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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*.

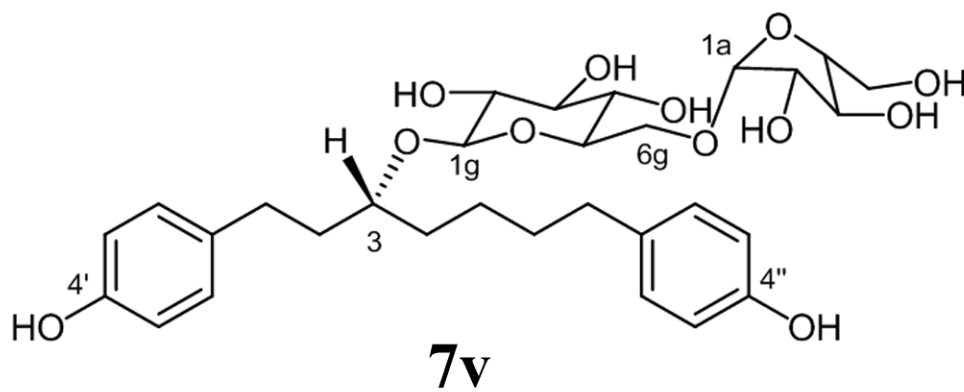
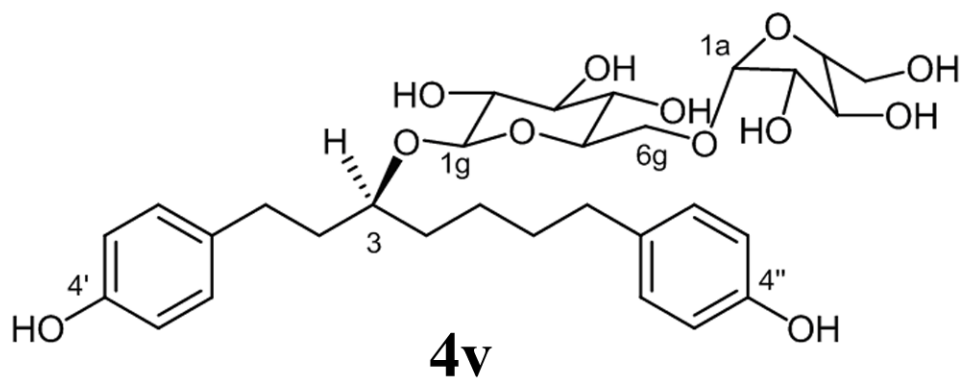
(3*S*)-1,7-di(4-hydroxyphenyl)-heptan-3-*O*- $\alpha$ -L-arabinofuranosyl(1 $\rightarrow$ 6)- $\beta$ -D-glucopyranoside (**v4**): brown amorphous solid;  $[\alpha]_D^{25}$ -51.0 (c 0.1, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 224 (3.83), 278 (3.55) nm; IR (MeOH)  $\nu_{\max}$  3357, 2929, 1614, 1513, 1450, 1361, 1233, 1076, 1042  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  data, see [Table S1](#); HR-ESI-MS  $m/z$  593,2617  $[\text{M} - \text{H}]^-$  (calcd. for  $\text{C}_{30}\text{H}_{42}\text{O}_{12}\text{-H}$ , 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 ( $^1\text{H}$ ,  $^{13}\text{C}$ , HSQC, HMBC) were recorded on a Bruker Avance III 500 spectrometer at 500.26 for  $^1\text{H}$  and 125.80 MHz for  $^{13}\text{C}$ , with  $\text{CD}_3\text{OD}$  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 ( $\text{SiO}_2$ ; under 0.063 mm, Merck) was used. Analytical TLC was carried out on silicagel 60 GF<sub>254</sub> 20  $\times$  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  $\times$  9.4 mm, 5  $\mu\text{m}$ ). All solvents used for column chromatography were freshly distilled and solvents for HPLC analysis were chromatographic grade.

