	7.35	2
M26	2.60	0.13	13.51	-0.14	-0.19	7.36	3
M27	4.53	0.03	92.82	0.05	0.08	–	3
M28	4.20	0.03	66.47	0.00	0.00	–	2
M29	3.58	0.06	36.04	-0.12	0.01	3.30	4
M30	2.96	0.09	19.28	-0.02	-0.09	5.46	4
M31	4.06	0.03	58.13	0.18	0.01	–	5
M32	3.88	0.06	48.66	0.08	-0.02	–	3
M33	4.16	0.05	64.06	0.18	0.09	–	5
M34	2.92	0.08	18.53	-0.11	-0.17	7.82	4
M35	3.36	0.08	28.80	-0.11	-0.11	9.74	7
M36	4.14	0.05	63.08	-0.04	0.01	–	2
M37	2.83	0.10	16.95	-0.07	-0.11	6.21	4
M38	4.36	0.03	78.39	0.04	0.15	–	4
M39	3.93	0.04	50.68	0.03	0.02	–	3
M40	4.30	0.04	73.76	-0.03	0.01	–	2
M41	4.13	0.05	62.29	0.03	0.00	–	5
M42	3.82	0.05	45.73	-0.01	-0.05	–	2
M43	2.05	0.08	7.79	-0.18	-0.23	9.13	3
M44	4.35	0.04	77.27	0.24	0.09	–	6
M45	2.77	0.15	15.92	-0.15	-0.15	7.68	4

Table S.2 provides information on the composition of mixtures tested. For modeling purposes, the mixture composition value for a given drug was assigned the value one if the fraction of drug in the mixture was greater than zero and assigned the value zero otherwise. Except for counts of drugs, values listed in the table are fractions of the total mixture content.

Table S.2. Mixture composition for 55 mixtures

Drug/ Mixture	Number of Drugs	Baicalein	Curcumin	Doxorubicin	EGCG	Juglone	Vitamin K3	Luteolin	Plumbagin	Quercetin	Rhein
Baicalein	1	1.00	-	-	-	-	-	-	-	-	-
Curcumin	1	-	1.00	-	-	-	-	-	-	-	-
Doxorubicin	1	-	-	1.00	-	-	-	-	-	-	-
EGCG	1	-	-	-	1.00	-	-	-	-	-	-
Juglone	1	-	-	-	-	1.00	-	-	-	-	-
Vitamin K3	1	-	-	-	-	-	1.00	-	-	-	-
Luteolin	1	-	-	-	-	-	-	1.00	-	-	-
Plumbagin	1	-	-	-	-	-	-	-	1.00	-	-
Quercetin	1	-	-	-	-	-	-	-	-	1.00	-
Rhein	1	-	-	-	-	-	-	-	-	-	1.00
M1	10	0.06	0.18	0.01	0.06	0.15	0.14	0.11	0.09	0.09	0.10
M2	6	0.10	0.28	-	0.10	-	0.22	0.16	0.13	-	-
M3	2	-	0.55	-	-	0.45	-	-	-	-	-
M4	2	-	-	0.17	0.83	-	-	-	-	-	-
M5	2	-	-	-	-	-	-	-	-	0.47	0.53
M6	3	0.19	-	-	-	0.48	-	-	-	-	0.33
M7	2	0.37	-	-	-	-	-	0.63	-	-	-
M8	7	0.10	0.30	0.02	0.11	-	-	-	0.14	0.15	0.17
M9	4	-	-	-	0.16	0.37	-	0.26	-	0.22	-
M10	7	0.10	-	0.02	0.10	0.24	0.23	0.17	-	0.14	-
M11	2	-	-	-	-	-	0.63	-	0.37	-	-
M12	2	-	-	-	-	-	-	-	0.49	0.51	-
M13	2	-	-	-	0.38	-	-	-	-	-	0.62
M14	5	-	-	-	0.12	0.27	0.26	-	0.16	-	0.19
M15	3	-	-	0.06	-	-	-	0.47	-	-	0.47
M16	2	-	-	-	-	-	-	0.55	0.45	-	-
M17	5	-	0.29	-	-	-	0.23	0.17	-	0.15	0.17
M18	2	0.40	-	-	-	-	-	-	-	0.60	-
M19	8	0.07	0.22	0.02	-	0.18	0.17	0.13	0.10	0.11	-
M20	3	-	0.53	0.04	-	-	0.43	-	-	-	-
M21	5	0.13	0.38	0.03	0.14	0.32	-	-	-	-	-
M22	2	-	-	-	0.43	-	-	-	0.57	-	-

