

Supplementary Materials

In Vitro Effects of Selective COX and LOX Inhibitors and Their Combinations with Antineoplastic Drugs in the Mouse Melanoma Cell Line B16F10

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1. Cytotoxicity studies

Table S1. Tabulated data for IC₅₀ and IC₂₀ values of single treatments.

		DTIC		TMZ	
		MTT	SRB	MTT	SRB
24 hours	IC ₅₀	-	-	-	-
48 hours	IC ₅₀	1.23*10 ³	1.27*10 ³	1.03*10 ³	-
72 hours	IC ₅₀	1.21*10 ³	1.33*10 ³	1.23*10 ³	1.01*10 ³

		Meloxicam		ASA	
		MTT	SRB	MTT	SRB
24 hours	IC ₂₀	0.16*10 ³	0.16*10 ³	1.00*10 ³	1.00*10 ³
	IC ₅₀	0.76*10 ³	0.68*10 ³	4.78*10 ³	8.64*10 ³
48 hours	IC ₂₀	0.16*10 ³	0.16*10 ³	1.00*10 ³	1.00*10 ³
	IC ₅₀	0.59*10 ³	0.54*10 ³	3.10*10 ³	3.80*10 ³
72 hours	IC ₂₀	0.16*10 ³	0.16*10 ³	1.00*10 ³	1.00*10 ³
	IC ₅₀	0.65*10 ³	0.61*10 ³	3.86*10 ³	3.89*10 ³

		AA-861		MK-886	
		MTT	SRB	MTT	SRB
24 hours	IC ₂₀	10.00	20.00	5-10.00	10.00
	IC ₅₀	41.03	39.45	20.98	24.99
48 hours	IC ₂₀	2.50	10.00	<5.00	5-10.00
	IC ₅₀	30.50	37.33	12.41	14.34
72 hours	IC ₂₀	2.50	10.00	5.00	5-10.00
	IC ₅₀	12.08	37.61	13.96	12.42

		Baicalein		PD-146176	
		MTT	SRB	MTT	SRB
24 hours	IC ₂₀	5.00	5.00	1.00	1.00
	IC ₅₀	30.98	41.06	13.89	13.64
48 hours	IC ₂₀	5.00	5.00	1.00	1.00
	IC ₅₀	11.43	34.14	11.24	10.69
72 hours	IC ₂₀	5.00	5.00	1.00	1.00
	IC ₅₀	33.68	30.53	16.85	12.65

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2. Cell cycle profiles for single drug treatments

