

Hofer et al.:

Contradictory Effects of Chemical Filters in UV/ROS-stressed Human Keratinocyte and Fibroblast Cells

Supplementary Data

Tab. S1: Table of the test compounds (generic names) including CAS numbers and IUPAC nomenclature

Generic name	CAS number	IUPAC name
ecamsule	92761-26-7	{3-[(4-[[7,7-dimethyl-3-oxo-4-(sulfomethyl)bicyclo[2.2.1]heptan-2-ylidene]methyl]phenyl)methylidene]-7,7-dimethyl-2-oxobicyclo[2.2.1]heptan-1-yl)methanesulfonic acid
oxybenzone	131-57-7	2-hydroxy-4-methoxyphenyl)phenylmethanone
menthyl anthranilate	134-09-8	2-isopropyl-5-methylcyclohexyl 2-aminobenzoate
quercetin	117-39-5	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one
N-acetylcysteine	616-91-1	(2R)-2-acetamido-3-sulfanylpropanoic acid

Tab. S2: Half maximal inhibitory concentrations (IC₅₀) for ROS-inhibition of antioxidant and UV filter treatments in HaCaT and WT Fibs E6/E7

Data was calculated either with the CalcuSyn software (for UV filters) or via linear regression due to the availability of only 2 data points (antioxidant controls); no treat., no treatment; C.I., 95% confidence interval; R², coefficient of determination.

Substance	Cell type	Stimulation	IC ₅₀ [μM]	(upper C.I. - lower C.I.)	R ²
quercetin	HaCaT	no treat.	18.47	18.47 - 18.47	1.0000
	HaCaT	UV	68.40	68.40 - 68.40	1.0000
	HaCaT	AAPH	9.18	9.18 - 9.18	1.0000
	WT Fibs E6/E7	no treat.	15.17	15.17 - 15.17	1.0000
	WT Fibs E6/E7	UV	65.83	65.83 - 65.83	1.0000
	WT Fibs E6/E7	AAPH	10.00	10.00 - 10.00	1.0000
N-acetylcysteine	HaCaT	no treat.	-	- - -	-
	HaCaT	UV	-	- - -	-
	HaCaT	AAPH	1692.73	1692.73 - 1692.73	1.0000
	WT Fibs E6/E7	no treat.	7029.65	7029.65 - 7029.65	1.0000
	WT Fibs E6/E7	UV	-	- - -	-
	WT Fibs E6/E7	AAPH	514.01	514.01 - 514.01	1.0000
oxybenzone	HaCaT	no treat.	2980.62	2657.65 - 3342.85	0.9992
	HaCaT	UV	3887.75	2944.58 - 5133.04	0.9954
	HaCaT	AAPH	7426.70	3536.06 - 1.56E+04	0.9867
	WT Fibs E6/E7	no treat.	1236.69	1070.36 - 1428.87	0.9981
	WT Fibs E6/E7	UV	1487.47	1257.30 - 1759.77	0.9978
	WT Fibs E6/E7	AAPH	1418.56	1260.10 - 1596.96	0.9991
menthyl anthranilate	HaCaT	no treat.	-	- - -	-
	HaCaT	UV	-	- - -	-
	HaCaT	AAPH	-	- - -	-
	WT Fibs E6/E7	no treat.	461.03	249.38 - 852.32	0.9866
	WT Fibs E6/E7	UV	-	- - -	-
	WT Fibs E6/E7	AAPH	480.37	184.77 - 1248.89	0.9694
ecamsule	HaCaT	no treat.	-	- - -	-
	HaCaT	UV	9893.71	6286.14 - 1.56E+04	0.9929
	HaCaT	AAPH	-	- - -	-
	WT Fibs E6/E7	no treat.	1.00E+04	6776.10 - 1.48E+04	0.9956
	WT Fibs E6/E7	UV	9163.67	5726.10 - 14665.00	0.9921
	WT Fibs E6/E7	AAPH	-	- - -	-

Tab. S3: Half-maximal inhibitory concentrations (IC₅₀) for the reduction of viability at 1h post-treatment of UV-filter treatments in HaCaT and WT Fibs E6/E7

Data was calculated with the CalcuSyn software. No IC₅₀ could be calculated for the antioxidant controls quercetin and N-acetylcysteine in the tested concentration range. (no treat = no treatment; C.I. = 95% confidence interval; R² = coefficient of determination.

