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SUPPLEMENTAL DATA

Butyl and isobutyl ortho-coumaric acid derivatives reduce self-renewal and MGMT expression in GBM cells with stem cell-like characteristics *in vitro*

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Full article is available at the following link: Butyl and isobutyl ortho-coumaric acid derivatives reduce self-renewal and MGMT expression in GBM cells with stem cell-like characteristics *in vitro*

Table S1. Inhibition of spheroid formation in U87-MG cells

Treatment	Estimated spheroids counts (EMM)	95% CI (lower-upper)
Untreated	45.3	38.3 – 53.6
TMZ	27.7	22.3 – 34.3
Butyl o-CA	26.7	21.4 – 33.2
Isobuthyl o-CA	30.3	24.7 – 37.3
Propyl o-CA	40.7	34.1 – 48.6
Ethyl o-CA	40.0	33.4 – 47.8
Methyl o-CA	37.0	30.7 – 44.6
Vehicle	42.3	35.6 – 50.4

Values were obtained through Poisson regression analysis. **Abbreviations**: EMM: Estimated marginal means (model-predicted spheroid counts); CI: Confidence interval.

Table S2. Inhibition of colony formation in U87-MG cells

Treatment	Estimated colony counts (EMM)	95% CI (lower-upper)
Untreated	81.9	72.3 – 92.8
TMZ	45.9	38.8 – 54.2
Butyl o-CA	21.9	17.2 – 27.9
Isobuthyl o-CA	14.8	11.0 – 19.8
Propyl o-CA	49.7	42.3 – 58.3
Ethyl o-CA	27.6	22.2 – 34.2
Methyl o-CA	29.1	23.6 – 35.9
Vehicle	74.2	65.1 – 84.6

Values were obtained through Poisson regression analysis. **Abbreviations**: EMM: Estimated marginal means (model-predicted spheroid counts); CI: Confidence interval.

Table S3. Inhibition of wound closure in U87-MG cells

Treatment	Estimated wound closure (%) (EMM)	95% CI (lower-upper)
Untreated	85.0	82.0 – 87.9
TMZ	63.3	59.3 – 67.3
Butyl o-CA	45.4	42.2 – 49.5
Isobutyl o-CA	71.3	67.6 – 75.1
Propyl o-CA	63.2	59.2 – 67.2
Ethyl o-CA	70.0	66.2 – 73.8
Methyl o-CA	69.0	65.2 – 72.8
Vehicle	83.3	80.0 – 86.4

Values were obtained from a beta regression model. **Abbreviations**: EMM: Estimated marginal probabilities (%); CI: Confidence interval.

Table S4. Impact of o-CA derivatives on cell cycle phases in U87-MG cells

Phase of cell cycle	Treatment	Estimated proportion of cells (%)	95% CI (lower-upper)
G0/G1	Untreated	65.35	64.76 – 65.95
	TMZ	45.68	45.03 – 46.33
	Butyl o-CA	80.40	79.79 – 81.0
	Isobutyl o-CA	77.02	76.47 – 77.57

	Propyl o-CA	77.20	76.61 – 77.79
	Ethyl o-CA	77.33	76.75 – 77.91
	Methyl o-CA	65.93	65.32 – 66.54
	Vehicle	65.14	64.5 – 65.78
S	Untreated	18.81	18.32 – 19.30
	TMZ	32.16	31.55 – 32.77
	Butyl o-CA	11.98	11.48 – 12.47
	Isobutyl o-CA	15.97	15.49 – 16.45
	Propyl o-CA	17.42	16.59 – 17.95
	Ethyl o-CA	15.35	14.85 – 15.85
	Methyl o-CA	17.19	16.70 – 17.37
	Vehicle	18.91	18.39 -19.44
G2/M	Untreated	15.84	15.38 – 16.30
	TMZ	22.16	21.62 – 22.70
	Butyl o-CA	7.63	7.22 – 8.03
	Isobutyl o-CA	7.01	6.68 – 7.35
	Propyl o-CA	5.38	5.06 – 5.70
	Ethyl o-CA	7.32	6.96 – 7.68
	Methyl o-CA	16.88	16.40 – 17.67
	Vehicle	15.95	15.45 – 16.44

Values were obtained from a multinomial regression model. **Abbreviations**: EMM: Estimated marginal probabilities (%); CI: Confidence interval.