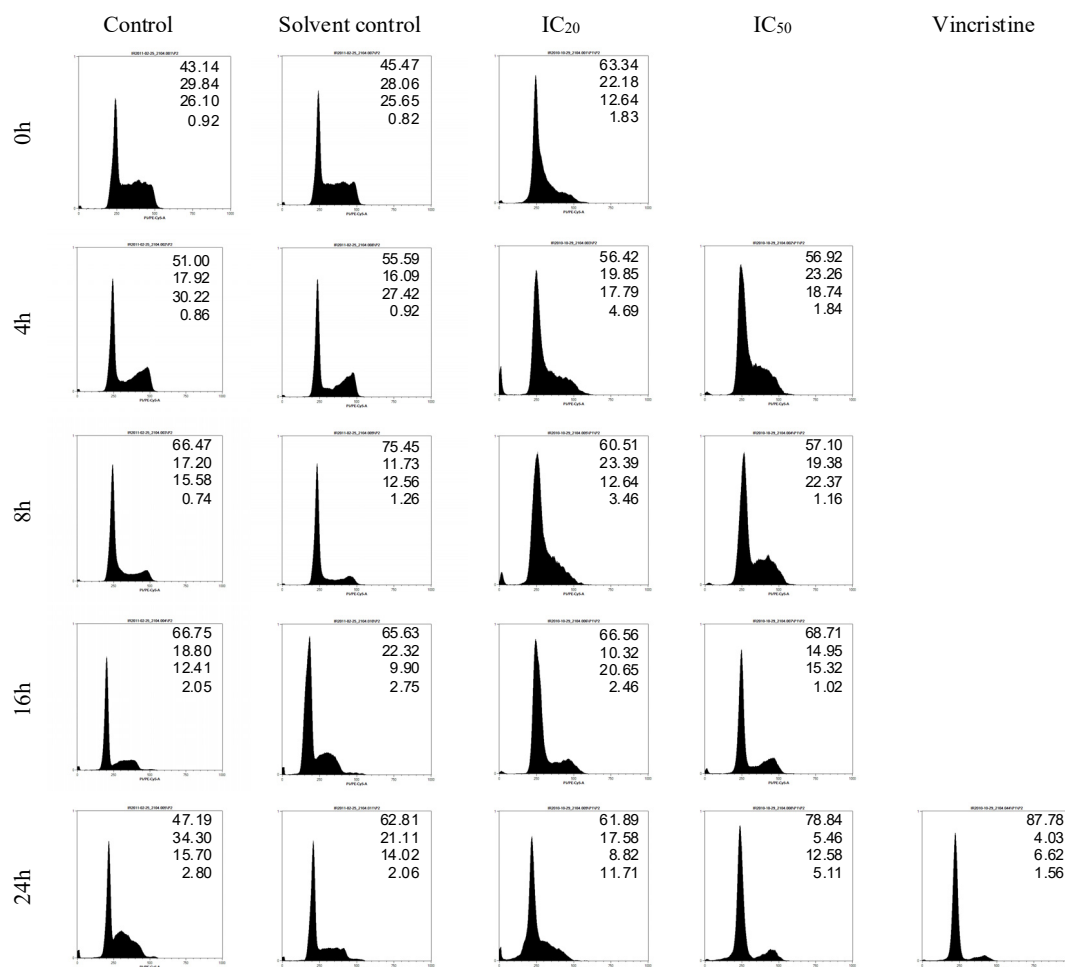


Figure S1. Effects of COX2 inhibitor on cell cycle status. B16F10 cells were treated with IC₂₀ and IC₅₀ concentration of Meloxicam inhibitor for 0, 4, 8, 16 and 24 hours. Flow cytometric analysis of DNA content was performed after propidium iodide staining. Percentage of cells in each phase of the cell cycle (sub-G₁, G₁, S, and G₂/M) is indicated. Representative data from three independent experiments are shown.

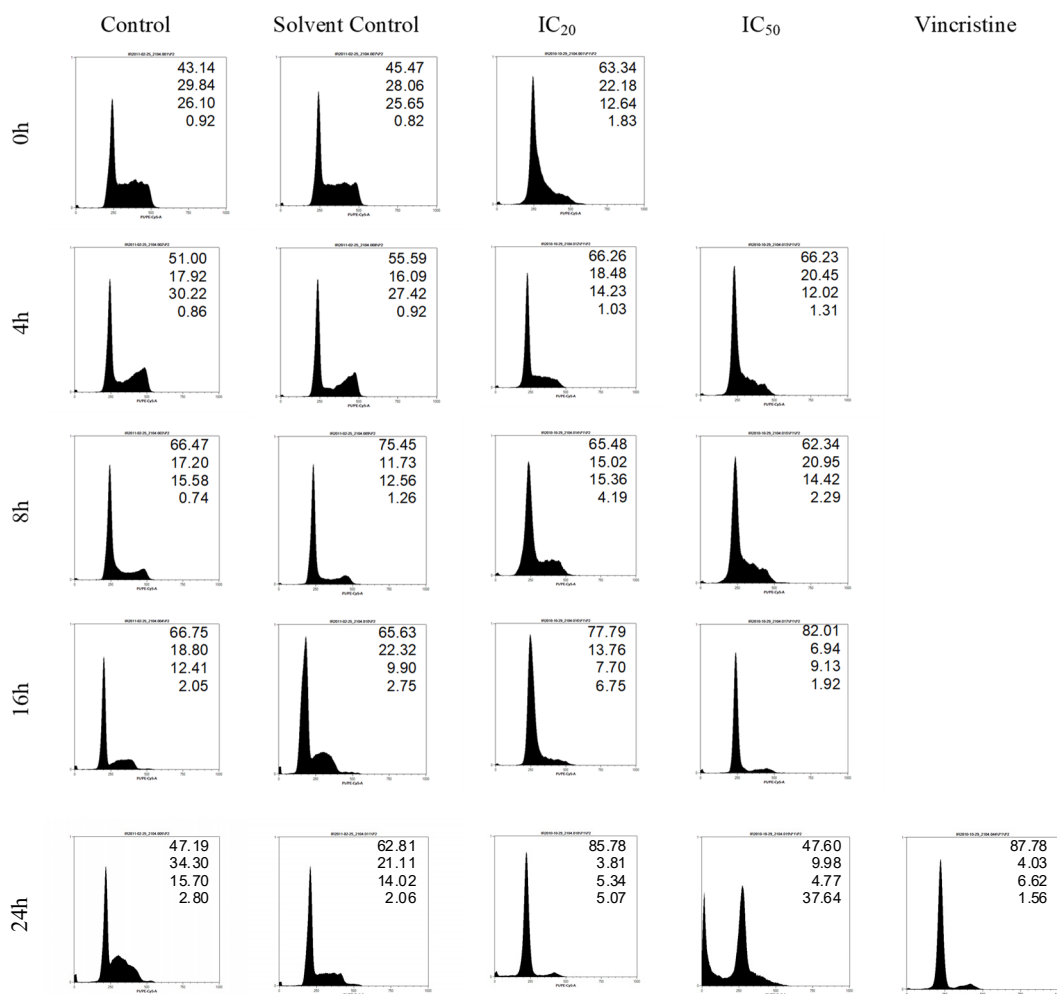


Figure S2. Effects of COX1 inhibitor on cell cycle status. B16F10 cells were treated with IC₂₀ and IC₅₀ concentration of Acetyl salicylic acid (ASA) inhibitor for 0, 4, 8, 16 and 24 hours. Flow cytometric analysis of DNA content was performed after propidium iodide staining. Percentage of cells in each phase of the cell cycle (sub-G₁, G₁, S, and G₂/M) is indicated. Representative data from three independent experiments are shown.