Substance	Cell type	Stimulation	IC ₅₀ [μM]	(upper C.I. - lower C.I.)	R ²
oxybenzone	HaCaT	no treat.	5.54E+04	2.92E+04 - 1.05E+05	0.9951
	HaCaT	UV	-	- - -	-
	HaCaT	AAPH	-	- - -	-
	WT Fibs E6/E7	no treat.	-	- - -	-
	WT Fibs E6/E7	UV	9.73E+04	7916.42 - 1.20E+06	0.9559
	WT Fibs E6/E7	AAPH	-	- - -	-
menthyl anthranilate	HaCaT	no treat.	-	- - -	-
	HaCaT	UV	-	- - -	-
	HaCaT	AAPH	-	- - -	-
	WT Fibs E6/E7	no treat.	-	- - -	-
	WT Fibs E6/E7	UV	-	- - -	-
	WT Fibs E6/E7	AAPH	-	- - -	-
ecamsule	HaCaT	no treat.	3.53E+05	1.04E+05 - 1.20E+06	0.9877
	HaCaT	UV	-	- - -	-
	HaCaT	AAPH	-	- - -	-
	WT Fibs E6/E7	no treat.	-	- - -	-
	WT Fibs E6/E7	UV	-	- - -	-
	WT Fibs E6/E7	AAPH	-	- - -	-

Tab. S4: Half maximal inhibitory concentrations (IC₅₀) for the reduction of viability at 24 h post-treatment of UV filter treatments in HaCaT and WT Fibs E6/E7

Data was calculated with the CalcuSyn software. No IC₅₀ could be calculated for the antioxidant controls quercetin and N-acetylcysteine in the tested concentration range; no treat, no treatment; C.I., 95% confidence interval; R², coefficient of determination.

Substance	Cell type	Stimulation	IC ₅₀ [μM]	(upper - lower)	R ²
oxybenzone	HaCaT	no treat.	-	- - -	-
	HaCaT	UV	-	- - -	-
	HaCaT	AAPH	-	- - -	-
	WT Fibs E6/E7	no treat.	9796.20	1376.72 - 6.97E+04	0.9556
	WT Fibs E6/E7	UV	2346.89	658.56 - 8363.48	0.9442
	WT Fibs E6/E7	AAPH	2468.87	1858.49 - 3279.71	0.9971
menthyl anthranilate	HaCaT	no treat.	-	- - -	-
	HaCaT	UV	794.09	346.10 - 1821.96	0.9830
	HaCaT	AAPH	1.00E+04	5115.33 - 1.96E+04	0.9979
	WT Fibs E6/E7	no treat.	218.21	78.98 - 602.94	0.9376
	WT Fibs E6/E7	UV	191.00	160.91 - 226.72	0.9978
	WT Fibs E6/E7	AAPH	147.60	126.98 - 171.57	0.9980
ecamsule	HaCaT	no treat.	-	- - -	-
	HaCaT	UV	-	- - -	-
	HaCaT	AAPH	-	- - -	-
	WT Fibs E6/E7	no treat.	-	- - -	-
	WT Fibs E6/E7	UV	-	- - -	-
	WT Fibs E6/E7	AAPH	-	- - -	-

Fig. S1: Impact of increasing concentrations of quercetin on intracellular ROS formation on (a) HaCat and (d) WT Fibs E6/E7 cells exposed to UV, AAPH, or without additional treatment (respective controls set to 1). Effect of quercetin on HaCaT and on WT Fibs E6/E7 cell viability (b,e) 1 h and (c,f) 24 h post-treatment

Metabolic activity was measured by estimating resazurin conversion to indicate viability; results were normalized to the activity in the unstimulated buffer control (set to 100%). A vehicle concentration of 0.08% (v/v) DMSO was used. Results are shown as mean values \pm S.E.M. of 3 independent experiments, each performed at least in triplicates (* $p \leq 0.05$, ** $p \leq 0.005$ compared to control).