M23	8	–	–	0.02	0.09	0.20	0.19	0.14	0.11	0.12	0.14
M24	6	–	0.28	0.02	–	0.23	–	0.17	0.13	–	0.16
M25	2	0.82	–	0.18	–	–	–	–	–	–	–
M26	3	–	–	0.05	–	0.59	–	–	–	0.36	–
M27	3	–	–	–	–	0.38	0.36	0.26	–	–	–
M28	2	–	–	–	–	0.64	–	–	0.36	–	–
M29	4	0.20	–	0.04	–	–	0.47	–	0.28	–	–
M30	4	–	–	0.05	0.24	–	–	0.39	0.32	–	–
M31	5	0.11	–	–	–	0.27	0.25	0.19	–	–	0.18
M32	3	–	0.51	–	0.18	–	–	0.30	–	–	–
M33	5	0.14	–	–	0.14	0.33	–	–	0.19	0.20	–
M34	4	–	0.49	0.04	–	–	–	–	0.23	0.25	–
M35	7	0.09	0.25	0.02	0.09	0.21	0.20	–	–	–	0.14
M36	2	–	–	–	0.30	0.70	–	–	–	–	–
M37	4	0.23	–	0.05	–	–	–	–	–	0.34	0.39
M38	4	0.14	0.42	–	–	–	–	–	0.20	–	0.24
M39	3	0.23	–	–	0.24	–	0.53	–	–	–	–
M40	2	–	0.66	–	–	–	–	–	–	0.34	–
M41	5	–	0.31	–	0.11	0.25	–	–	–	0.15	0.18
M42	2	–	–	–	–	–	0.61	–	–	0.39	–
M43	3	–	–	0.07	0.36	–	–	–	–	–	0.57
M44	6	0.09	0.26	–	–	0.22	–	0.15	–	0.13	0.15
M45	4	–	–	0.04	0.21	–	0.46	–	–	0.29	–
M46	2	–	0.93	0.07	–	–	–	–	–	–	–
M47	3	–	0.52	0.04	–	0.44	–	–	–	–	–
M48	4	–	0.37	0.03	–	0.31	0.29	–	–	–	–
M49	5	–	0.30	0.02	–	0.25	0.24	–	–	–	0.18
M50	6	–	0.27	0.02	0.10	0.23	0.22	–	–	–	0.16
M51	7	–	0.24	0.02	0.09	0.20	0.19	–	–	0.12	0.14
M52	8	–	0.22	0.02	0.08	0.18	0.17	–	0.10	0.11	0.12
M53	3	–	0.38	–	–	0.32	0.30	–	–	–	–
M54	4	–	0.31	–	–	0.26	0.25	–	–	–	0.18
M55	5	–	0.28	–	0.10	0.23	0.22	–	–	–	0.16

Data for the 10 additional mixtures tested are listed in Table S.3.

Table S.3. Data for 10 additional mixtures

Drug/ Mixture	Log IC50 (μL)	SE Log IC50 (μL)	IC50 (μL)	Observed Responses	Predicted Responses	Doxorubicin Dose Reduction	Number of Drugs
M46	2.50	0.07	12.23	-0.22	-0.24	6.19	2
M47	2.51	0.07	12.36	-0.26	-0.22	10.87	3
M48	2.93	0.07	18.80	-0.22	-0.22	10.13	4
M49	3.23	0.09	25.30	-0.16	-0.20	9.14	5
M50	3.17	0.08	23.86	-0.16	-0.19	10.75	6
M51	3.60	0.09	36.52	-0.05	-0.13	7.99	7
M52	3.89	0.06	48.68	0.00	-0.08	6.69	8
M53	4.85	0.03	127.59	0.17	-0.06	-	3
M54	4.91	0.02	135.81	0.28	-0.04	-	4
M55	4.40	0.05	81.62	0.04	-0.03	-	5

Data for the drug concentrations in stock solutions are listed in Table S.4.

Table S.4. Drug concentration in stock solutions

Drug	Wavelength (nm)	Retention time (min)	Drug concentration in stock solution ($\mu\text{g/ml}$)
Baicalein	276	12.6	107
Curcumin	424	14.9	80
Doxorubicin	–	–	500
EGCG	–	–	10,000
Juglone	251	11.2	78
Luteolin	351	10.9	302
Plumbagin	266	14.2	130
Quercetin	255	10.2	360
Rhein	258	17.1	616
Vitamin K3	250	12.4	536

Out of the 7,809 unique proteins downloaded from the Protein Data Bank (PDB) and discussed in the text, 286 were successfully docked to their ligands using eHits and were found to bind with at least one of the 10 drugs used. These proteins are listed in Table S.5.