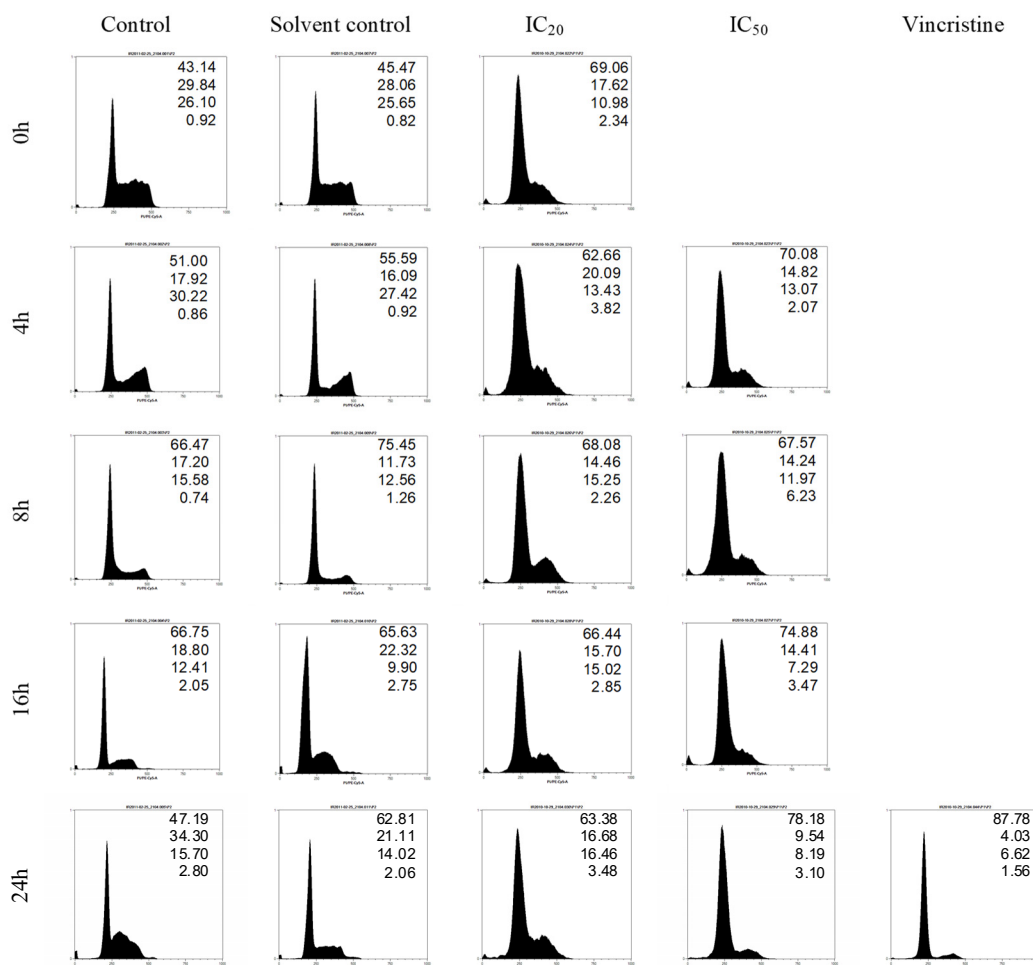


Figure S3. Effects of the 5-LOX inhibitor AA-861 on cell cycle status. B16F10 cells were treated with IC₂₀ and IC₅₀ concentration of AA-861 for 0, 4, 8, 16 and 24 hours. Flow cytometric analysis of DNA content was performed after propidium iodide staining. Percentage of cells in each phase of the cell cycle (sub-G₁, G₁, S, and G₂/M) is indicated. Representative data from three independent experiments are shown.

