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Supplementary Data

c-Myc Protein Level Affected by Unsymmetrical Bisacridines Influences Apoptosis and Senescence Induced in HCT116 Colorectal and H460 Lung Cancer Cells

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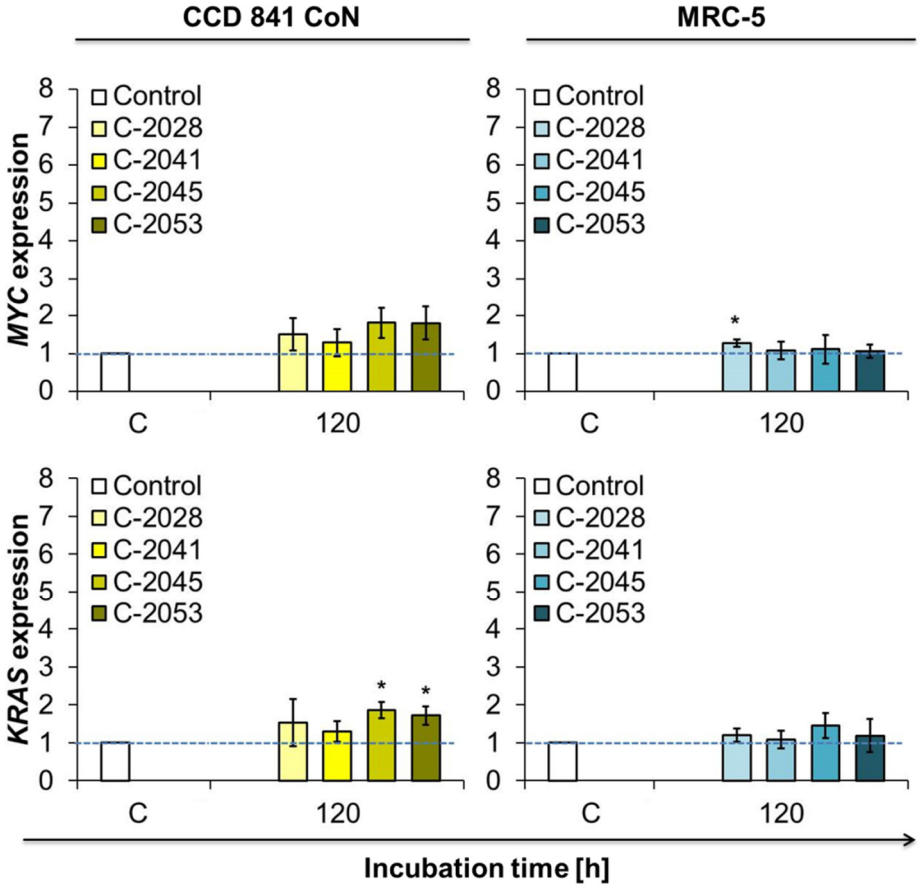

Figure S1. *MYC* and *KRAS* expression in CCD 841 CoN colon and MRC-5 lung normal cells. Cells were incubated with IC_{90} doses of UAs for 120 h, total mRNA was isolated, transcribed to cDNA, and real-time PCR analysis was performed with the appropriate primers for the *MYC* and *KRAS* genes. *ACTB* (β-actin) was used as housekeeping gene standard. Relative gene expression was calculated using 2^-ΔΔCt method [Livak]. Significantly different from the control at: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, n = 3).