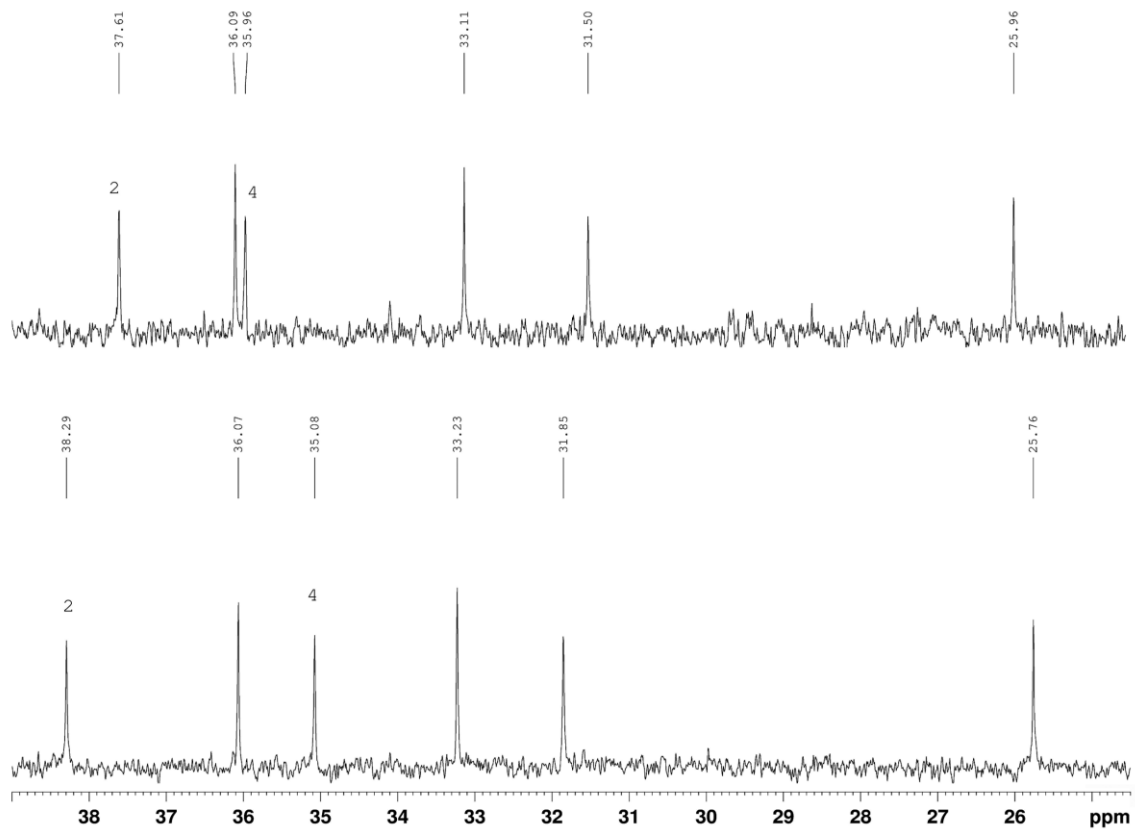
*Plant Material.* The plant material was collected at Stara planina in south-eastern Serbia in July 2010 and was identified by Professor Petar Marin, Faculty of Biology, University of Belgrade, Serbia. A voucher specimen No. 16681 was deposited at the Herbarium of the Institute of Botany and Botanical 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  $\text{CHCl}_3/\text{MeOH}$  1:1 (4  $\times$  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%  $\text{CH}_2\text{Cl}_2$  and gradually increasing

