

Table S1: IC₅₀ values for seriniquinone (SQ1) and its synthetic analogs for 72h treatment determined by MTT assay on cell lines (n=3).

Compounds	SK-MEL-28	SK-MEL-19	SK-MEL-147	WM293A	501mel	MM200	MCF7	HCT-116	MRC-5	FDH
SQ1	IC ₅₀ (μM)	0.15	0.06	0.13	1.05	0.32	1.36	3.3	1.0	0.64
	95% CI	n.d.	0.05 – 0.09	0.08 – 0.21	0.73 – 1.49	0.27 – 0.39	0.1 – 1.85	2.9 – 3.7	n.d.	0.32 – 1.27
	R ²	0.96	0.89	0.86	0.79	0.94	0.81	0.92	0.89	0.87
SQ2	IC ₅₀ (μM)	0.04	0.04	0.1	0.74	0.11	0.75	0.73	0.23	1.57
	95% CI	n.d.	0.03 – 0.06	0.07 – 0.15	0.51 – 1.08	0.05 – 0.24	0.6 – 0.93	0.63 – 0.84	n.d.	0.83 – 2.95
	R ²	0.92	0.9	0.87	0.8	0.9	0.92	0.97	0.92	0.85
SQ3	IC ₅₀ (μM)	0.24	0.11	0.26	1.5	0.2	1.43	1.9	1.02	1.43
	95% CI	n.d.	0.06 – 0.2	0.15 – 0.44	1.12 – 1.97	n.d.	1.19 – 1.72	1.6 – 2.3	n.d.	1.01 – 2.63
	R ²	0.9	0.8	0.75	0.84	0.62	0.95	0.92	0.88	0.96
SQ4	IC ₅₀ (μM)	0.19	0.32	0.4	1.75	>5	1.16	1.0	1.02	2.45
	95% CI	0.14 – 0.27	0.24 – 0.42	0.26 – 0.61	1.32 – 2.31	n.d.	n.d.	0.89 – 1.17	n.d.	1.36 – 4.41
	R ²	0.83	0.9	0.8	0.85	0.7	0.9	0.95	0.87	0.86
SQ5	IC ₅₀ (μM)	0.15	0.04	0.07	1.65	1.71	0.3	0.49	0.22	0.56
	95% CI	0.09 – 0.24	0.03 – 0.06	0.05 – 0.11	1.3 – 2.1	n.d.	n.d.	0.41 – 0.57	n.d.	0.37 – 0.85
	R ²	0.86	0.84	0.74	0.9	0.73	0.97	0.95	0.97	0.96
DOX	IC ₅₀ (μM)	1.22	0.39	0.18	0.06	0.26	0.1	0.56	0.15	0.05
	95% CI	0.83 – 1.8	0.23 – 0.66	0.12 – 0.29	0.04 – 0.08	0.2 – 0.35	0.07 – 0.13	0.11 – 0.3	0.1 – 0.22	n.d.
	R ²	0.94	0.85	0.94	0.92	0.93	0.96	0.95	0.96	0.48

LEGEND: n.d. = not determinated; CI = confidence intervals (μM); DOX = doxorubicin.

Table S2: IC₅₀ values for seriniquinone (SQ1) and its synthetic analog 2 (SQ2) by MTT assay on melanoma cell lines SK-MEL-28 and SK-MEL-147 in different times of treatments (n=3).

		SK-MEL-28			SK-MEL-147		
		24h	48h	72h	24h	48h	72h
SQ1	IC ₅₀ (μM)	0.56	0.69	0.15	1.79	1.61	0.13
	95% CI	0.42 – 0.74	0.55 – 0.86	n.d.	1.36 – 2.34	1.19 – 2.16	0.08 – 0.21
	R ²	0.93	0.94	0.96	0.89	0.80	0.86
SQ2	IC ₅₀ (μM)	0.14	0.26	0.04	1.26	0.89	0.1
	95% CI	0.1 – 0.19	0.19 – 0.36	n.d.	0.83 – 1.93	0.76 – 1.03	0.07 – 0.15
	R ²	0.94	0.87	0.92	0.83	0.94	0.88
DOX	IC ₅₀ (μM)	1.39	2.11	1.22	0.79	0.48	0.18
	95% CI	0.98 – 1.97	1.59 – 2.81	0.83 – 1.78	0.46 – 1.34	0.27 – 0.87	0.12 – 0.29
	R ²	0.86	0.91	0.94	0.93	0.87	0.94

LEGEND: n.d. = not determined; CI = confidence intervals (μM); DOX = doxorubicin.

Table S3: Primer sequences and concentrations for quantitative PCR.