Table S.5. List of PDB proteins used in models

1N51	1QJ3	1SWX	2GJ6	1EEF	1JH9	1VJA	1Z57	2ANO
1NAI	1QP0	1T4V	2GPP	1EFI	1JQE	1WSS	1ZD5	2AOU
1NHT	1QP7	1TA6	2H42	1EK4	1JTQ	1WSV	1ZFK	2AOV
1O5F	1QPZ	1TB7	2H44	1EUY	1JWT	1X2H	1ZGE	2AWH
1OOQ	1QQB	1TF0	2HBY	1F4E	1JYX	1X76	1ZGF	2AX6
1ORK	1QXK	1TGV	2HU6	1F4F	1K3L	1X7R	1ZGY	2B1V
1OSF	1R0P	1TKY	2IMG	1F91	1K3Y	1X97	1ZHM	2B50
1OTY	1R1J	1TR7	2IPW	1FJ4	1K5Q	1XF0	1ZHP	2B52
1OUM	1R1X	1TRG	2IPX	1FQG	1K5S	1XGJ	1ZHR	2B7D
1OV4	1R55	1TT6	2IS7	1FYF	1K97	1XM4	1ZJ2	2BAL
1OV6	1R9O	1TZ8	2ITM	1G1B	1KCE	1XMU	1ZJ3	2BDG
1OYN	1RBQ	1U1C	2J1N	1G27	1KE9	1XMY	1ZJP	2BH3
1P57	1RC2	1U3Q	2PUE	1G81	1KEC	1XNZ	1ZKN	2D0T
1P60	1RHU	1U3U	2TSC	1GM8	1KKB	1XON	1ZLT	2D5Z
1P61	1RKP	1U3V	4PRG	1GSJ	1KMN	1XOQ	1ZML	2DN1
1PF8	1RMT	1U3W	1AHF	1HDX	1KQU	1XOS	1ZRK	2ETK
1PG2	1RMY	1U6Q	1AI4	1HO5	1KRU	1XWK	1ZYS	2ETR
1PJ2	1RMZ	1U71	1AIB	1I2Z	1KSN	1Y2C	1ZZ2	2EU2
1PJ4	1RO6	1U72	1AIQ	1I30	1KW0	1Y2D	2A0W	2EVT
1PMV	1ROS	1U9E	1AJN	1ICR	1L6Y	1Y2J	2A0X	2EXM
1PNR	1RRM	1UHO	1AJP	1ICU	1LI4	1Y8J	2A0Y	2F6W
1PVS	1RSZ	1UKI	1AMR	1IF4	1LLB	1YCI	2A2Q	2F7I
1PWM	1RT9	1UWF	1AMS	1IF5	1LO6	1YDB	2A2R	
1PWY	1RWK	17GS	1AN5	1IF6	1LT8	1YDK	2A2S	
1PXI	1RWO	19GS	1AXW	1IF7	1M9M	1YKR	2A4Z	
1PXJ	1RWW	20GS	1AZ1	1IKT	1M9Q	1YMX	2A5U	
1PXK	1S14	2FAI	1BDH	1IQG	1MD3	1YOE	2A8U	
1PYE	1S1D	2FES	1BJ0	1IQN	1MD4	1YOL	2AAC	
1Q0B	1S8C	2FHY	1BQ1	1IRJ	1MLW	1YPJ	2AB6	
1Q3A	1SB1	2FLS	1DAH	1J7E	1MMK	1YRY	2AER	
1Q4N	1SC8	2FTO	1DDU	1J99	1MMT	1YTA	2AGT	
1Q5K	1SD1	2G8O	1DJR	1JFT	1MUO	1YXX	2AI8	
1Q6K	1SQT	2GG3	1DNP	1JH1	1VJ9	1Z11	2ANM	

Short mathematical description of the MixLow method

In typical *in-vitro* cytotoxicity experiments, responses are measured at different drug concentrations. Such studies commonly utilize multi-well incubation trays, where groups of wells within each tray receive different drug concentrations, and control wells receive no drug treatment. To assess drug interactions in a mixture, four steps are typically followed:

1. Individual drugs and the mixture are assayed for cytotoxicity against a target cancer cell line.
2. Parameters of the concentration-response curves are estimated.
3. The estimated parameters are used in a null interaction model to estimate drug interaction. For the MixLow method, the null interaction model is based on Loewe additivity. The Loewe additivity index produces the intuitively reasonable result that a *sham* mixture, a mixture of a drug with itself, is additive.
4. Confidence intervals of the index are calculated.