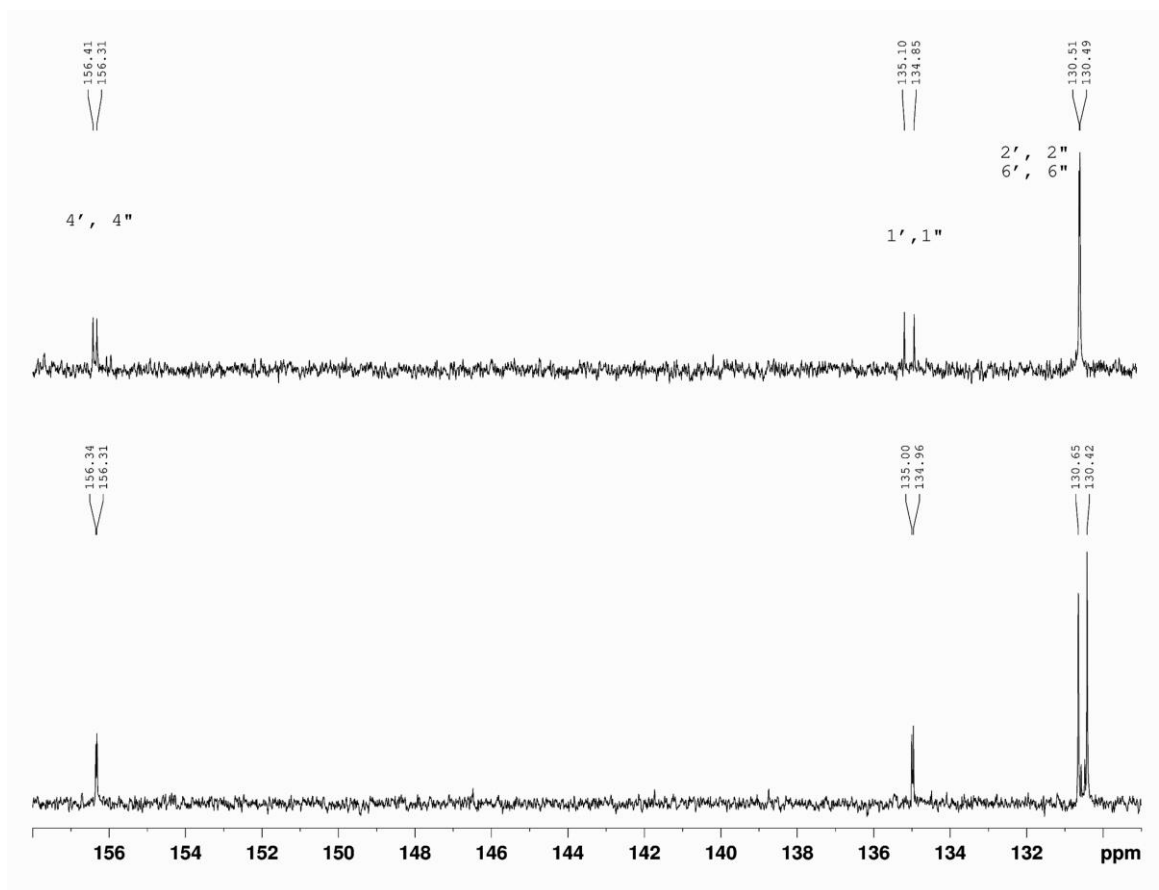
polarity by addition of MeOH (up to 40% MeOH) to yield 200 fractions of approximately 15 ml. Similar fractions were combined after TLC (carried out with different CH<sub>2</sub>Cl<sub>2</sub>/MeOH solvent systems) and further fractionated by semipreparative HPLC-DAD into pure compounds using 0.025% HCOOH/MeCN 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.



