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Supporting information

Structural differences in diarylheptanoids analogues from *Alnus viridis* and *Alnus glutinosa* influence their activity and selectivity towards cancer cells

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Spectroscopic data for new compound v4 isolated from *A. viridis*.

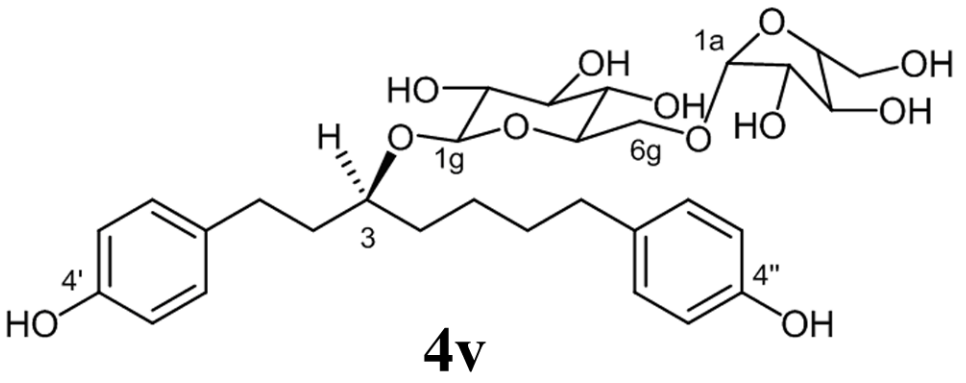
(3*S*)-1,7-di(4-hydroxyphenyl)-heptan-3-*O*-*α*-L-arabinofuranosyl(1→6)-*β*-D-glucopyranoside (**v4**): brown amorphous solid; $[\alpha]_D^{22}$-51.0 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 224 (3.83), 278 (3.55) nm; IR (MeOH) ν_{max} 3357, 2929, 1614, 1513, 1450, 1361, 1233, 1076, 1042 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR data, see Table S1; HR-ESI-MS *m/z* 593,2617 $[M - H]^-$ (calcd. for $C_{30}H_{42}O_{12}$-H, 593.2604).

Experimental procedure for isolation and structure elucidation. Optical rotations were measured on a Rudolph Research Analytical AUTOPOL IV automatic polarimeter. UV spectra were recorded using GBC Cintra 40 UV/VIS spectrometer. IR spectra were recorded at a ThermoScientific Nicolet 6700 FT-IR spectrometer using capilar film technique. All NMR spectra (^{1}H, ^{13}C, HSQC, HMBC) were recorded on a Bruker Avance III 500 spectrometer at 500.26 for ^{1}H and 125.80 MHz for ^{13}C, with CD_3OD as solvent and TMS as reference. Mass spectral (HR-ESI-MS) data were obtained from Agilent Technologies 6210 time-of-flight LC/MS system. For column chromatography (CC) silicagel 60 (SiO_2; under 0.063 mm, Merck) was used. Analytical TLC was carried out on silicagel 60 GF_{254} 20 × 20 cm plates, layer thickness 0.25 mm (Merck). Semipreparative HPLC was performed on an Agilent 1100 Series instrument equipped with DAD and using a Zorbax Eclipse XDB C-18 column (250 mm × 9.4 mm, 5 μm). All solvents used for column chromatography were freshly distilled and solvents for HPLC analysis were chromatographic grade.

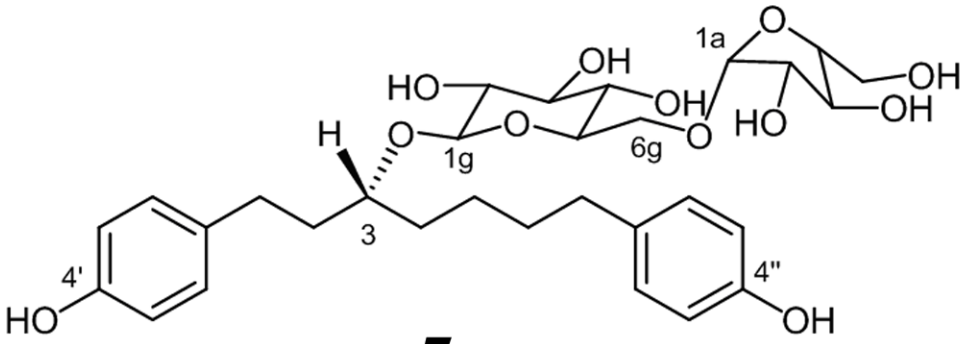
Plant Material. The plant material was collected at Stara planina in south-eastern Serbia in July 2010 and was identified by Professor Petar Marin, Faculty of Biology, University of Belgrade, Serbia. A voucher specimen No. 16681 was deposited at the Herbarium of the Institute of Botany and Botanical Garden 'Jevremovac', Faculty of Biology, University of Belgrade (BEOU), Serbia.