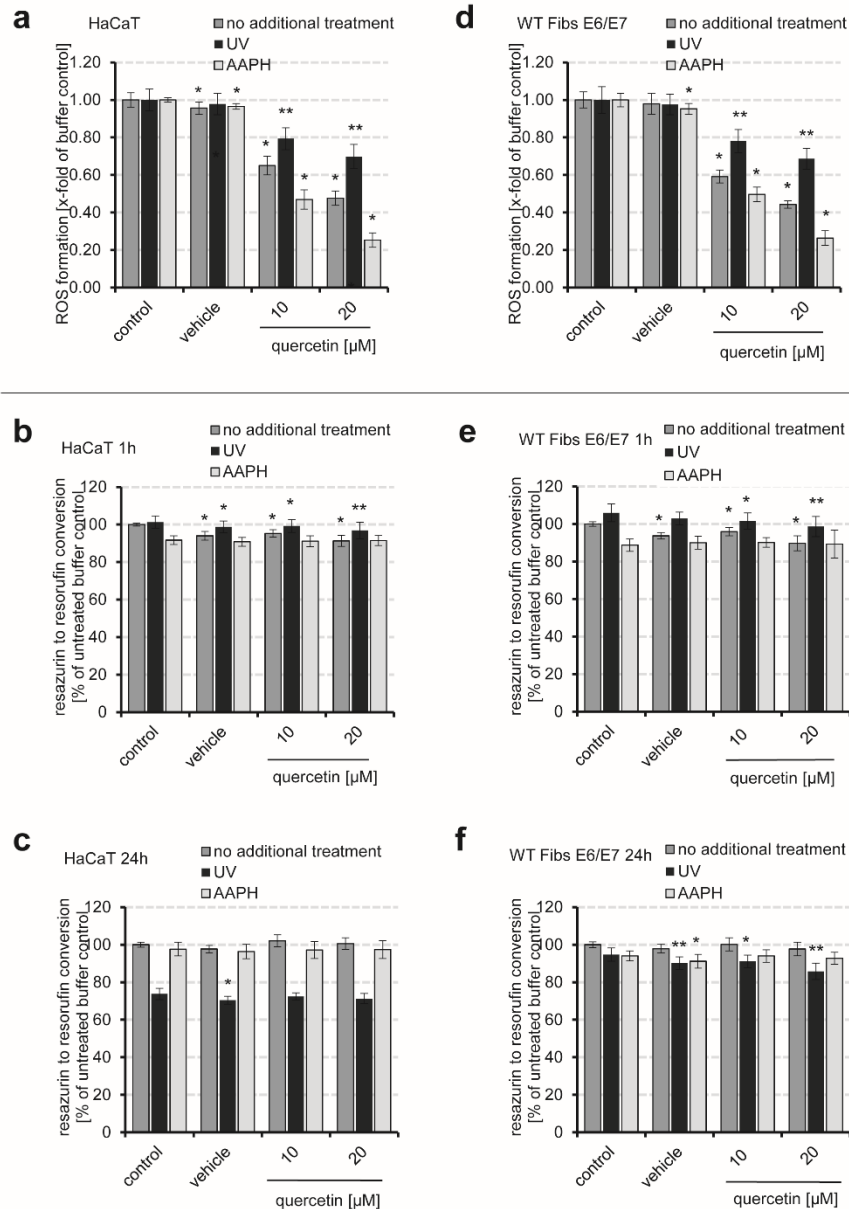


Fig. S2: Impact of increasing concentrations of N-acetylcysteine on intracellular ROS formation in (a) HaCaT and (d) WT Fibs E6/E7 cells exposed to UV, AAPH or without additional treatment (respective controls set to 1). Effect of N-acetylcysteine on HaCaT and on WT Fibs E6/E7 cell viability (b,e) 1 h and (c,f) 24 h post-treatment, estimated by measuring resazurin reduction

Results were normalized to the activity in the unstimulated buffer control (set to 100%). Results are shown as mean values \pm S.E.M. of 3 independent experiments, each performed at least in triplicates (* $p \leq 0.05$, ** $p \leq 0.005$ compared to control).