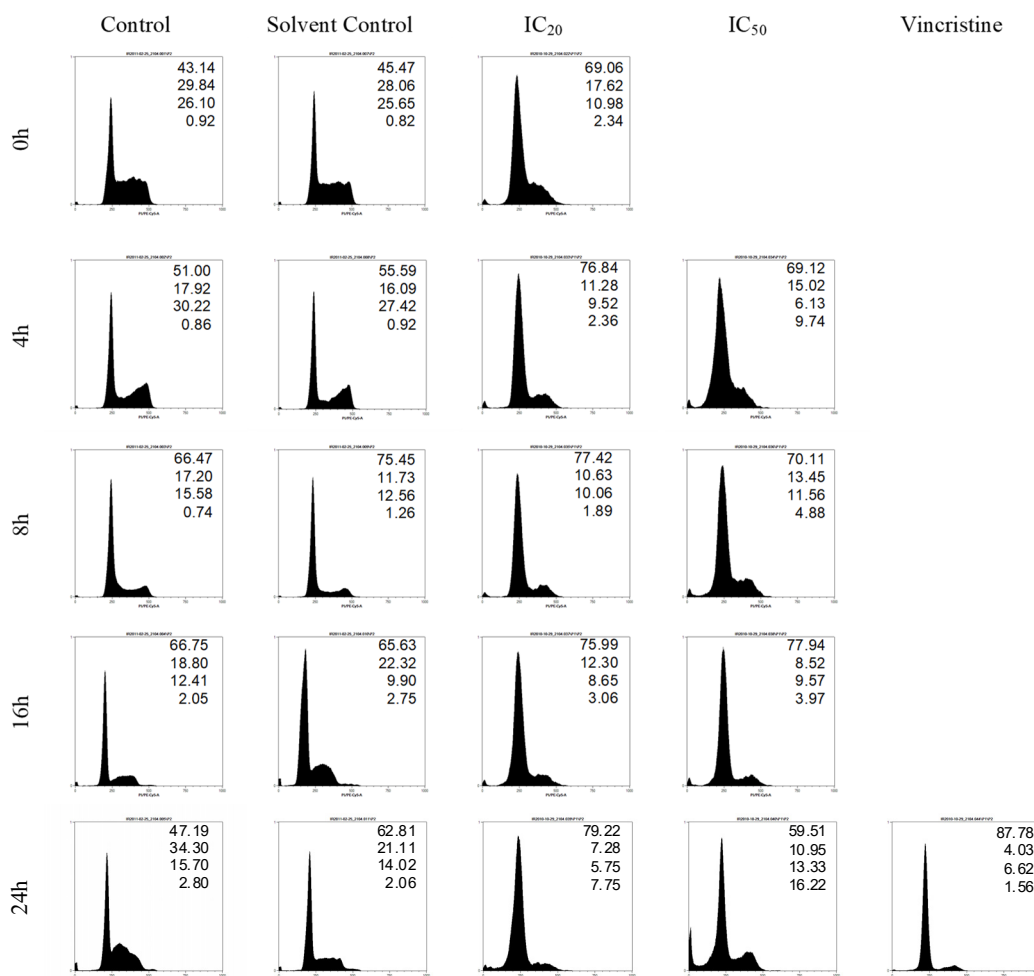


Figure S4. Effects of the FLAP inhibitor MK-886 on cell cycle status. B16F10 cells were treated with IC₂₀ and μ M IC₅₀ concentration of MK-886 for 0, 4, 8, 16 and 24 hours. Flow cytometric analysis of DNA content was performed after propidium iodide staining. Percentage of cells in each phase of the cell cycle (sub-G₁, G₁, S, and G₂/M) is indicated. Representative data from three independent experiments are shown.