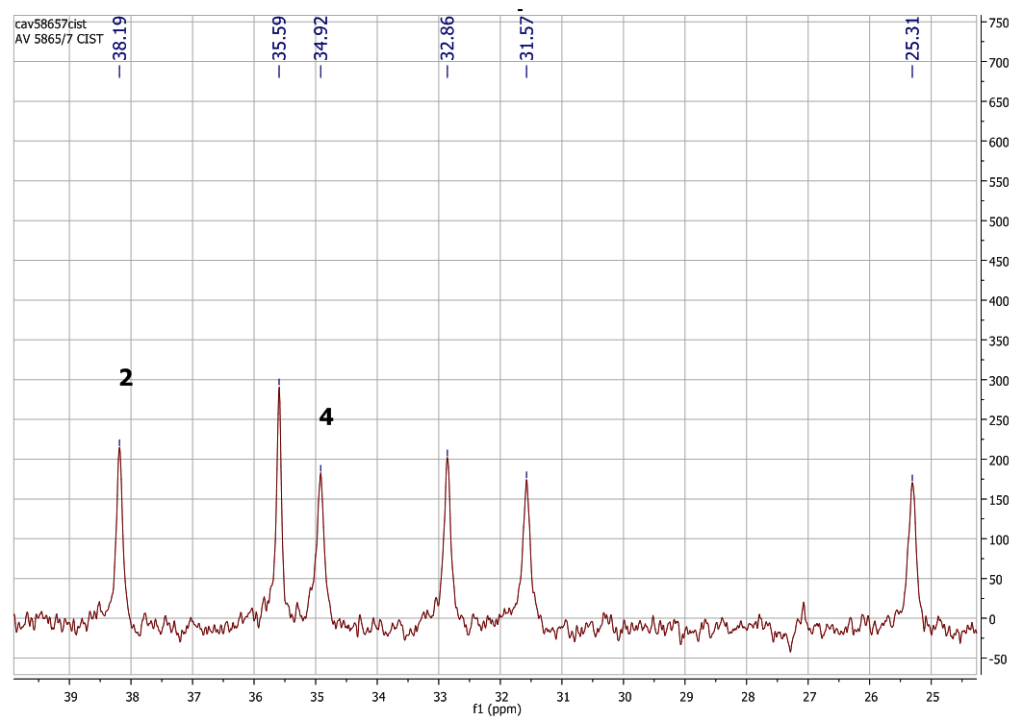
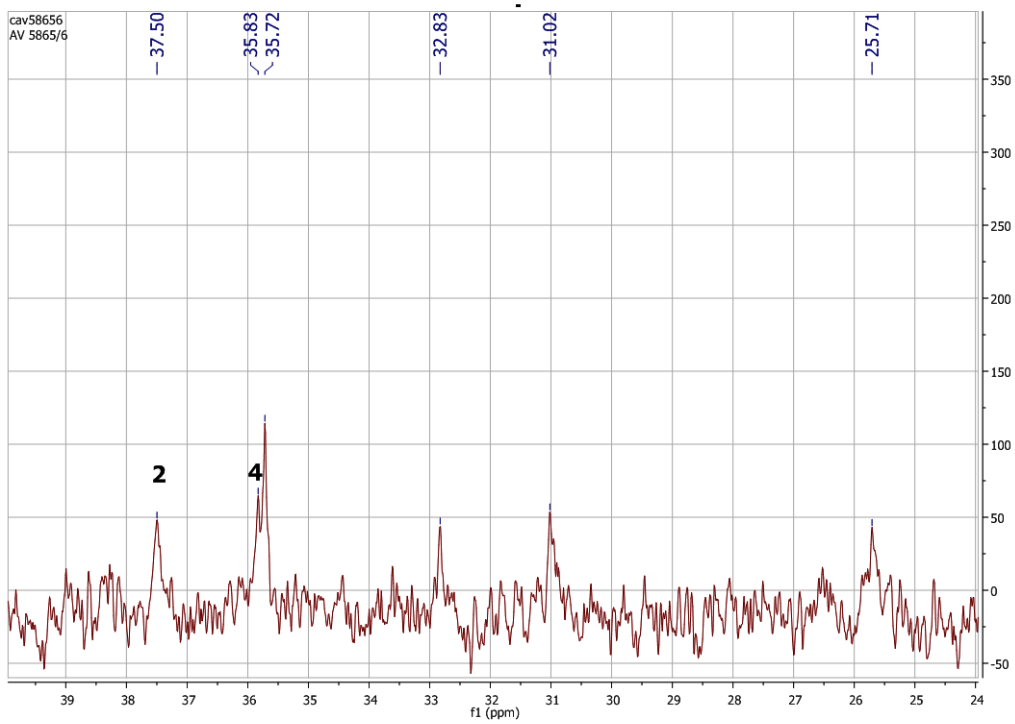
**Figure S1.** Chemical structures of compounds **4v** and **7v** from *A. viridis*.



**Figure S2.** Heptane parts of the  $^{13}\text{C}$  NMR spectra ( $\text{CD}_3\text{OD}$ ) of **4v** (up) and **7v** (down).



**Figure S3.** Aromatic parts of the  $^{13}\text{C}$  NMR spectra ( $\text{CD}_3\text{OD}$ ) of **4v** (up) and **7v** (down).



**Figure S4.** Heptane parts of the  $^{13}\text{C}$  NMR spectra (pyridine- $d_5$ ) of **4v** (up) and **7v** (down) used for comparing with (-)-centrololol literature data.