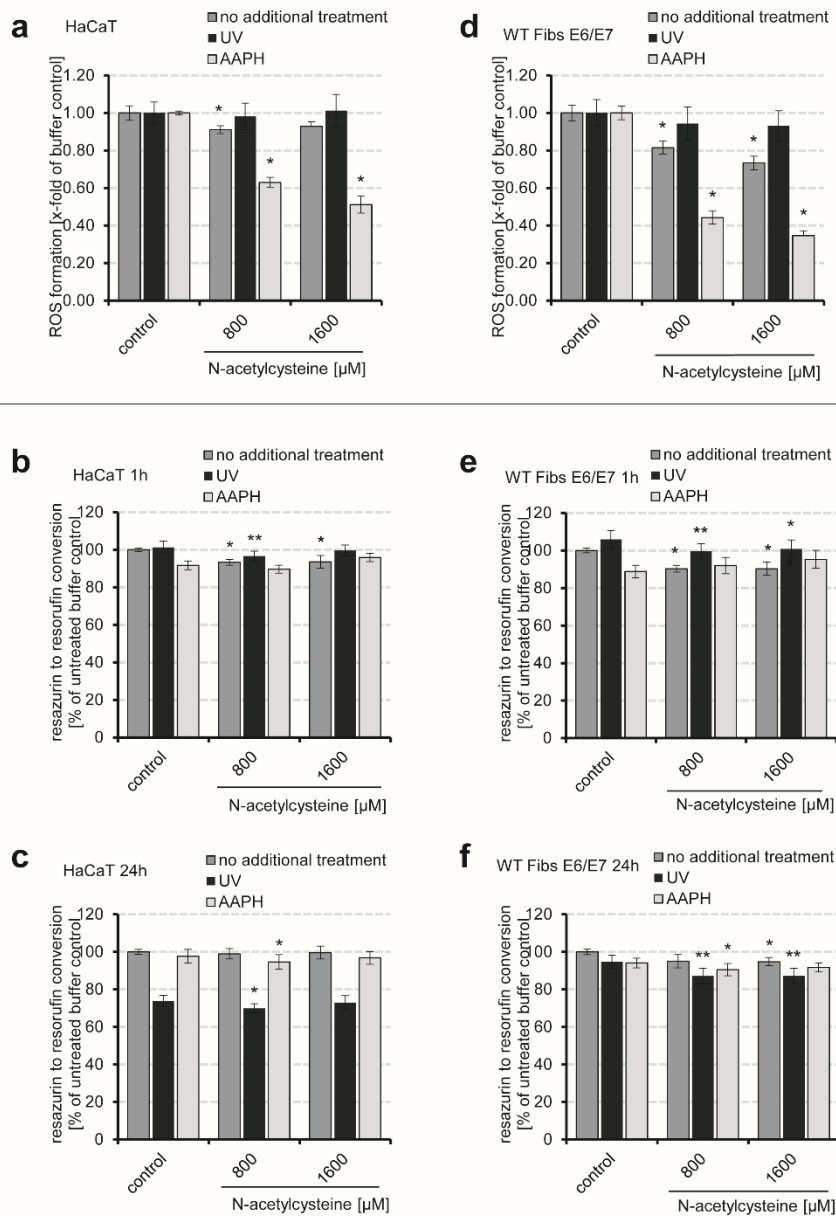


Fig. S3: Impact of increasing concentrations of oxybenzone on intracellular ROS formation in (a) HaCat and (d) WT Fibs E6/E7 cells exposed to UV, AAPH or without additional treatment (respective controls set to 1). Effect of oxybenzone on HaCaT and on WT Fibs E6/E7 cell viability (b,e) 1 h and (c,f) 24 h post-treatment, estimated by reduction of resazurin
Results were normalized to the activity in the unstimulated buffer control (set to 100%). Vehicle concentrations of 2% (v/v) DMSO (for HaCaT) and 1% (v/v) (for WT Fibs E6/E7) were used. Results are shown as mean values \pm S.E.M. of 3 independent experiments, each performed at least in triplicates (* $p \leq 0.05$, ** $p \leq 0.005$ compared to control, ° $p \leq 0.05$, °° $p \leq 0.005$ compared to vehicle).

