

Supporting information for

The effect of sulindac on redox homeostasis and apoptosis-related proteins in melanotic and amelanotic cells

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Table 1S. Superoxide dismutase activity in COLO829 melanotic melanoma cells cultured in the presence of sulindac and dacarbazine.

Drug	Concentration [μM]	Average SOD activity		
		[U/ml] ± SD	[U/mg protein] ± SD	[%]
Sulindac	control	3.92 ± 0.13	1.74 ± 0.06	100.0 ± 3.3
	0.1	3.96 ± 0.18	1.80 ± 0.08	103.7 ± 4.6
	1.0	4.09 ± 0.19	1.84 ± 0.08	106.0 ± 4.8
	10	4.35 ± 0.11	1.92 ± 0.05	110.3 ± 2.7
	50	4.81 ± 0.20	2.16 ± 0.09	124.4 ± 5.1
	100	5.60 ± 0.34	2.46 ± 0.15	141.4 ± 8.7
Dacarbazine	control	3.92 ± 0.13	1.74 ± 0.06	100.0 ± 3.3
	0.1	4.08 ± 0.23	1.82 ± 0.10	104.8 ± 6.0
	1.0	4.27 ± 0.18	1.95 ± 0.08	112.3 ± 4.8
	10	4.85 ± 0.21	2.17 ± 0.09	124.7 ± 5.4
	50	5.06 ± 0.24	2.30 ± 0.11	132.0 ± 6.2
	100	5.40 ± 0.22	2.40 ± 0.10	137.9 ± 5.7

Table 2S. Superoxide dismutase activity in C32 amelanotic melanoma cells cultured in the presence of sulindac and dacarbazine.

Drug	Concentration [μ M]	Average SOD activity		
		[U/ml] $\pm$ SD	[U/mg protein] $\pm$ SD	[%]
Sulindac	control	5.96 $\pm$ 0.33	3.08 $\pm$ 0.17	100.0 $\pm$ 5.6
	10	6.17 $\pm$ 0.35	3.18 $\pm$ 0.18	103.3 $\pm$ 5.9
	50	6.00 $\pm$ 0.18	3.09 $\pm$ 0.09	100.6 $\pm$ 3.0
	100	5.76 $\pm$ 0.25	2.97 $\pm$ 0.13	96.4 $\pm$ 4.2
Dacarbazine	control	5.96 $\pm$ 0.33	3.08 $\pm$ 0.17	100.0 $\pm$ 5.6
	10	6.73 $\pm$ 0.14	3.47 $\pm$ 0.07	112.7 $\pm$ 2.4
	50	6.92 $\pm$ 0.33	3.57 $\pm$ 0.17	116.0 $\pm$ 5.5
	100	7.10 $\pm$ 0.17	3.67 $\pm$ 0.09	119.2 $\pm$ 2.9

Table 3S. Catalase activity in COLO829 melanotic melanoma cells cultured in the presence of sulindac and dacarbazine.

Drug	Concentration [μ M]	Average CAT activity		
		[nmol/min/ml] $\pm$ SD	[nmol/min/mg protein] $\pm$ SD	[%]
Sulindac	control	9.28 $\pm$ 0.56	4.12 $\pm$ 0.25	100.0 $\pm$ 6.0
	0.1	9.03 $\pm$ 0.57	3.99 $\pm$ 0.26	99.8 $\pm$ 6.3
	1.0	8.04 $\pm$ 0.46	3.54 $\pm$ 0.21	87.8 $\pm$ 5.1
	10	7.82 $\pm$ 0.61	3.45 $\pm$ 0.27	83.8 $\pm$ 6.6
	50	7.36 $\pm$ 0.40	3.31 $\pm$ 0.18	80.4 $\pm$ 4.4
	100	5.85 $\pm$ 0.50	2.57 $\pm$ 0.22	62.4 $\pm$ 5.3
Dacarbazine	control	9.28 $\pm$ 0.56	4.12 $\pm$ 0.25	100.0 $\pm$ 6.0
	0.1	8.74 $\pm$ 0.48	3.75 $\pm$ 0.21	94.7 $\pm$ 5.2
	1.0	8.32 $\pm$ 0.39	3.80 $\pm$ 0.18	92.4 $\pm$ 4.4
	10	8.14 $\pm$ 0.38	3.64 $\pm$ 0.17	88.5 $\pm$ 4.2
	50	7.45 $\pm$ 0.42	3.38 $\pm$ 0.19	82.0 $\pm$ 4.6
	100	7.39 $\pm$ 0.38	3.28 $\pm$ 0.17	79.7 $\pm$ 4.1

Table 4S. Catalase activity in C32 amelanotic melanoma cells cultured in the presence of sulindac and dacarbazine.