Extraction and isolation. The air-dried bark (150.0 g) was powdered and extracted with $CHCl_3$/MeOH 1:1 (4 × 1 l, 24 h) at room temperature, with the use of an ultrasonic bath in the last hour of extraction. The yield of the extraction was 22 %. The half of the crude extract (16.5 g) was fractionated by gradient CC (60 cm length and 5 cm diameter column size), starting elution with 100% CH_2Cl_2 and gradually increasing

polarity by addition of MeOH (up to 40% MeOH) to yield 200 fractions of approximately 15 ml. Similar fractions were combined after TLC (carried out with different CH_2Cl_2/MeOH solvent systems) and further fractionated by semipreparative HPLC-DAD into pure compounds using 0.025% HCOOH/MeCN elution system with flow rate of 4 ml/min and gradient program: 0-20 min, 15-25% MeCN; 20-25 min, 25-40% MeCN; 25-28 min, 40-70% MeCN; 28-31 min, 70-100%. The detection wavelength was 280 nm.



4v



7v

Figure S1. Chemical structures of compounds **4v** and **7v** from *A. viridis*.

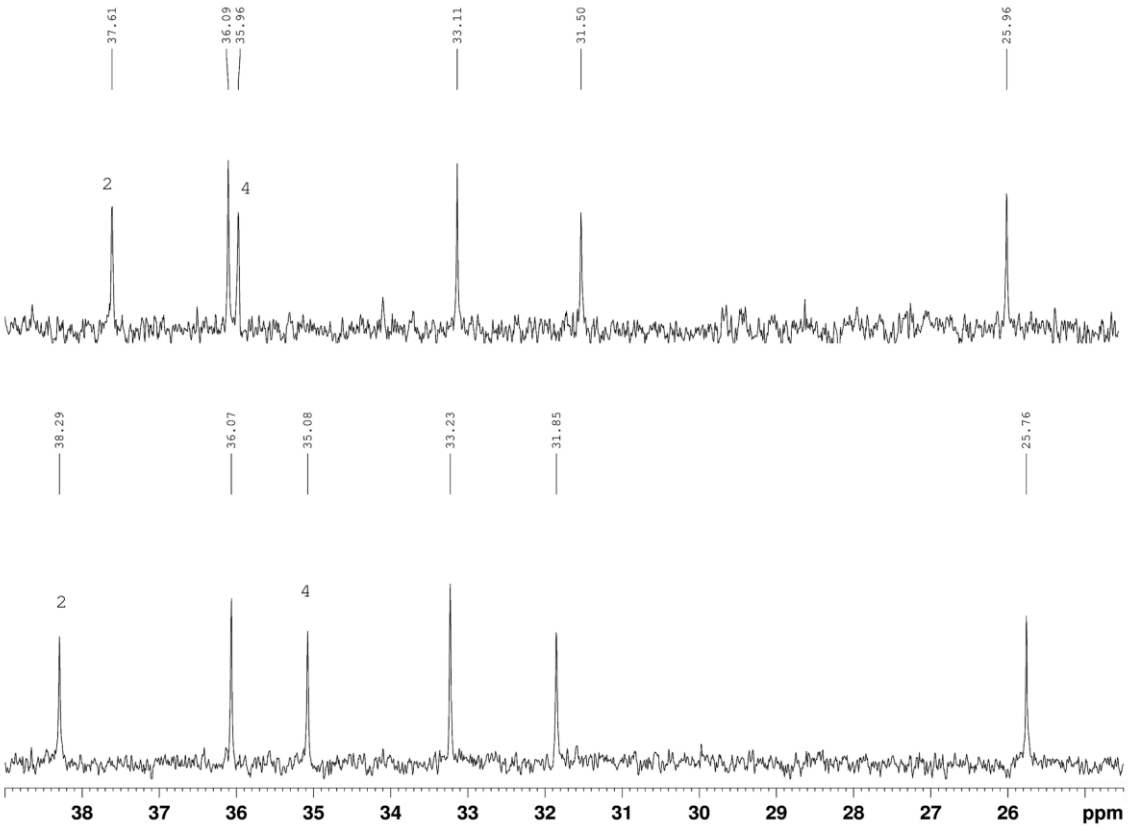


Figure S2. Heptane parts of the ^{13}C NMR spectra (CD_3OD) of **4v** (up) and **7v** (down).

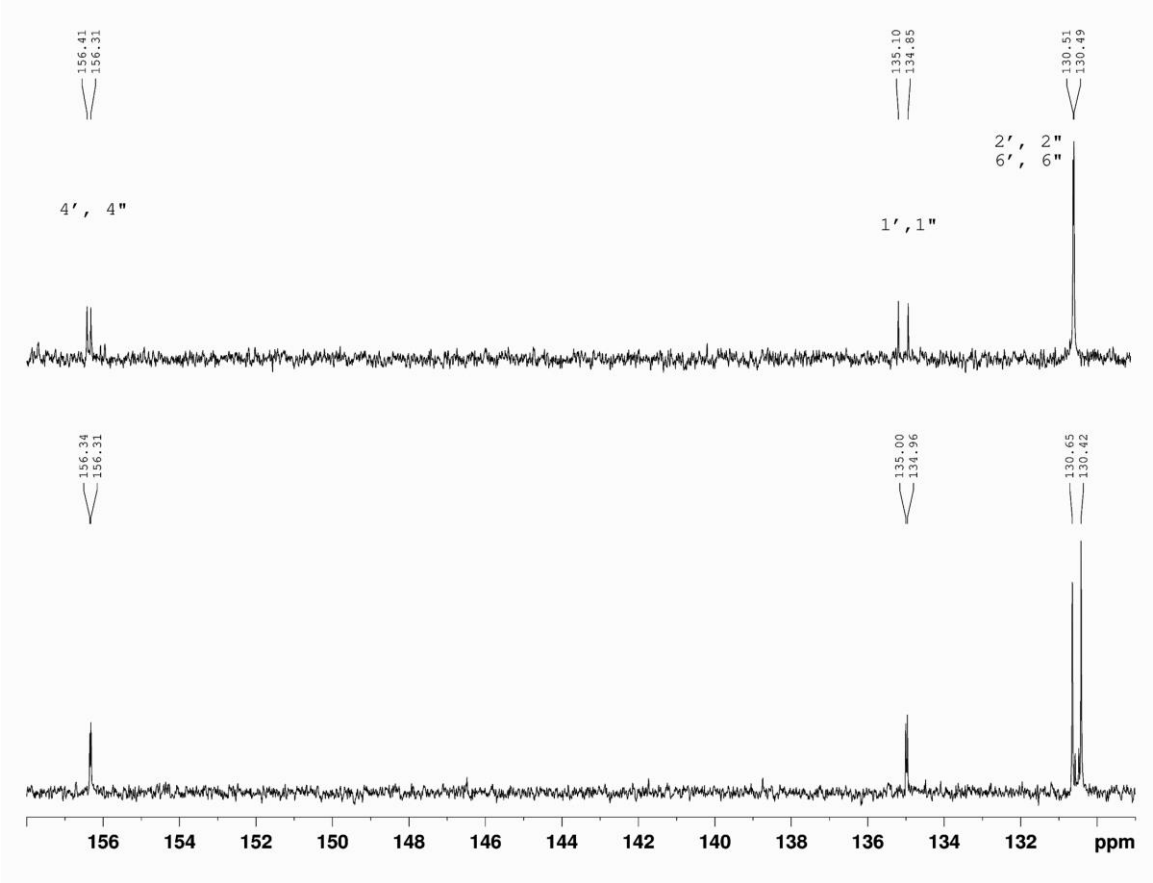


Figure S3. Aromatic parts of the ^{13}C NMR spectra (CD_3OD) of **4v** (up) and **7v** (down).