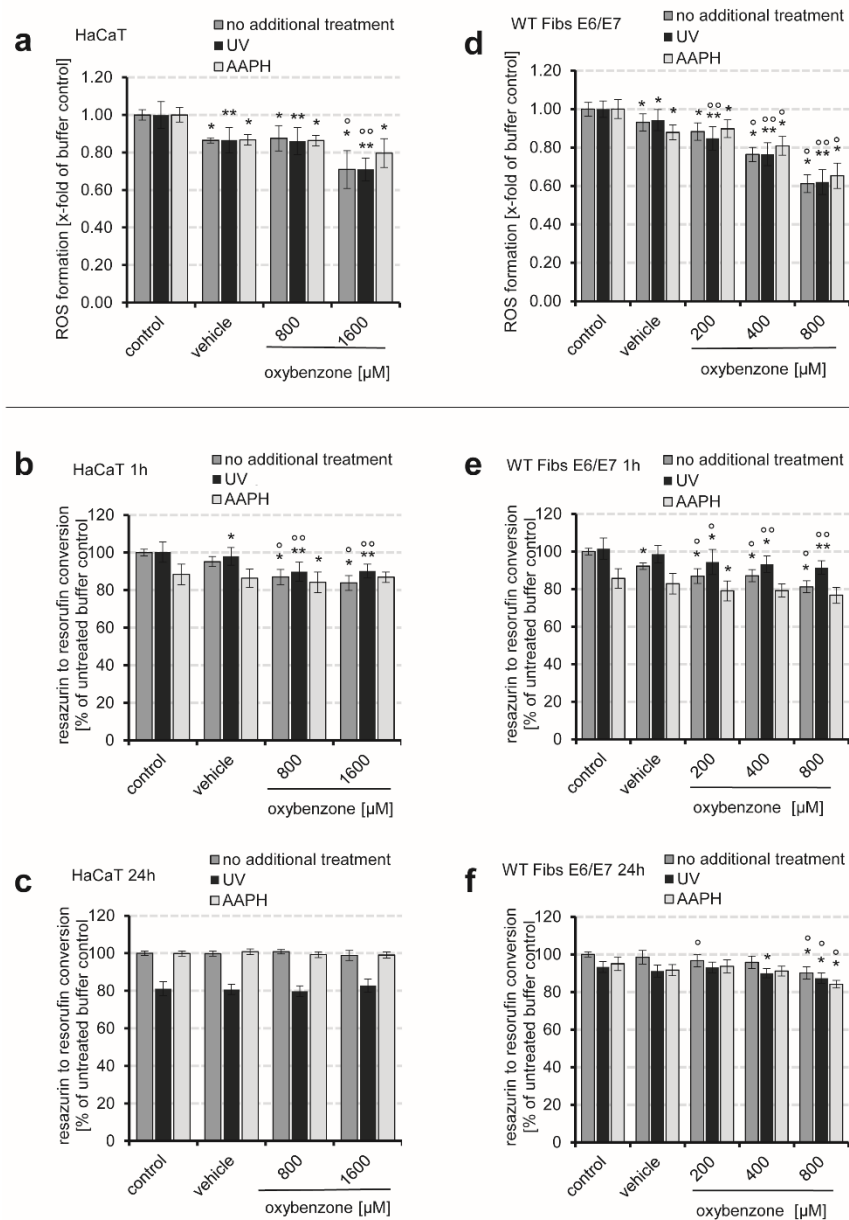


Fig. S4: Effect of menthyl anthranilate on intracellular ROS formation in (a) HaCat and (d) WT Fibs E6/E7 cells exposed to UV, AAPH or without additional treatment (respective controls set to 1). Effect of menthyl anthranilate on HaCaT and on WT Fibs E6/E7 viability at (b,e) 1 h and (c,f) 24 h post-treatment
Resazurin conversion is shown relative to the activity in the unstimulated buffer control (set to 100%). DMSO was used with 0.25% (v/v) as vehicle in both cell lines. Results are shown as mean values \pm S.E.M. of at least 3 independent experiments, each performed at least in triplicates (* $p \leq 0.05$, ** $p \leq 0.005$ compared to control, ° $p \leq 0.05$, °° $p \leq 0.005$ compared to vehicle)