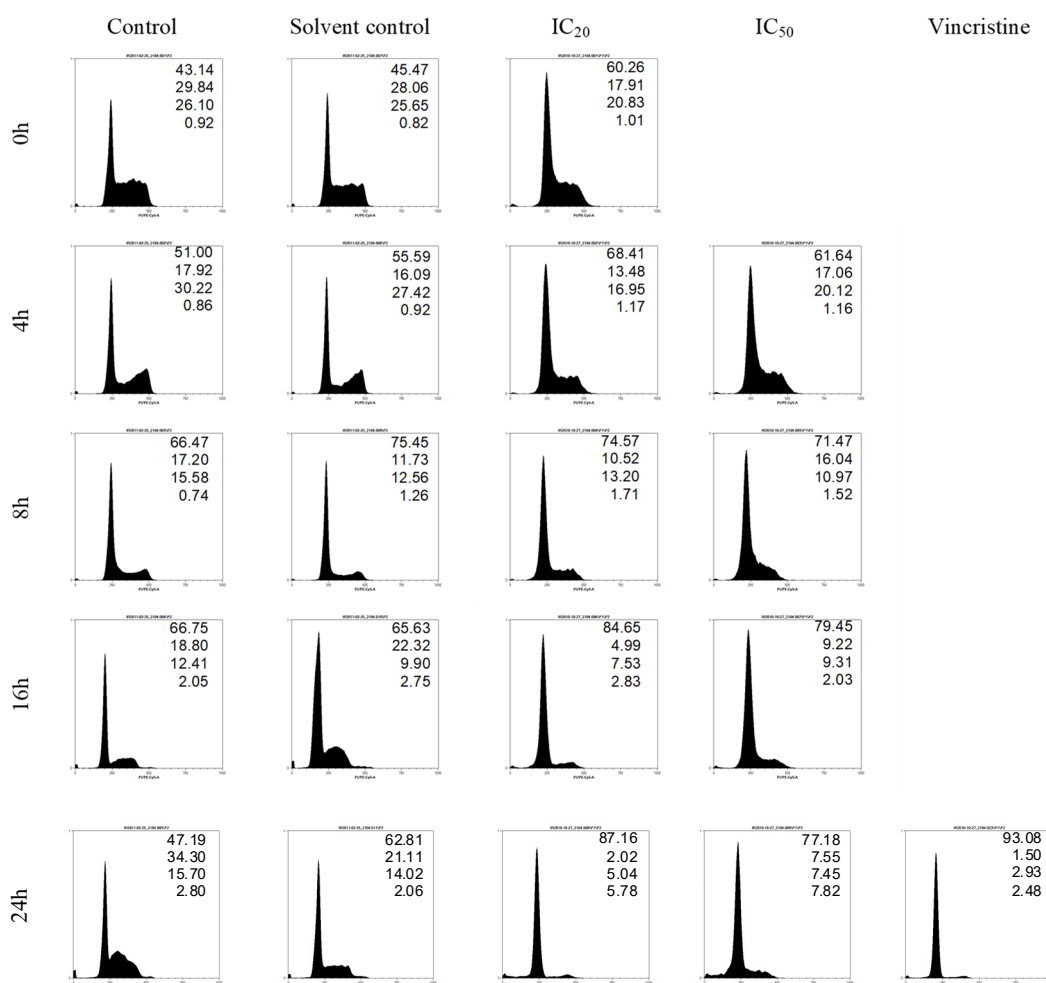


Figure S5. Effects of the 12-LOX inhibitor baicalein on cell cycle status. B16F10 cells were treated with IC₂₀ and IC₅₀ concentration of Baicalein for 0, 4, 8, 16 and 24 hours. Flow cytometric analysis of DNA content was performed after propidium iodide staining. Percentage of cells in each phase of the cell cycle (sub-G₁, G₁, S, and G₂/M) is indicated. Representative data from three independent experiments are shown.