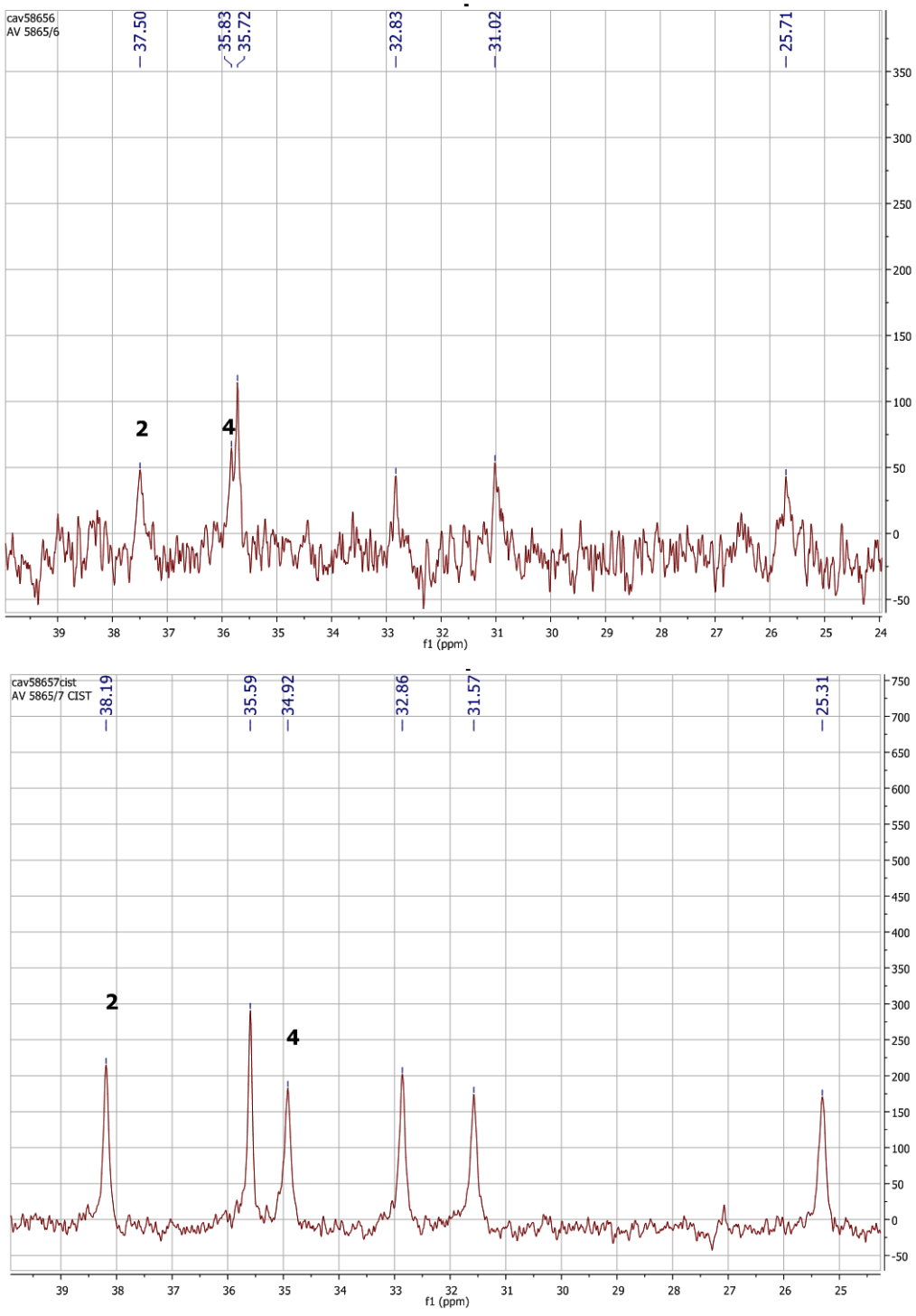


Figure S4. Heptane parts of the ^{13}C NMR spectra (pyridine-d5) of **4v** (up) and **7v** (down) used for comparing with (-)-centrolobol literature data.

Table S1. ^{13}C and ^{1}H NMR data (CD_3OD) for **4v** and **7v**.