Table S5. Expression of MGMT in U87-MG SLGCs

Treatment	Mean IF (NFU-log10)	95% CI (lower–upper)
Untreated	0.645	0.434 – -0.856
TMZ	0.686	0.475 – 0.897
Butyl o-CA	-0.467	-0.677 – -0.256
Isobutyl o-CA	0.689	0.478 / 0.899
Propyl o-CA	0.863	0.652 – 1.074
Ethyl o-CA	0.486	0.275 – 0.697
Methyl o-CA	0.250	0.039 – 0.461
Vehicle o-CA	0.697	0.486 – 0.908

Abbreviations: MGMT: O6-methylguanine DNA methyltransferase; U87-MG: U87-MG glioblastoma cell line; SLGCs: Stem-like glioblastoma cells; TMZ: Temozolomide; o-CA: ortho-coumaric acid; IF: Immunofluorescence; NFU: Normalized fluorescence units (log10-transformed); CI: Confidence interval; ANOVA: Analysis of variance.

Table S6. Expression levels of LC3-B in U87-MG SLGCs

Treatment	Mean IF (NFU-log10)	95% CI (lower/upper)
Untreated	-0.139	-0.269 – -0.008
TMZ	0.422	0.311 – 0.572
Butyl o-CA	0.203	0.072 – 0.331
Isobutyl o-CA	0.485	0.355 – 0.615
Propyl o-CA	0.786	0.655 – 0.916
Ethyl o-CA	0.552	0.422 – 0.682

Methyl o-CA	0.300	0.170 – 0.431
Vehicle o-CA	0.274	0.144 – 0.404

Abbreviations: LC3-B: Microtubule-associated protein 1 light chain 3B; U87-MG: U87-MG glioblastoma cell line; SLGCs: Stem-like glioblastoma cells; TMZ: Temozolomide; o-CA: ortho-coumaric acid; IF: Immunofluorescence; NFU: Normalized fluorescence units (log10-transformed); CI: Confidence interval; ANOVA: Analysis of variance.

Table S7. Expression levels of NeuN, CD133, GFAP and Nest in U87-MG SLGCs

Stemness markers	**Treatment**	**Mean IF (NFU-log10)**	**95% CI (low-upper)**
NeuN	Untreated	0.136	-0.092 – 0.366
	TMZ	0.019	-0.209 – 0.249
	Butyl o-CA	0.287	0.058 –0.516
	Isobutyl o-CA	0.363	0.134 – 0.593
	Propyl o-CA	0.306	0.076 – 0.535
	Ethyl o-CA	0.053	-0.176 – 0.282
	Methyl o-CA	0.063	-0.166 – 0.292
CD133	Untreated	0.590	0.361 – 0.819
	TMZ	-0.938	-0.577 – -0.119
	Butyl o-CA	-2.018	-2.247 – -1.788
	Isobutyl o-CA	-0.883	-1.112 – -0.653
	Propyl o-CA	-0.621	-0.850 – -0.392
	Ethyl o-CA	-1.387	-1.617 – -1.158
	Methyl o-CA	-0.938	-1.167 – -0.708

GFAP	Untreated	-0.035	-0.265 – 0.193
	TMZ	0.286	0.056 – 0.515
	Butyl o-CA	0.619	0.389 – 0.848
	Isobutyl o-CA	-0.143	-0.373 – 0.085
	Propyl o-CA	0.180	-0.048 – 0.409
	Ethyl o-CA	0.219	-0.009 – 0.449
	Methyl o-CA	0.371	0.141 – 0.600
Nest	Untreated	0.267	0.037 – 0.496
	TMZ	-2.241	-2.470 – -2.012
	Butyl o-CA	-0.770	-1.000 – -0.541
	Isobutyl o-CA	-0.826	-1.055 – -0.596
	Propyl o-CA	-1.488	-1.718 – -1.259
	Ethyl o-CA	-1.592	-1.821 – -1.362
	Methyl o-CA	-1.161	-1.390 – -0.931

Abbreviations: NeuN: Neuronal nuclei; GFAP: Glial fibrillary acidic protein; U87-MG: U87-MG glioblastoma cell line; SLGCs: Stem-like glioblastoma cells; TMZ: Temozolomide; o-CA: ortho-coumaric acid; IF: Immunofluorescence; NFU: Normalized fluorescence units (log10-transformed); CI: Confidence interval; ANOVA: Analysis of variance.