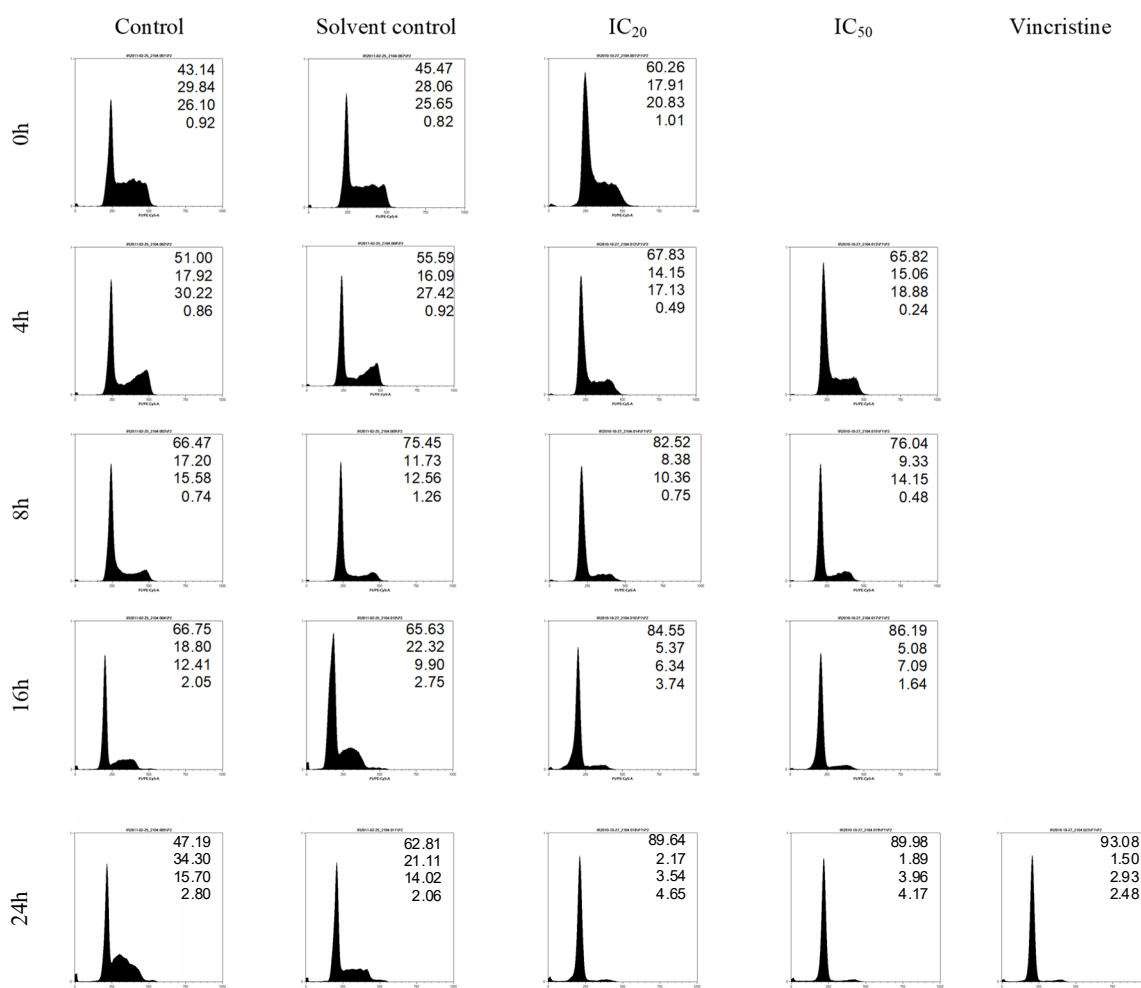


Figure S6. Effects of the 15-LOX inhibitor PD-146176 on cell cycle status. B16F10 cells were treated with IC₂₀ and IC₅₀ concentration of PD-146176 for 0, 4, 8, 16 and 24 hours. Flow cytometric analysis of DNA content was performed after propidium iodide staining. Percentage of cells in each phase of the cell cycle (sub-G₁, G₁, S, and G₂/M) is indicated. Representative data from three independent experiments are shown.

3. Numerical results from the combination values

Table S2. Combination index (CI) and fraction affected (f_a) values in B16F10 cells for DTIC or TMZ and meloxicam in combination based on the Calcsyn software.

DTIC (μM)	Meloxicam (μM)	f_a	CI	Effect	TMZ (μM)	Meloxicam (μM)	f_a	CI	Effect
100	0.31	0.643	0.011	Very strong synergism	100	0.31	0.136	0.255	Strong synergism
100	0.63	0.628	0.012	Very strong synergism	100	0.63	0.125	0.276	Strong synergism
100	3.13	0.644	0.012	Very strong synergism	100	3.13	0.159	0.295	Strong synergism
100	6.25	0.634	0.013	Very strong synergism	100	6.25	0.181	0.321	Synergism
100	31.25	0.637	0.020	Very strong synergism	100	31.25	0.260	0.461	Synergism
100	62.50	0.658	0.025	Very strong synergism	100	62.50	0.332	0.506	Synergism
100	312.50	0.832	0.018	Very strong synergism	100	312.50	0.317	2.097	Antagonism
250	0.31	0.362	0.064	Strong synergism	250	0.31	0.275	0.431	Synergism
250	0.63	0.358	0.065	Strong synergism	250	0.63	0.309	0.404	Synergism
250	3.13	0.361	0.070	Strong synergism	250	3.13	0.389	0.358	Synergism
250	6.25	0.379	0.072	Strong synergism	250	6.25	0.398	0.364	Synergism
250	31.25	0.489	0.097	Strong synergism	250	31.25	0.423	0.430	Synergism
250	62.50	0.409	0.104	Strong synergism	250	62.50	0.464	0.470	Synergism
250	312.50	0.685	0.083	Strong synergism	250	312.50	0.486	1.027	Moderate antagonism
500	0.31	0.136	0.330	Synergism	500	0.31	0.668	0.430	Synergism
500	0.63	0.129	0.354	Synergism	500	0.63	0.687	0.415	Synergism
500	3.13	0.155	0.345	Synergism	500	3.13	0.646	0.451	Synergism
500	6.25	0.168	0.364	Synergism	500	6.25	0.636	0.462	Synergism
500	31.25	0.161	0.802	Moderate synergism	500	31.25	0.603	0.521	Synergism
500	62.50	0.212	0.779	Moderate synergism	500	62.50	0.598	0.566	Synergism
500	312.50	0.485	0.365	Synergism	500	312.50	0.597	0.885	Slight synergism

Table S3. Combination index (CI), fraction affected (f_a) values and effect of combination therapy with ASA and DTIC or TMZ.

DTIC (μ M)	ASA (μ M)	f _a	CI	Effect	TMZ (μ M)	ASA (μ M)	f _a	CI	Effect
100	5	-	-	No effect	100	5	0.061	0.453	Synergism
100	10	-	-	No effect	100	10	0.051	0.706	Moderate synergism
100	50	-	-	No effect	100	50	0.070	0.993	Nearly additive
100	100	-	-	No effect	100	100	0.092	0.896	Slightly synergism
100	500	-	-	No effect	100	500	0.190	0.534	Synergism
100	1000	-	-	No effect	100	1000	0.313	0.271	Strong synergism
100	5000	0.380	0.996	Nearly additive	100	5000	0.289	0.924	Nearly additive
250	5	-	-	No effect	250	5	0.081	0.826	Slightly synergism
250	10	-	-	No effect	250	10	0.056	1.170	Moderate antagonism
250	50	-	-	No effect	250	50	0.121	0.785	Moderate synergism
250	100	-	-	No effect	250	100	0.132	0.830	Moderate synergism
250	500	0.012	1.266	Moderate antagonism	250	500	0.312	0.455	Synergism
250	1000	0.079	0.538	Synergism	250	1000	0.441	0.343	Synergism
250	5000	0.475	0.945	Nearly additive	250	5000	0.356	0.702	Moderate synergism
500	5	-	-	No effect	500	5	0.203	1.013	Nearly additive
500	10	-	-	No effect	500	10	0.191	1.049	Nearly additive
500	50	-	-	No effect	500	50	0.234	0.953	Nearly additive
500	100	-	-	No effect	500	100	0.254	0.920	Nearly additive
500	500	0.148	0.425	Synergism	500	500	0.406	0.691	Synergism
500	1000	0.249	0.406	Synergism	500	1000	0.482	0.608	Synergism
500	5000	0.528	0.940	Nearly additive	500	5000	0.479	0.682	Synergism