C/H	**4v**		**7v**	
	δ_C, tip	δ_H (*J* u Hz)	δ_C, tip	δ_H (*J* u Hz)
1	31,5; CH_2	2,61 m	31,9; CH_2	2,60 m
2	37,6; CH_2	1,77 m	38,3; CH_2	1,75 m
3	80,4; CH	3,67 t (6,0)	80,2; CH	3,66 m
4	36,0; CH_2	1,59 m	35,1; CH_2	1,55 m; 1,61 m
5	26,0; CH_2	1,40 m	25,8; CH_2	1,39 m
6	33,1; CH_2	1,59 m	33,2; CH_2	1,54 m
7	36,1; CH_2	2,51 t (7,0)	36,1; CH_2	2,50 t (7,5)
1'	135,1; C	-	135,0; C	-
2'	130,5; CH	7,02 d (8,5)	130,6; CH	7,02 d (8,5)
3'	116,2; CH	6,68 d (8,5)	116,2; CH	6,69 d (8,5)
4'	156,4; C	-	156,3; C	-
5'	116,2; CH	6,68 d (8,5)	116,2; CH	6,69 d (8,5)
6'	130,5; CH	7,01 d (8,5)	130,6; CH	7,02 d (8,5)
1"	134,9; C	-	135,0; C	-
2"	130,5; CH	7,01 d (8,5)	130,4; CH	6,97 d (8,5)
3"	116,2; CH	6,68 d (8,5)	116,2; CH	6,68 d (8,5)
4"	156,3; C	-	156,3; C	-
5"	116,2; CH	6,68 d (8,5)	116,2; CH	6,68 d (8,5)
6"	130,5; CH	6,99 d (8,5)	130,4; CH	6,97 d (8,5)
Glc*p*				
1g	103,9; CH	4,29 d (8,0)	103,7; CH	4,29 d (8,0)
2g	75,5; CH	3,18 dd (8,0; 1,5)	75,4; CH	3,18 dd (8,0; 1,5)
3g	78,3; CH	3,34 m	78,2; CH	3,34 m
4g	72,3; CH	3,34 m	72,1; CH	3,34 m
5g	76,6; CH	3,36 m	76,6; CH	3,39 m
6g	68,3; CH_2	3,61 m 3,99 m	68,2; CH_2	3,64 dd (11,0; 5,5) 4,03 dd (11,0; 2,0)
Arab*f*				
1a	110,0; CH	4,96 d (1,0)	110,0; CH	4,97 d (1,0)
2a	83,2; CH	4,00 m	83,2; CH	4,01 dd (3,5; 1,5)
3a	79,1; CH	3,84 m	79,0; CH	3,84 dd (5,5; 3,5)
4a	86,2; CH	3,96 m	86,1; CH	3,96 m
5a	63,1; CH_2	3,58 m 3,72 dd (12,0; 3,5)	63,0; CH_2	3,59 dd (12,0; 5,5) 3,69 dd (12,0; 3,5)

Glc*p* – glucopyranosyl group; **Arab*f*** – arabinofuranosyl group

Table S2. Statistical analysis of growth inhibition activity of *A. viridis* and *A. glutinosa* diarylheptanoids in NCI-H460 and HaCaT cells.