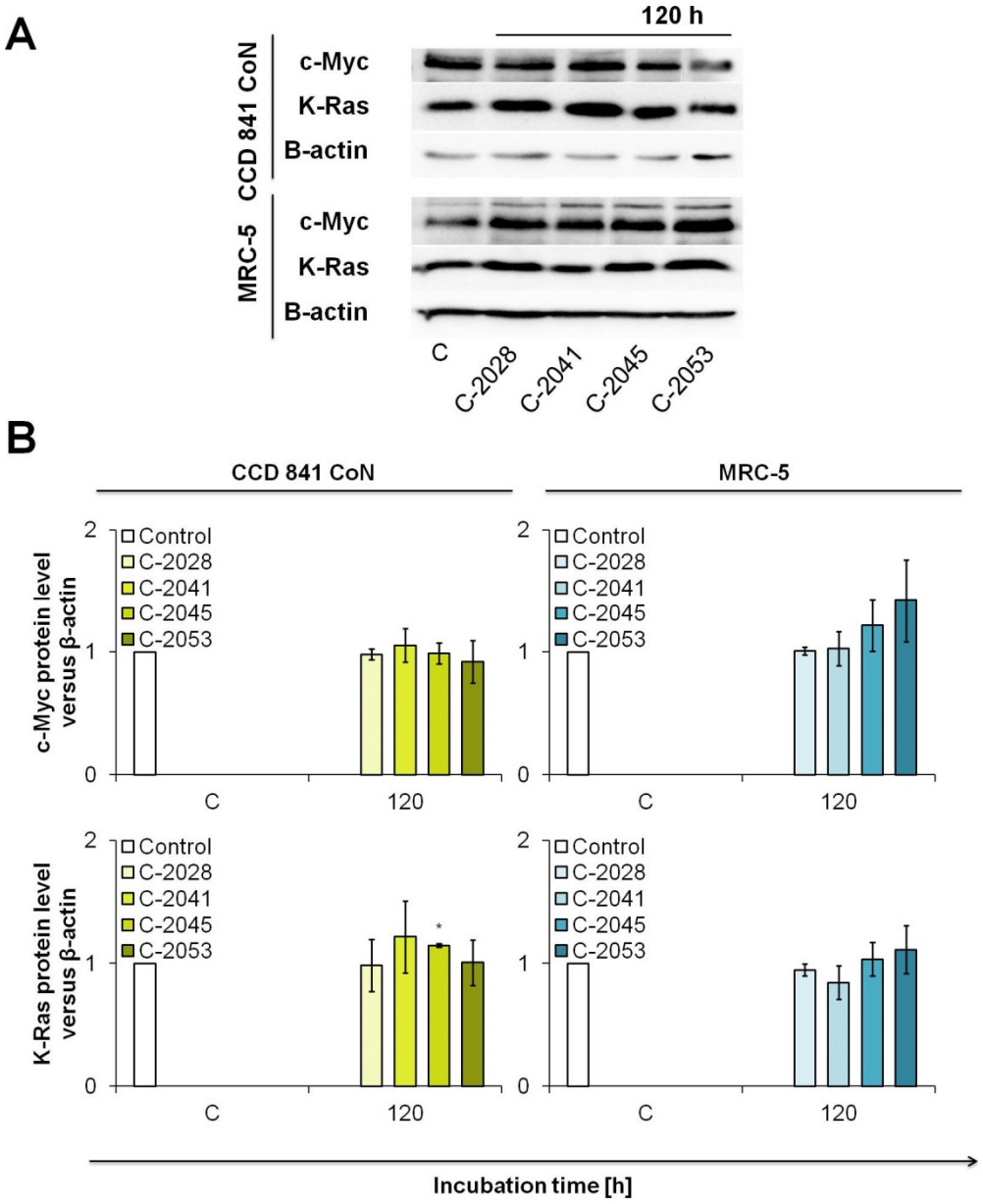

Figure S2. Western blot analysis of c-Myc and K-Ras protein levels in CCD841 CoN colon and MRC-5 lung normal cells. Cells were incubated with IC_{90} doses of UAs for 120 h, whole cell extracts were prepared, 20 μg of proteins/lane were separated by polyacrylamide gel electrophoresis and semi-dry transferred on a membrane. Protein levels were detected after immunostaining the membrane with appropriate antibodies and ECL developing. (**A**) Representative Western blot analysis of c-Myc and K-Ras in CCD 841 CoN and MRC-5 cells and (**B**) their relative densitometry quantification performed using ImageJ Software. Significantly different from the control at: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; (n = 3).

Table S1. Cell cycle distribution in UAs treated HCT116 cells. Flow cytometry analysis of cell cycle distribution of HCT116 untreated (control) and treated cells with C-2028, C-2041, C-2045 and C-2053 at concentrations corresponding to IC_{90} doses for the times indicated. Data shows percentages of cells in the sub-G1, G1, S, G2/M, and Poli (polyploid cells) phases of the cell cycle. Data represents the averages of at least three independent experiments with standard deviation ($n \geq 3$).