Drug	Concentration [μ M]	Average CAT activity		
		[nmol/min/ml] $\pm$ SD	[nmol/min/mg protein] $\pm$ SD	[%]
Sulindac	control	1.13 $\pm$ 0.09	0.59 $\pm$ 0.05	100.0 $\pm$ 8.1
	10	1.11 $\pm$ 0.09	0.57 $\pm$ 0.05	98.0 $\pm$ 8.1
	50	1.11 $\pm$ 0.09	0.57 $\pm$ 0.05	98.2 $\pm$ 8.1
	100	1.15 $\pm$ 0.07	0.59 $\pm$ 0.03	101.6 $\pm$ 5.7
Dacarbazine	control	1.13 $\pm$ 0.09	0.59 $\pm$ 0.05	100.0 $\pm$ 8.1
	10	1.03 $\pm$ 0.07	0.53 $\pm$ 0.03	90.8 $\pm$ 5.7
	50	0.97 $\pm$ 0.07	0.50 $\pm$ 0.03	85.3 $\pm$ 5.7
	100	0.89 $\pm$ 0.09	0.46 $\pm$ 0.05	78.1 $\pm$ 8.1

Table 5S. Glutathione peroxidase activity in COLO829 melanotic melanoma cells cultured in the presence of sulindac and dacarbazine.

Drug	Concentration [μ M]	Average GPx activity		
		[nmol/min/ml] $\pm$ SD	[nmol/min/mg protein] $\pm$ SD	[%]
Sulindac	control	9.82 $\pm$ 0.43	4.36 $\pm$ 0.19	100.0 $\pm$ 4.4
	0.1	9.54 $\pm$ 0.50	4.34 $\pm$ 0.23	99.4 $\pm$ 5.2
	1.0	9.12 $\pm$ 0.49	4.11 $\pm$ 0.22	94.1 $\pm$ 5.1
	10	8.74 $\pm$ 0.51	3.85 $\pm$ 0.23	88.2 $\pm$ 5.2
	50	7.67 $\pm$ 0.45	3.44 $\pm$ 0.20	78.8 $\pm$ 4.6
	100	7.21 $\pm$ 0.45	3.16 $\pm$ 0.20	72.5 $\pm$ 4.5
Dacarbazine	control	9.82 $\pm$ 0.43	4.36 $\pm$ 0.19	100.0 $\pm$ 4.4
	0.1	9.38 $\pm$ 0.37	4.19 $\pm$ 0.16	96.1 $\pm$ 3.8
	1.0	8.80 $\pm$ 0.30	4.02 $\pm$ 0.14	92.3 $\pm$ 3.1
	10	8.15 $\pm$ 0.59	3.65 $\pm$ 0.26	83.7 $\pm$ 6.0
	50	7.33 $\pm$ 0.45	3.32 $\pm$ 0.21	76.2 $\pm$ 4.7
	100	7.29 $\pm$ 0.40	3.24 $\pm$ 0.18	74.3 $\pm$ 4.0

Table 6S. Glutathione peroxidase activity in C32 amelanotic melanoma cells cultured in the presence of sulindac and dacarbazine.

Drug	Concentration [μ M]	Average GPx activity		
		[nmol/min/ml] $\pm$ SD	[nmol/min/mg protein] $\pm$ SD	[%]
Sulindac	control	26.06 $\pm$ 1.04	13.45 $\pm$ 0.53	100.0 $\pm$ 4.0
	10	26.09 $\pm$ 1.57	13.45 $\pm$ 0.81	100.0 $\pm$ 6.0
	50	25.33 $\pm$ 1.86	13.08 $\pm$ 0.96	97.2 $\pm$ 7.1
	100	24.84 $\pm$ 1.56	12.79 $\pm$ 0.80	95.1 $\pm$ 6.0
Dacarbazine	control	26.06 $\pm$ 1.04	13.45 $\pm$ 0.53	100.0 $\pm$ 4.0
	10	24.40 $\pm$ 0.64	12.58 $\pm$ 0.33	93.5 $\pm$ 2.4
	50	23.46 $\pm$ 0.63	12.10 $\pm$ 0.33	89.9 $\pm$ 2.4
	100	22.16 $\pm$ 1.37	11.45 $\pm$ 0.71	85.1 $\pm$ 5.3

Table 7S. Content of hydrogen peroxide in COLO829 melanotic melanoma cells cultured in the presence of sulindac and dacarbazine.