**Table S1.**  $^{13}\text{C}$  and  $^1\text{H}$  NMR data ( $\text{CD}_3\text{OD}$ ) for **4v** and **7v**.

C/H	<b>4v</b>		<b>7v</b>	
	$\delta_{\text{C}}$ , tip	$\delta_{\text{H}}$ (J u Hz)	$\delta_{\text{C}}$ , tip	$\delta_{\text{H}}$ (J u Hz)
1	31,5; CH <sub>2</sub>	2,61 m	31,9; CH <sub>2</sub>	2,60 m
2	37,6; CH <sub>2</sub>	1,77 m	38,3; CH <sub>2</sub>	1,75 m
3	80,4; CH	3,67 t (6,0)	80,2; CH	3,66 m
4	36,0; CH <sub>2</sub>	1,59 m	35,1; CH <sub>2</sub>	1,55 m; 1,61 m
5	26,0; CH <sub>2</sub>	1,40 m	25,8; CH <sub>2</sub>	1,39 m
6	33,1; CH <sub>2</sub>	1,59 m	33,2; CH <sub>2</sub>	1,54 m
7	36,1; CH <sub>2</sub>	2,51 t (7,0)	36,1; CH <sub>2</sub>	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)
<b>Glc<sub>p</sub></b>				
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 <sub>2</sub>	3,61 m 3,99 m	68,2; CH <sub>2</sub>	3,64 dd (11,0; 5,5) 4,03 dd (11,0; 2,0)
<b>Arab<sub>f</sub></b>				
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 <sub>2</sub>	3,58 m 3,72 dd (12,0; 3,5)	63,0; CH <sub>2</sub>	3,59 dd (12,0; 5,5) 3,69 dd (12,0; 3,5)

**Glc<sub>p</sub>** – glucopyranosyl group; **Arab<sub>f</sub>** – 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 $\mu$ M	5v 45 $\mu$ M	5g 15 $\mu$ M	5g 45 $\mu$ M	9v 15 $\mu$ M	9v 45 $\mu$ M	9g 15 $\mu$ M	9g 45 $\mu$ M
<b>viable cells</b>	<b>untreated</b>	ns	****	ns	****	*	****	ns	****
<b>early apoptosis</b>		ns	**	ns	ns	ns	ns	ns	***
<b>late apoptosis</b>		ns	ns	ns	*	ns	****	ns	**
<b>necrosis</b>		ns	ns	ns	*	ns	ns	ns	*
HaCaT									
		5v 15 $\mu$ M	5v 45 $\mu$ M	5g 15 $\mu$ M	5g 45 $\mu$ M	9v 15 $\mu$ M	9v 45 $\mu$ M	9g 15 $\mu$ M	9g 45 $\mu$ M
<b>viable cells</b>	<b>untreated</b>	***	****	ns	**	****	****	ns	****
<b>early apoptosis</b>		ns	ns	ns	ns	ns	ns	ns	ns
<b>late apoptosis</b>		ns	****	ns	ns	*	****	ns	****
<b>necrosis</b>		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 $\mu$ M	5v 45 $\mu$ M	5g 15 $\mu$ M	5g 45 $\mu$ M	9v 15 $\mu$ M	9v 45 $\mu$ M	9g 15 $\mu$ M	9g 45 $\mu$ M
subG <sub>0</sub>	untreated	ns	**	ns	ns	*	*	ns	ns
G <sub>0</sub> /G <sub>1</sub>		ns	****	ns	ns	***	****	ns	ns
S		ns	ns	ns	ns	ns	ns	ns	ns
G <sub>2</sub> /M		ns	ns	ns	ns	ns	ns	ns	ns
HaCaT									
		5v 15 $\mu$ M	5v 45 $\mu$ M	5g 15 $\mu$ M	5g 45 $\mu$ M	9v 15 $\mu$ M	9v 45 $\mu$ M	9g 15 $\mu$ M	9g 45 $\mu$ M
subG <sub>0</sub>	untreated	*	*	ns	ns	ns	ns	ns	ns
G <sub>0</sub> /G <sub>1</sub>		ns	**	ns	ns	**	*	ns	**
S		ns	ns	ns	ns	ns	ns	ns	ns
G <sub>2</sub> /M		*	ns	ns	ns	ns	ns	ns	ns

\* p<0.05

\*\* p<0.01

\*\*\* p<0.001

\*\*\*\* p<0.0001

ns = non significant