		Phase of Cell Cycle				
Compound	**Incubation Time [h]**	**Sub-G1**	**G1**	**S**	**G2/M**	**Poli**
Control	**24**	2.03 ± 0.43	48.54 ±2.58	16.72 ± 2.01	22.88 ± 2.27	9.98 ± 1.81
C-2028	**24**	3.37 ± 0.69	24.89 ± 3.90	19.33 ± 1.58	45.89 ± 3.13	5.73 ± 0.93
	72	36.97 ± 8.93	28.63 ± 5.96	7.67 ± 2.54	21.02 ± 4.86	5.11 ± 1.54
	120	37.46 ± 9.35	25.39 ± 3.25	4.95 ± 1.90	26.26 ± 4.90	5.73 ±2.33
C-2041	**24**	11.81 ± 1.94	28.75 ± 3.88	25.97 ± 2.80	29.92 ± 4.56	4.22 ± 2.00
	72	48.83 ± 7.71	22.16 ± 5.06	9.59 ± 2.24	14.29 ± 1.52	4.47 ± 2.13
	120	68.86 ± 9.48	13.63 ± 3.71	3.25 ± 1.05	9.44 ± 3.88	4.64 ± 2.56
C-2045	**24**	3.90 ± 0.83	24.61 ± 2.63	16.33 ± 1.34	50.18 ± 2.39	4.91 ± 2.33
	72	17.38 ± 4.00	28.51 ± 4.31	6.15 ± 0.70	34.06 ± 3.35	12.20 ± 1.85
	120	35.40 ± 5.84	22.11 ± 2.54	4.56 ± 1.19	22.94 ± 2.48	15.17 ± 3.74
C-2053	**24**	3.06 ± 1.09	22.34 ± 2.79	19.19 ± 1.77	44.81 ± 7.10	8.89 ± 1.38
	72	10.45 ± 2.56	35.18 ± 2.63	5.43 ± 1.59	36.32 ± 3.14	11.02 ± 2.20
	120	23.64 ± 4.86	30.06 ± 4.35	4.10 ± 0.56	27.20 ± 4.16	14.62 ± 4.62

Table S2. Cell cycle distribution in UAs treated H460 cells Flow cytometry analysis of cell cycle distribution of H460 untreated (control) and treated cells with C-2028, C-2041, C-2045 and C-2053 at concentrations corresponding to IC_{90} doses for the times indicated. Data shows percentages of cells in the sub-G1, G1, S, G2/M, and Poli (polyploid cells) phases of the cell cycle. Data represents the averages of at least three independent experiments with standard deviation ($n \geq 3$).

		Phase of Cell Cycle				
Compound	**Incubation Time [h]**	**Sub-G1**	**G1**	**S**	**G2/M**	**Poli**
Control	**24**	2.39 ± 0.73	60.09 ± 1.73	16.46 ± 0.55	18.37 ± 1.08	2.66 ± 0.64
C-2028	**24**	11.09 ± 3.80	32.92 ± 3.77	32.74 ± 2.09	21.64 ± 4.23	1.70 ± 0.42
	72	25.45 ± 7.20	37.15 ± 4.13	15.80 ± 2.33	19.21 ± 6.60	2.11 ± 0.55
	120	26.84 ± 1.92	29.87 ± 2.31	9.93 ± 2.46	28.98 ± 3.64	4.03 ± 0.95
C-2041	**24**	8.43 ± 3.14	31.30 ± 2.48	34.76 ± 4.78	23.89 ± 5.34	1.78 ± 0.42
	72	17.43 ± 2.40	42.86 ± 1.87	16.09 ± 2.94	21.24 ± 2.69	2.52 ± 0.71
	120	36.47 ± 3.18	35.08 ± 3.65	11.59 ± 1.16	14.84 ± 4.06	2.30 ± 0.60
C-2045	**24**	9.84 ± 4.46	32.25 ± 3.01	26.56 ± 3.44	28.15 ± 2.14	1.78 ± 0.33
	72	18.66 ± 3.61	39.94 ± 4.65	18.53 ± 5.99	20.87 ± 5.18	2.05 ± 0.60
	120	42.42 ± 8.29	23.89 ± 3.28	9.03 ± 1.37	21.49 ± 5.96	2.28 ± 1.33
C-2053	**24**	6.28 ± 1.52	30.44 ± 2.27	33.77 ± 3.66	27.37 ± 2.20	2.76 ± 0.30
	72	22.37 ± 2.06	36.08 ± 5.84	14.09 ± 2.46	24.96 ± 3.05	1.73 ± 0.52
	120	41.51 ± 5.77	25.01 ± 3.97	10.78 ± 1.76	19.77 ± 6.06	2.39 ± 1.17