Gene	Sequence (5' → 3')	Concentration
<i>DCD</i>	FW: AAGCCAAGGAAGCAGAGATCC RV: GCTCCTTACCCACGCTTCT	300 nM
<i>BCL2</i>	FW: ATGTGTGTGGAGAGCGTCAA RV: ACAGTTCCACAAAGGCATCC	300 nM
<i>BCL2L1</i>	FW: CTTGGATGCCACTTACCTGAA RV: GCTGCTGCATTGTTCCCATA	300 nM
<i>MCL1</i>	FW: GTAATAACACCAGTACGGACGG RV: TCCCGAAGGTACCGAGAGAT	300 nM
<i>BAX</i>	FW: GAGCTGCAGAGGATGATTGC RV: CAGCTGCCACTCGGAAAA	300 nM
<i>BAK1</i>	FW: TGAGTACTTCACCAAGATTGCCA RV: AGTCAGGCCATGCTGGTAGAC	300 nM
<i>BAD</i>	FW: CACCAGCAGGAGCAGCCAAC RV: CGACTCCGGATCTCCACAGC	300 nM
<i>BCL2L11</i>	FW: ATGTCTGACTCTGACTCTCG RV: CCTTGTGGCTCTGTCTGTAG	300 nM
<i>BID</i>	FW: ATGGACCGTAGCATCCCTCC RV: GTAGGTGCGTAGGTTCTGGT	300 nM
<i>PMAIP1</i>	FW: CGCGCAAGAACGCTCAACC RV: CACACTCGACTTCCAGCTCTGCT	300 nM
<i>BBC3</i>	FW: GACCTAACGCACAGTACGAG RV: AGGAGTCCCCATGATGAGATTGT	300 nM
<i>BNIP3</i>	FW: ATATGGGATTGGTCAAGTCGG RV: CGCTCGTGTTCCTCATGCT	300 nM
<i>BECN1</i>	FW: TCTGAAGAGGACCTGGACCCCT RV: GGCTCACGTCCATCTCGTC	300 nM
<i>ATG5</i>	FW: GGGCCATCAATCGGAAACAG RV: AGCCACAGGACGAAACAG	300 nM
<i>ATG7</i>	FW: CGTTGCCACAGCATCATCTTC RV: TCCCATGCCTCCTTCTGGTTC	300 nM
<i>MAP1LC3B</i>	FW: AAGGCCTTACAGCTCAATG RV: CTGGGAGGCATAGACCATGT	300 nM
<i>XIAP</i>	FW: GACAGTATGCAAGATGAGTCAGTC RV: GCAAAGCTTCTCCTCTTGCAAG	300 nM
<i>BIRC5</i>	FW: GCCCAGTGTCTCTCTGCTTC RV: GCACTTCTCCGCAGTTCTC	300 nM
<i>HPRT1</i>	FW: GAACGTCTGCTCGAGATGTGA RV: TCCAGCAGGTCAGCAAAGAAT	300 nM
<i>ACTB</i>	FW: AGGCCAACCGCGAGAAG RV: ACAGCCTGGATAGCAACGTACA	300 nM
<i>RPLPO</i>	FW: GCAATGTTGCCAGTGTCTG RV: GCCTTGACCTTTCAGCAA	300 nM

FW: Forward; RV: Reverse.

Table S4: IC₅₀ (μM) of seriniquinone (SQ1), its synthetic analog 2 (SQ2), rapamycin and doxorubicin by MTT assay in SK-MEL-28 and SK-MEL-147 melanoma cell lines of 24h treatment. Cells were 1h pre-treated with 30 nM baflomycin A1 and 20 μM Z-VAD-FMK (n=3).

SK-MEL-28					
Combinations	-	BAFILOMYCIN A1		Z-VAD-FMK	
RAPAMYCIN	17.75		15.65		31.79
R ² / CI 95%	0.80	11.0-28.5	0.39	8.9-27.4	0.75
DOXORUBICIN	0.59		0.33		1.15
R ² / CI 95%	0.95	0.5-0.7	0.92	0.27-0.4	0.89
SQ1	0.35		0.14		0.7
R ² / CI 95%	0.95	0.3-0.4	0.92	0.1-0.2	0.84
SQ2	0.24		0.06		0.37
R ² / CI 95%	0.96	0.2-0.3	0.95	0.05-0.08	0.89
SK-MEL-147					
Combinations	-	BAFILOMYCIN A1		Z-VAD-FMK	
RAPAMYCIN	17.47		39.1		22.8
R ² / CI 95%	0.89	11.0-27.8	0.69	n.d.	0.87
DOXORUBICIN	0.8		2.48		1.88
R ² / CI 95%	0.87	0.6-1.1	0.51	1.3-4.7	0.73
SQ1	1.64		2.26		1.89
R ² / CI 95%	0.78	1.2-2.3	0.44	1.3-4.0	0.72
SQ2	0.91		1.18		1.28
R ² / CI 95%	0.86	0.7-1.2	0.55	0.5-2.6	0.7

LEGEND: CI = confidence intervals (μM).