Drug	Concentration [μ M]	Content of H ₂ O ₂		
		[μ mol/ml] $\pm$ SD	[μ mol/mg protein] $\pm$ SD	[%]
Sulindac	control	47.12 $\pm$ 1.51	20.24 $\pm$ 0.65	100.0 $\pm$ 3.2
	0.1	49.94 $\pm$ 1.32	20.90 $\pm$ 0.55	103.2 $\pm$ 2.7
	1.0	49.28 $\pm$ 1.91	21.35 $\pm$ 0.83	105.4 $\pm$ 4.1
	10	52.96 $\pm$ 2.19	22.50 $\pm$ 0.93	111.1 $\pm$ 4.6
	50	63.90 $\pm$ 2.50	26.46 $\pm$ 1.05	130.7 $\pm$ 5.2
	100	70.42 $\pm$ 3.06	29.82 $\pm$ 1.30	147.3 $\pm$ 6.4
Dacarbazine	control	47.12 $\pm$ 1.51	20.24 $\pm$ 0.65	100.0 $\pm$ 3.2
	0.1	58.35 $\pm$ 2.98	25.50 $\pm$ 1.30	123.9 $\pm$ 6.3
	1.0	61.38 $\pm$ 2.51	27.22 $\pm$ 1.11	132.2 $\pm$ 5.4
	10	68.60 $\pm$ 3.66	30.53 $\pm$ 1.63	148.3 $\pm$ 7.9
	50	72.38 $\pm$ 2.36	32.23 $\pm$ 1.05	156.6 $\pm$ 5.1
	100	77.42 $\pm$ 2.88	33.87 $\pm$ 1.26	164.6 $\pm$ 6.1

Table 8S. Content of hydrogen peroxide in C32 amelanotic melanoma cells cultured in the presence of sulindac and dacarbazine.

Drug	Concentration [μ M]	Content of H ₂ O ₂		
		[μ mol/ml] $\pm$ SD	[μ mol/mg protein] $\pm$ SD	[%]
Sulindac	control	639.15 $\pm$ 22.20	132.00 $\pm$ 4.58	100.0 $\pm$ 3.5
	10	653.43 $\pm$ 14.86	134.69 $\pm$ 3.06	102.0 $\pm$ 2.3
	50	639.25 $\pm$ 30.48	132.00 $\pm$ 6.29	100.0 $\pm$ 4.8
	100	651.39 $\pm$ 21.02	134.21 $\pm$ 4.33	101.7 $\pm$ 3.3
Dacarbazine	control	639.15 $\pm$ 22.20	132.00 $\pm$ 4.58	100.0 $\pm$ 3.5
	10	769.44 $\pm$ 22.00	158.73 $\pm$ 4.54	120.3 $\pm$ 3.4
	50	816.55 $\pm$ 21.41	168.48 $\pm$ 4.42	127.6 $\pm$ 3.3
	100	854.54 $\pm$ 27.59	176.53 $\pm$ 5.70	133.7 $\pm$ 4.3

Table 9S. Content of protein p53 in COLO829 melanotic melanoma cells cultured in the presence of sulindac and dacarbazine.

Drug	Concentration [μ M]	Content of p53	
		[U/mg protein] $\pm$ SD	[%]
Sulindac	control	2.74 $\pm$ 0.17	100.0 $\pm$ 6.2
	0.1	3.25 $\pm$ 0.15	118.7 $\pm$ 5.5
	1.0	3.30 $\pm$ 0.15	120.5 $\pm$ 5.4
	10	3.52 $\pm$ 0.22	128.5 $\pm$ 7.9
	50	4.20 $\pm$ 0.23	153.4 $\pm$ 8.6
	100	5.43 $\pm$ 0.40	198.1 $\pm$ 4.7
Dacarbazine	control	2.74 $\pm$ 0.17	100.0 $\pm$ 6.2
	0.1	3.11 $\pm$ 0.10	113.4 $\pm$ 3.7
	1.0	3.38 $\pm$ 0.26	123.4 $\pm$ 9.3
	10	3.64 $\pm$ 0.24	132.8 $\pm$ 8.8
	50	4.28 $\pm$ 0.25	156.3 $\pm$ 9.2
	100	5.12 $\pm$ 0.31	186.7 $\pm$ 11.3

Table 10S. Content of protein p53 in C32 amelanotic melanoma cells cultured in the presence of sulindac and dacarbazine.