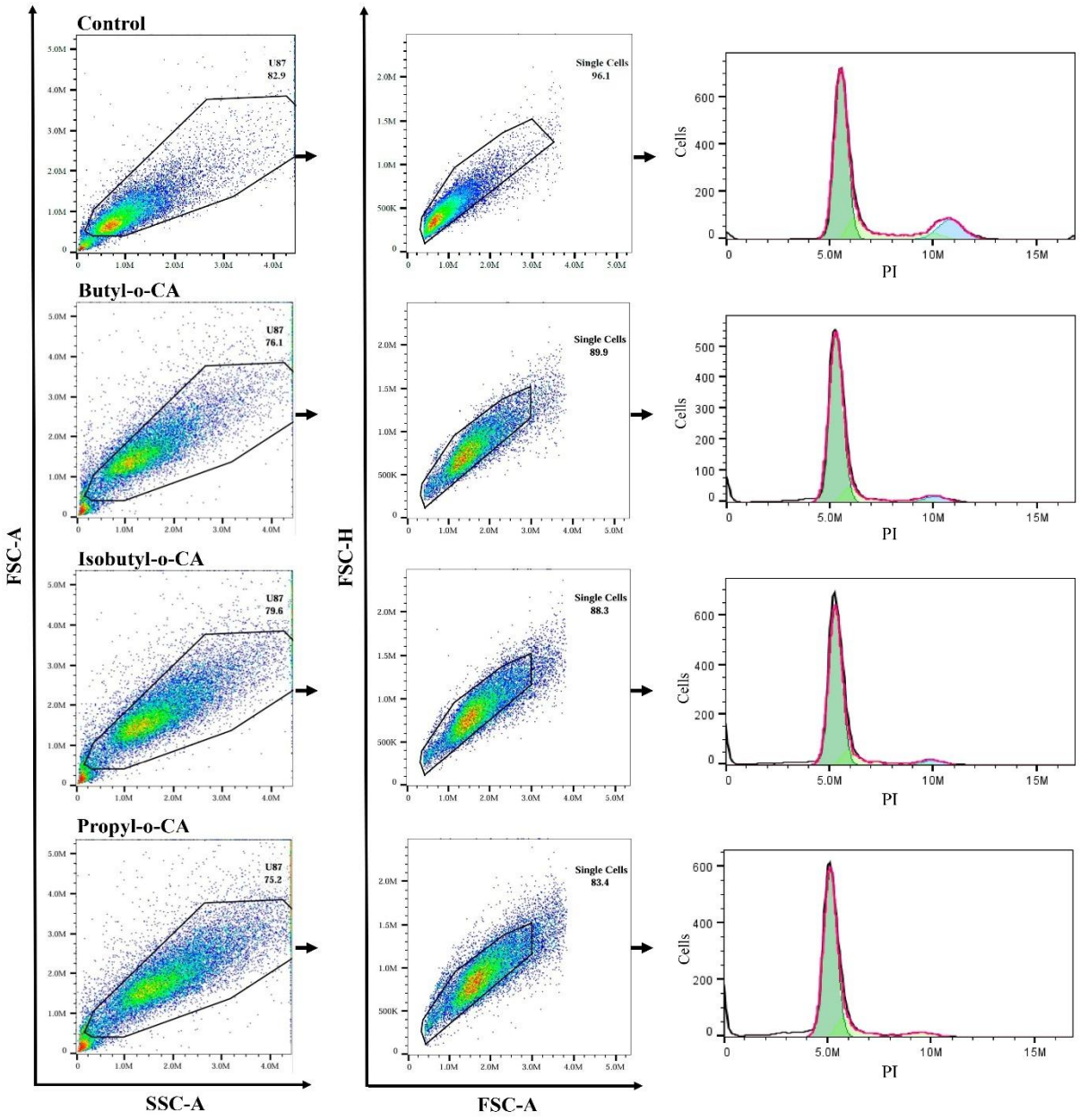

Figure S1. Flow cytometric analysis of the effects of o-CA derivatives (Butyl o-CA, Isobutyl o-CA, and Propyl o-CA) and untreated controls on the cell cycle in U87-MG cells. Dot plots illustrate the populations of U87-MG cells, while histograms depict the proportions of cells in each phase of the cell cycle (G0/G1, bright green; S phase, light green; G2 phase, light blue). Data are presented as mean fluorescence indices. **Abbreviations**: o-CA: ortho-coumaric acid; U87-MG: U87-MG glioblastoma cell line; G0/G1: Gap 0/Gap 1 phase; S: Synthesis phase; G2: Gap 2 phase; PI: Propidium iodide; FSC-A: Forward scatter area; FSC-H: Forward scatter height; SSC-A: Side scatter area.