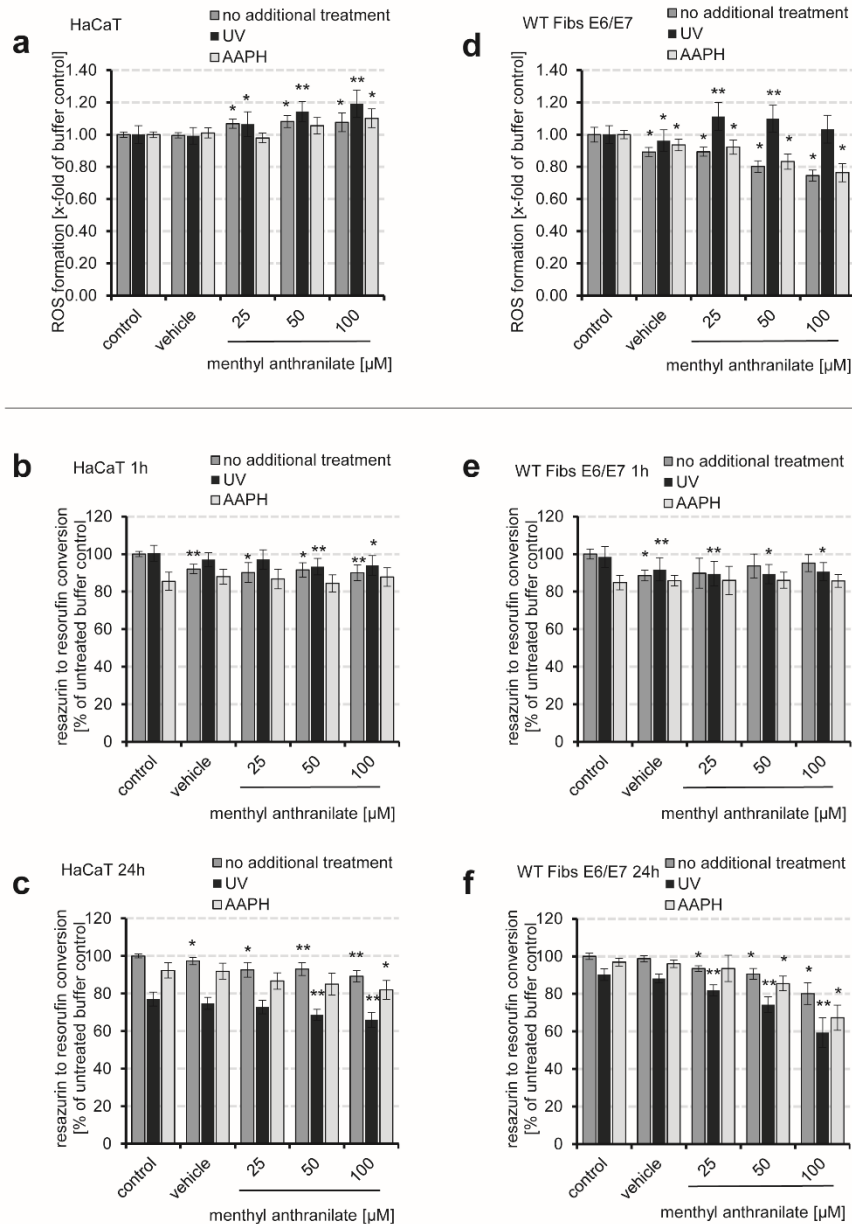


Fig. S5: Impact of increasing concentrations of ecamsule on ROS levels in (a) HaCat and (d) WT Fibs E6/E7 cells exposed to UV, AAPH or without additional treatment (respective controls set to 1). Viability of HaCat and WT Fibs E6/E7 (b,e) 1 h and (c,f) 24 h after the treatment

Viability data were normalized to the unstimulated buffer control (set to 100%). Results are shown as mean values \pm S.E.M. of 3 independent experiments, each performed at least in triplicates (* $p \leq 0.05$, ** $p \leq 0.005$ compared to control, ° $p \leq 0.05$, °° $p \leq 0.005$ compared to vehicle).