The MixLow method [1] uses a nonlinear mixed-effects model to estimate parameters of the concentration-effect curves. Random effects are commonly associated with observations sharing the same level of a classification factor, and in the case of the MixLow method this classification factor is tray. The MixLow method can be used to quantify drug interactions in any fixed-ratio drug combination study that includes within-group and between-group replicates, and where responses follow a sigmoidal pattern.

Let the random variable Fa signify the fraction of cells affected by a drug concentration. Define $\phi = E[Fa]$, where $E[\bullet]$ is the expected value. In some contexts ϕ is estimated based on concentration-response data and in other contexts a concentration is estimated that results in a fixed value of ϕ . Denote by $\psi_{d,\phi}$ the ϕ -effective log concentration of drug d . This is the log concentration that produces a fraction affected equal to a fixed ϕ . For example, the log concentration of drug d that inhibits proliferation of a cell population by 10 percent relative to controls is denoted by $\psi_{d,0.1}$. By convention, $\exp(\psi_{d,0.1})$ is called the IC10 (10 percent inhibitory concentration).

The Loewe index provides a measure of drug interaction. For two drugs, the Loewe index and its estimator¹ are

$$L_\phi = \sum_{d=1}^2 \frac{\exp(m_{d,\phi})}{\exp(\psi_{d,\phi})} \text{ and } \hat{L}_\phi = \sum_{d=1}^2 \frac{\exp(\hat{m}_{d,\phi})}{\exp(\hat{\psi}_{d,\phi})}, \quad (1)$$

respectively, where $m_{d,\phi}$ is an unknown constant signifying the log concentration of drug d in the mixture when the mixture is at its ϕ -effective log concentration, and $\psi_{d,\phi}$ is the unknown ϕ -effective log concentration of drug d alone. The mixture is synergistic,

¹ The hat notation is used here to denote parameter estimators and estimates.

additive, or antagonistic at ϕ depending on whether the value of the Loewe index is less than 1, equal to 1, or greater than 1, respectively.

The MixLow model uses a nonlinear mixed-effects framework to represent the concentration-response curve. As a skeleton description, responses are modeled as the expected mean of control wells times a sigmoidal function, plus an error term. Sigmoidal models of this type are sometimes referred to as Hill models. Formally, responses, $\{Y_{d,t,w}\}$, obtained from unprocessed data are modeled as a sigmoidal function of the concentration:

$$Y_{d,t,w} = \exp(\mu + b_t)(1 - \phi_{d,t,w}) + \varepsilon_{d,t,w}, \text{ where} \quad (2)$$

$$\phi_{d,t,w} = 1 - \frac{1}{1 + \left(\frac{\exp(c_{d,t,w})}{\exp(\psi_{d,0.5})} \right)^{\beta_d}} \quad (3)$$

and the subscripts d, t, w refer to the d^{th} drug, t^{th} tray, and w^{th} well, respectively. Here, drug d could refer to a single drug or a mixture, and $c_{d,t,w}$ refers to the log of the drug concentration for the d, t, w^{th} observation, a known constant. The expected value of $\exp(\mu + b_t)$ refers to the expected mean of control wells from all trays, where b_t is a random deviate specific to tray t . The notation $(1 - \phi)$ is used to denote the expected fraction unaffected, rather than introducing a new symbol for the latter. The exponent term in Model (2) is used to ensure that the expected response in control wells is always positive.

Values $\{b_t\}$ are independently distributed as $b_t \sim N(0, \sigma_b^2)$. The error terms in Model (2) are independently distributed as $\varepsilon_{d,t,w} \sim N\left(0, f(\sigma^2, E[Y_{d,t,w} | b_t])\right)$, where $f(\cdot)$ is an error function such as $\sigma E[Y_{d,t,w} | b_t]^{\beta_d}$. Here, the power parameter, β_d , is drug-dependent.

Discussions on implementation of the method, modifications to Model (2), and procedures used to calculate confidence intervals of the interaction index are provided in Boik et al. [1].

References

1. Boik J, Newman R, Boik R: **Quantifying synergism/antagonism using nonlinear mixed-effects modeling: A simulation study.** *Statistics in Medicine*, 2008. **27(7):1040-61.**