Table S4. Combination index (CI) and fraction affected (f_a) values in B16F10 cells for DTIC or TMZ and AA-861 in combination based on the Calcsyn software.

DTIC (μ M)	AA-861 (μ M)	f_a	CI	Effect	TMZ (μ M)	AA-861 (μ M)	f_a	CI	Effect
100	0.02	-	-	No effect	100	0.02	-	-	No effect
100	0.04	0.018	1.909	Antagonism	100	0.04	-	-	No effect
100	0.20	0.059	0.817	Moderate synergism	100	0.20	-	-	No effect
100	0.40	0.016	20.608	Very strong antagonism	100	0.40	0.028	4.529	Strong antagonism
100	2.00	0.021	58.668	Very strong antagonism	100	2.00	0.104	2.485	Antagonism
100	4.00	0.105	4.051	Strong antagonism	100	4.00	0.178	1.855	Antagonism
100	20.00	0.363	0.880	Moderate synergism	100	20.00	0.343	2.176	Antagonism
250	0.02	-	-	No effect	250	0.02	-	-	No effect
250	0.04	-	-	No effect	250	0.04	-	-	No effect
250	0.20	0.003	293.922	Very strong antagonism	250	0.20	-	-	No effect
250	0.40	0.008	83.245	Very strong antagonism	250	0.40	-	-	No effect
250	2.00	0.029	30.765	Very strong antagonism	250	2.00	0.054	7.744	Strong antagonism
250	4.00	0.102	4.439	Strong synergism	250	4.00	0.139	3.222	Antagonism
250	20.00	0.364	0.910	Nearly additive	250	20.00	0.302	3.136	Antagonism
500	0.02	0.119	0.376	Synergism	500	0.02	0.557	0.523	Synergism
500	0.04	0.159	0.297	Synergism	500	0.04	0.628	0.463	Synergism
500	0.20	0.162	0.351	Synergism	500	0.20	0.335	0.783	Moderate synergism
500	0.40	0.638	0.057	Very strong synergism	500	0.40	0.539	0.549	Synergism
500	2.00	0.638	0.064	Very strong synergism	500	2.00	0.501	0.645	Synergism
500	4.00	0.649	0.069	Very strong synergism	500	4.00	0.426	0.881	Slight synergism
500	20.00	0.662	0.124	Strong synergism	500	20.00	0.515	1.216	Moderate antagonism

Table S5. Combination index (CI) and fraction affected (f_a) values in B16F10 cells for DTIC or TMZ and MK-886 in combination based on the Calcsyn software.

DTIC (μ M)	MK-886 (μ M)	f_a	CI	Effect	DTIC (μ M)	MK-886 (μ M)	f_a	CI	Effect
100	0.015	-	-	No effect	100	0.015	-	-	No effect
100	0.030	-	-	No effect	100	0.030	-	-	No effect
100	0.150	0.029	3.271	Strong antagonism	100	0.150	-	-	No effect
100	0.300	0.045	2.879	Antagonism	100	0.300	0.009	48.036	Very strong antagonism
100	1.500	0.067	6.569	Strong antagonism	100	1.500	0.046	10.351	Very strong antagonism
100	3.0000	0.133	3.432	Strong antagonism	100	3.0000	0.174	1.432	Antagonism
100	15.000	0.505	0.604	Synergism	100	15.000	0.340	1.263	Moderate antagonism
250	0.015	-	-	No effect	250	0.015	-	-	No effect
250	0.030	-	-	No effect	250	0.030	-	-	No effect
250	0.150	0.036	2.518	Antagonism	250	0.150	-	-	No effect
250	0.300	0.034	5.048	Very strong antagonist	250	0.300	0.020	11.600	Very strong antagonist
250	1.500	0.050	11.551	Very strong antagonist	250	1.500	0.065	5.834	Strong antagonist
250	3.0000	0.068	12.835	Very strong antagonist	250	3.0000	0.163	1.973	Antagonist
250	15.000	0.409	1.222	Moderate antagonism	250	15.000	0.327	1.630	Antagonist
500	0.015	-	-	No effect	500	0.015	-	-	No effect
500	0.030	-	-	No effect	500	0.030	-	-	No effect
500	0.150	0.034	3.249	Antagonism	500	0.150	-	-	No effect
500	0.300	0.043	3.770	Strong antagonism	500	0.300	0.073	2.425	Antagonism
500	1.500	0.091	4.033	Strong antagonism	500	1.500	0.077	5.119	Strong antagonism
500	3.0000	0.074	11.213	Very strong antagonism	500	3.0000	0.161	2.584	Antagonism
500	15.000	0.289	3.157	Antagonism	500	15.000	0.292	2.524	Antagonism