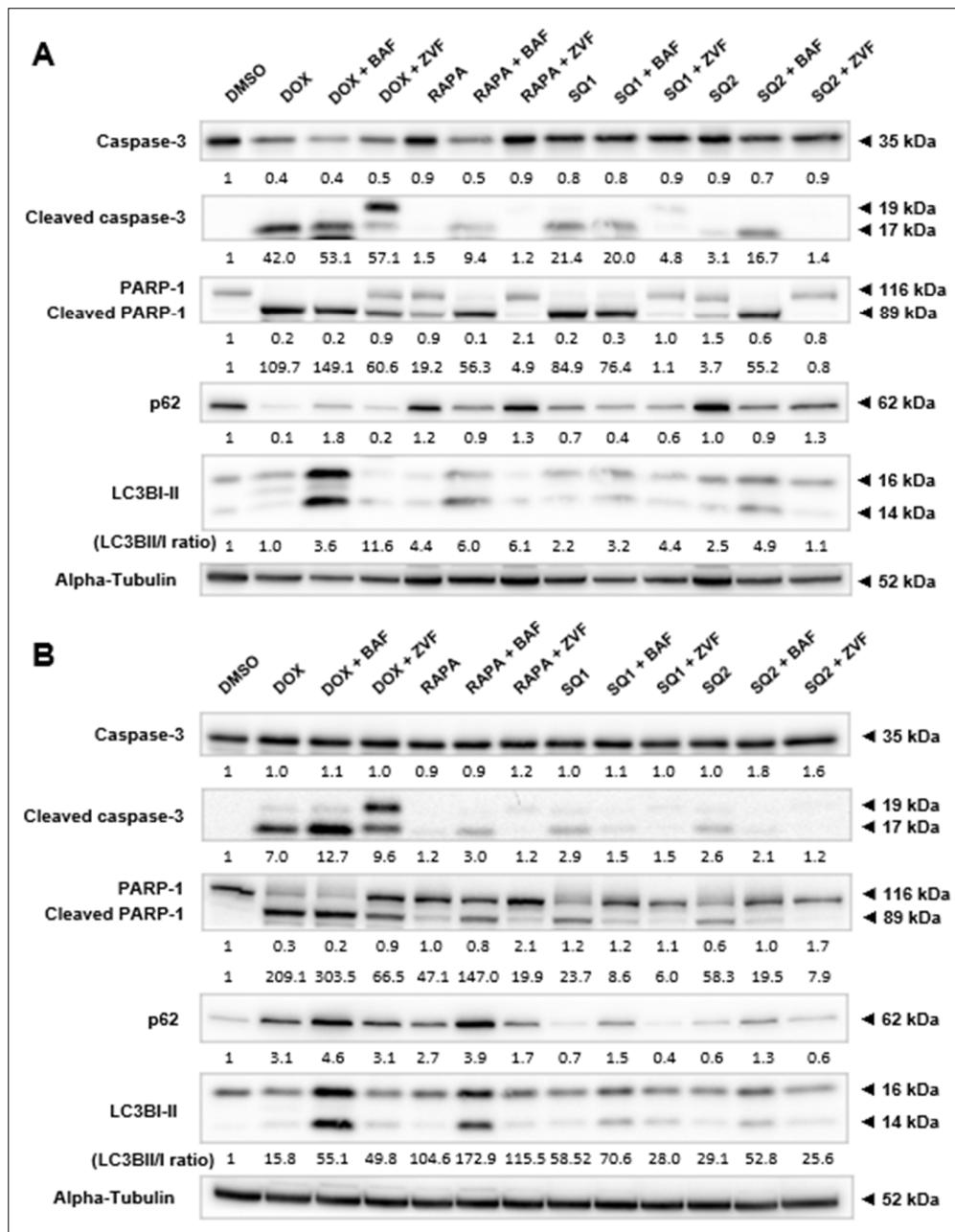


Figure S1: Quantifications from Western blotting analysis with inhibitor treatments. SK-MEL-28 (**A**) and SK-MEL-147 (**B**) were submitted to 1h pre-treatments of 30 nM baflomycin A1 (BAF) and 20 μ M Z-VAD-FMK (ZVF). Protein expression (PARP-1, cleaved PARP-1, caspase 3 and cleaved caspase 3, p62 and LC3B) was evaluated by western blotting after 24h of incubation with: DMSO (negative control: 0.2%), doxorubicin - DOX (positive control: 1.4 μ M for SK-MEL-28 and 0.8 μ M for SK-MEL-147), rapamycin - RAPA (18 μ M), **SQ1** (0.6 μ M for SK-MEL-28 and 1.79 μ M for SK-MEL-147) and **SQ2** (0.1 μ M for SK-MEL-28 and 1.26 μ M for SK-MEL-147). Quantifications were determined by the UN-SCAN-IT Gel 6.1 (Silk Scientific), normalized by alpha-tubulin and compared with the negative control of each experiment, showing the mean values (n=3).