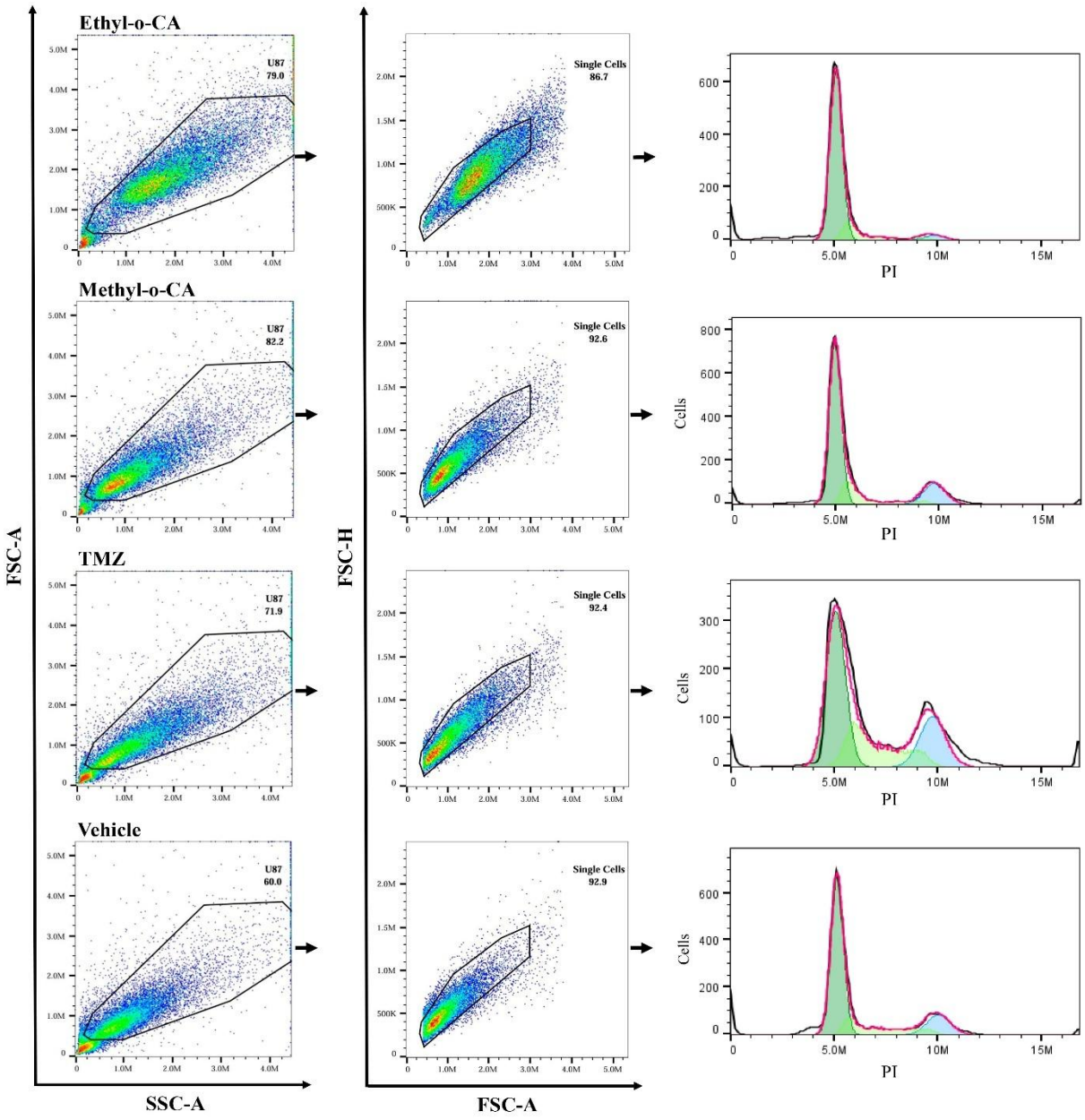

Figure S2. Flow cytometric analysis of the effects of o-CA derivatives (Ethyl o-CA and Methyl o-CA), TMZ, and vehicle control on the cell cycle in U87-MG cells. Dot plots depict U87-MG cell populations, while histograms illustrate the proportions of U87-MG cells in each phase of the cell cycle (G0/G1, bright green; S phase, light green; G2 phase, light blue). Data are expressed as mean fluorescence indexes. **Abbreviations**: o-CA: ortho-coumaric acid; TMZ: Temozolomide; U87-MG: U87-MG glioblastoma cell line; G0/G1: Gap 0/Gap 1 phase; S: Synthesis phase; G2: Gap 2 phase; PI: Propidium iodide; FSC-A: Forward scatter area; FSC-H: Forward scatter height; SSC-A: Side scatter area.

Graphical abstract