Table S6. Combination index (CI) and fraction affected (f_a) values in B16F10 cells for DTIC or TMZ and Baicalein in combination based on the CalcuSyn software.

DTIC (μM)	Baicalein (μM)	f_a	CI	Effect	TMZ (μM)	Baicalein (μM)	f_a	CI	Effect
100	0.025	-	-	No effect	100	0.025	-	-	No effect
100	0.050	-	-	No effect	100	0.050	-	-	No effect
100	0.250	-	-	No effect	100	0.250	-	-	No effect
100	0.500	-	-	No effect	100	0.500	0.01	18.264	Very strong antagonism
100	2.500	-	-	No effect	100	2.500	0.034	9.992	Strong antagonism
100	5.000	0.017	5.136	Strong antagonism	100	5.00	0.079	4.238	Strong antagonism
100	25.000	0.301	1.917	Antagonism	100	25.00	0.299	1.300	Moderate antagonism
250	0.025	-	-	No effect	250	0.025	-	-	No effect
250	0.050	-	-	No effect	250	0.050	-	-	No effect
250	0.250	-	-	No effect	250	0.250	-	-	No effect
250	0.500	-	-	No effect	250	0.500	0.003	153.422	Very strong antagonism
250	2.500	-	-	No effect	250	2.500	0.031	12.486	Very strong antagonism
250	5.000	0.074	1.697	Antagonism	250	5.000	0.098	3.295	Antagonism
250	25.000	0.359	1.596	Antagonism	250	25.000	0.362	1.047	Slight antagonism
500	0.025	0.099	0.425	Synergism	500	0.025	-	-	No effect
500	0.050	0.113	0.386	Synergism	500	0.050	-	-	No effect
500	0.250	0.104	0.456	Synergism	500	0.250	-	-	No effect
500	0.500	0.115	0.469	Synergism	500	0.500	0.036	3.950	Strong antagonism
500	2.500	0.088	1.078	Nearly additive	500	2.500	0.131	1.975	Antagonism
500	5.000	0.126	1.241	Moderate antagonism	500	5.000	0.238	1.324	Moderate antagonism
500	25.000	0.596	0.774	Nearly additive	500	25.000	0.384	1.277	Moderate antagonism

Table S7. Combination index (CI) and fraction affected (f_a) values in B16F10 cells for DTIC or TMZ and PD-146176 in combination based on the Calcsyn software.

DTIC (μM)	PD-146176 (μM)	f _a	CI	Effect	TMZ (μM)	PD-146176 (μM)	f _a	CI	Effect
100	0.005	-	-	No effect	100	0.005	-	-	No effect
100	0.010	-	-	No effect	100	0.010	-	-	No effect
100	0.050	-	-	No effect	100	0.050	-	-	No effect
100	0.100	-	-	No effect	100	0.100	-	-	No effect
100	0.500	-	-	No effect	100	0.500	0.045	2.318	Antagonism
100	1.000	0.027	1.255	Moderate antagonism	100	1.000	0.149	1.246	Moderate antagonism
100	5.000	0.206	1.590	Moderate antagonism	100	5.000	0.358	1.707	Antagonism
250	0.005	-	-	No effect	250	0.005	-	-	No effect
250	0.010	-	-	No effect	250	0.010	-	-	No effect
250	0.050	-	-	No effect	250	0.050	-	-	No effect
250	0.100	-	-	No effect	250	0.100	-	-	No effect
250	0.500	-	-	No effect	250	0.500	0.084	1.744	Antagonism
250	1.000	0.041	1.232	Moderate antagonism	250	1.000	0.132	1.791	Antagonism
250	5.000	0.168	1.902	Antagonism	250	5.000	0.339	2.077	Antagonism
500	0.005	-	-	No effect	500	0.005	-	-	No effect
500	0.010	-	-	No effect	500	0.010	-	-	No effect
500	0.050	-	-	No effect	500	0.050	-	-	No effect
500	0.100	-	-	No effect	500	0.100	-	-	No effect
500	0.500	-	-	No effect	500	0.500	0.082	2.557	Antagonism
500	1.000	0.031	1.987	Antagonism	500	1.000	0.097	3.101	Antagonism
500	5.000	0.192	1.858	Antagonism	500	5.000	0.322	2.618	Antagonisms