NCI-H460													
	1v	**2v**	**3v**	**4v**	**5v**	**6v**	**7v**	**8v**	**9v**	**3g**	**5g**	**8g**	**9g**
1v	/	****	****	****	****	****	****	****	****	****	****	****	****
2v	****	/	****	****	****	****	*	****	****	****	****	****	****
3v	****	****	/	****	ns	****	****	ns	ns	ns	ns	ns	ns
4v	****	****	****	/	****	****	****	****	****	****	****	****	****
5v	****	****	ns	****	/	****	****	*	ns	ns	*	ns	ns
6v	****	****	****	****	****	/	****	****	****	****	****	****	****
7v	****	*	****	****	****	****	/	****	****	****	****	****	****
8v	****	****	ns	****	*	****	****	/	****	ns	ns	ns	ns
9v	****	****	ns	****	ns	****	****	****	/	ns	***	**	ns
3g	****	****	ns	****	ns	****	****	ns	ns	/	ns	ns	ns
5g	****	****	ns	****	*	****	****	ns	***	ns	/	ns	ns
8g	****	****	ns	****	ns	****	****	ns	**	ns	ns	/	ns
9g	****	****	ns	****	ns	****	****	ns	ns	ns	ns	ns	/
HaCaT													
	1v	**2v**	**3v**	**4v**	**5v**	**6v**	**7v**	**8v**	**9v**	**3g**	**5g**	**8g**	**9g**
1v	/	ns	****	ns	****	****	****	****	****	****	***	****	****
2v	ns	/	****	ns	****	ns	**	****	****	****	ns	****	****
3v	****	****	/	****	ns	****	****	****	ns	****	****	ns	****
4v	ns	ns	****	/	****	**	***	****	****	****	ns	****	****
5v	****	****	ns	****	/	****	****	****	ns	***	****	ns	****
6v	****	ns	****	**	****	/	ns	****	****	****	ns	****	****
7v	****	**	****	***	****	ns	/	****	****	****	ns	****	****
8v	****	****	****	****	****	****	****	/	****	ns	****	****	ns
9v	****	****	ns	****	ns	****	****	****	/	***	****	ns	****
3g	****	****	****	****	***	****	****	ns	***	/	****	***	ns
5g	***	ns	****	ns	****	ns	ns	****	****	****	/	****	****
8g	****	****	ns	****	ns	****	****	****	ns	***	****	/	****
9g	****	****	****	****	****	****	****	ns	****	ns	****	****	/

* $p<0.05$
** $p<0.01$
*** $p<0.001$
**** $p<0.0001$
ns = non significant

Table S3. Statistical analysis of cell death inducing activity of *A. viridis* and *A. glutinosa* diarylheptanoids in NCI-H460 and HaCaT cells.

NCI-H460									
		5v 15 μM	**5v 45 μM**	**5g 15 μM**	**5g 45 μM**	**9v 15 μM**	**9v 45 μM**	**9g 15 μM**	**9g 45 μM**
viable cells	**untreated**	ns	****	ns	****	*	****	ns	****
early apoptosis		ns	**	ns	ns	ns	ns	ns	***
late apoptosis		ns	ns	ns	*	ns	****	ns	**
necrosis		ns	ns	ns	*	ns	ns	ns	*
HaCaT									
		5v 15 μM	**5v 45 μM**	**5g 15 μM**	**5g 45 μM**	**9v 15 μM**	**9v 45 μM**	**9g 15 μM**	**9g 45 μM**
viable cells	**untreated**	***	****	ns	**	****	****	ns	****
early apoptosis		ns	ns	ns	ns	ns	ns	ns	ns
late apoptosis		ns	****	ns	ns	*	****	ns	****
necrosis		ns	****	ns	ns	ns	*	ns	***

* $p<0.05$
** $p<0.01$
*** $p<0.001$
**** $p<0.0001$
ns = non significant

Table S4. Statistical analysis of cell cycle arresting activity of *A. viridis* and *A. glutinosa* diarylheptanoids in NCI-H460 and HaCaT cells.

NCI-H460									
		5v 15 µM	**5v 45 µM**	**5g 15 µM**	**5g 45 µM**	**9v 15 µM**	**9v 45 µM**	**9g 15 µM**	**9g 45 µM**
subG$_0$	**untreated**	ns	**	ns	ns	*	*	ns	ns
G$_0$/G$_1$		ns	****	ns	ns	***	****	ns	ns
S		ns	ns	ns	ns	ns	ns	ns	ns
G$_2$/M		ns	ns	ns	ns	ns	ns	ns	ns
HaCaT									
		5v 15 µM	**5v 45 µM**	**5g 15 µM**	**5g 45 µM**	**9v 15 µM**	**9v 45 µM**	**9g 15 µM**	**9g 45 µM**
subG$_0$	**untreated**	*	*	ns	ns	ns	ns	ns	ns
G$_0$/G$_1$		ns	**	ns	ns	**	*	ns	**
S		ns	ns	ns	ns	ns	ns	ns	ns
G$_2$/M		*	ns	ns	ns	ns	ns	ns	ns

* $p<0.05$
** $p<0.01$
*** $p<0.001$
**** $p<0.0001$
ns = non significant