Drug	Concentration [μ M]	Content of p53	
		[U/mg protein] $\pm$ SD	[%]
Sulindac	control	6.35 $\pm$ 0.19	100.0 $\pm$ 3.0
	10	6.37 $\pm$ 0.29	100.3 $\pm$ 4.5
	50	6.60 $\pm$ 0.58	104.0 $\pm$ 9.2
	100	8.01 $\pm$ 0.34	126.1 $\pm$ 5.4
Dacarbazine	control	6.35 $\pm$ 0.19	100.0 $\pm$ 3.0
	10	6.46 $\pm$ 0.34	101.8 $\pm$ 5.4
	50	6.83 $\pm$ 0.33	107.6 $\pm$ 5.2
	100	8.59 $\pm$ 0.51	135.3 $\pm$ 8.0

Table 11S. Content of protein Bcl-2 in COLO829 melanotic melanoma cells cultured in the presence of sulindac and dacarbazine.

Drug	Concentration [μ M]	Content of Bcl-2	
		[ng/mg protein] $\pm$ SD	[%]
Sulindac	control	10.15 $\pm$ 0.75	100.0 $\pm$ 7.4
	0.1	9.62 $\pm$ 0.63	94.7 $\pm$ 6.2
	1.0	8.47 $\pm$ 0.38	83.4 $\pm$ 3.7
	10	7.36 $\pm$ 0.72	72.5 $\pm$ 7.1
	50	7.00 $\pm$ 0.81	68.9 $\pm$ 8.0
	100	6.81 $\pm$ 0.42	67.1 $\pm$ 4.2
Dacarbazine	control	10.15 $\pm$ 0.75	100.0 $\pm$ 7.4
	0.1	8.38 $\pm$ 0.68	82.5 $\pm$ 6.7
	1.0	7.21 $\pm$ 0.48	71.0 $\pm$ 4.7
	10	4.11 $\pm$ 0.57	40.5 $\pm$ 5.6
	50	3.52 $\pm$ 0.51	34.7 $\pm$ 5.1
	100	3.33 $\pm$ 0.33	32.8 $\pm$ 3.3

Table 12S. Content of protein Bcl-2 in C32 amelanotic melanoma cells cultured in the presence of sulindac and dacarbazine.

Drug	Concentration [μ M]	Content of Bcl-2	
		[ng/mg protein] $\pm$ SD	[%]
Sulindac	control	3.57 ± 0.13	100.0 ± 3.8
	10	4.94 ± 0.18	138.5 ± 5.0
	50	5.64 ± 0.32	158.2 ± 9.1
	100	7.84 ± 0.28	219.9 ± 7.7
Dacarbazine	control	3.57 ± 0.13	100.0 ± 3.8
	10	5.30 ± 0.18	148.5 ± 5.0
	50	5.54 ± 0.22	155.3 ± 6.1
	100	6.00 ± 0.22	168.2 ± 6.3

Table 13S. Content of protein Bax in COLO829 melanotic melanoma cells cultured in the presence of sulindac and dacarbazine.

Drug	Concentration [μ M]	Content of Bax	
		[pg/mg protein] $\pm$ SD	[%]
Sulindac	control	393.24 ± 12.87	100.0 ± 3.3
	0.1	397.55 ± 8.76	101.1 ± 2.2
	1.0	402.01 ± 12.48	102.2 ± 3.2
	10	410.27 ± 15.96	104.3 ± 4.1
	50	411.26 ± 10.40	104.6 ± 2.6
	100	431.48 ± 16.21	109.7 ± 4.1
Dacarbazine	control	393.24 ± 12.87	100.0 ± 3.3
	0.1	402.81 ± 12.71	102.4 ± 3.2
	1.0	403.92 ± 17.13	102.7 ± 4.4
	10	410.27 ± 18.74	104.3 ± 4.8
	50	417.17 ± 10.83	106.1 ± 2.8
	100	443.31 ± 13.41	112.7 ± 3.4

Table 14S. Content of protein Bax in C32 amelanotic melanoma cells cultured in the presence of sulindac and dacarbazine.

Drug	Concentration [μ M]	Content of Bax	
		[pg/mg protein] $\pm$ SD	[%]
Sulindac	control	465.01 $\pm$ 14.90	100.0 $\pm$ 3.2
	10	468.95 $\pm$ 19.79	100.8 $\pm$ 4.3
	50	466.16 $\pm$ 15.84	100.2 $\pm$ 3.4
	100	496.58 $\pm$ 26.40	106.8 $\pm$ 5.7
Dacarbazine	control	465.01 $\pm$ 14.90	100.0 $\pm$ 3.2
	10	470.89 $\pm$ 14.38	101.3 $\pm$ 3.1
	50	497.00 $\pm$ 15.76	106.9 $\pm$ 3.4
	100	502.37 $\pm$ 16.53	108.0 $\pm$ 3.6