Supplementary Materials

Table S1. The characteristic spectral data for tested compounds **1**, **2** and **3**.

Compound	^{1}H NMR (600 MHz) δ_H [ppm]	^{13}C NMR (600 MHz) δ_C [ppm]
1	1.69 (s, 3H, H-30) 2.45 (m, 1H, H-19) 2.91 (s, 1H, C≡C) 3.21 (m, 1H, H-3) 4.02 (d, *J* = 10.8 Hz, H-28) 4.41 (d, *J* = 10.8 Hz, H-28) 4.61 (s, 1H, H-29) 4.71 (s, 1H, H-29)	19.11 (C-30) 47.66 (C-19) 64.90 (C-28) 74.67 (C≡C) 74.79 (C≡C) 78.98 (C-3) 110.03 (C-29) 149.92 (C-20) 153.31 (O-C=O)
2	1.69 (s, 3H, H-30) 2.45 (m, 1H, H-19) 2.55 (t, 1H, *J* = 2.4 Hz, C≡C) 3.21 (m, 1H, H-3) 3.98 (d, *J* = 10.8 Hz, H-28) 4.41 (d, *J* = 10.8 Hz, H-28) 4.61 (s, 1H, H-29) 4.71 (s, 1H, H-29) 4.75 (d, *J* = 2.4 Hz, 2H, OCH_2)	19.12 (C-30) 47.67 (C-19) 67.31 (C-28) 75.67 (C≡C) 78.97 (C-3) 109.97 (C-29) 149.99 (C-20) 155.10 (O-C=O)
3	1.69 (s, 3H, H-30) 2.21 (m, 2H, OCH_2CH_2) 2.53 (t, 1H, *J* = 2.4 Hz, C≡C) 3.01 (m, 1H, H-19) 3.18 (m, 1H, H-3) 4.16 (m, 2H, OCH_2CH_2) 4.59 (s, 1H, H-29) 4.73 (s, 1H, H-29)	19.06 (C-30) 47.02 (C-19) 69.75 (C≡C) 79.00 (C-3) 109.57 (C-29) 150.57 (C-20) 175.92 (C-28)

Ad.2

The first step was to calculate the retardation factor (R_f). This parameter defines the ratio of the length of the path traveled by the substance a to the length of the path of the mobile phase front b (in cm).

$$R_f = \frac{a}{b}$$

The obtained values of retardation factor (R_f) were converted to R_M parameters according to equation:

$$R_M = log\frac{1}{R_f} - 1$$

The R_M parameter was calculated for every concentration of acetone and extrapolated to zero concentration of organic component in the mobile phase. The chromatographic parameter of lipophilicity (R_{M0}) was calculated using equation:

$$R_M = R_{M0} + bc$$

where C is the concentration of acetone in the mobile phase, while b is the slope of the regression plot.

Table S2. R_{M0}, b, R parameters for betulin and compounds **1**-**3** in acetone: Tris buffer aqueous solution

Substance	R_{M0}	b	R
Betulin	3,765	-0,062	0,988
1	5,763	-0,064	0,992
2	6,413	-0,073	0,983
3	6,197	-0,07	0,984

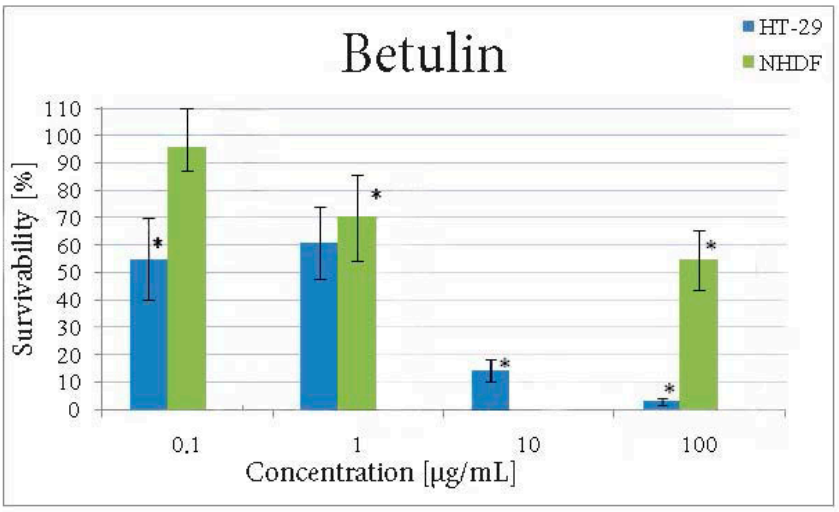

Figure S1. Viability of HT-29, NHDF after exposure to **betulin** at concentrations of 0.1 µg/mL; 1 µg/mL; 10 µg/mL; 100 µg/mL compared to control cells (0 µM). The absorbance value obtained in control cell cultures was taken as 100%. Cell survival is shown as a percentage of control values ± standard deviation of % cell growth. ANOVA, post hoc Tukey;

* - statistical significance, $p<0.05$

- no result for concentration 10 µg/mL on the NHDF cell line

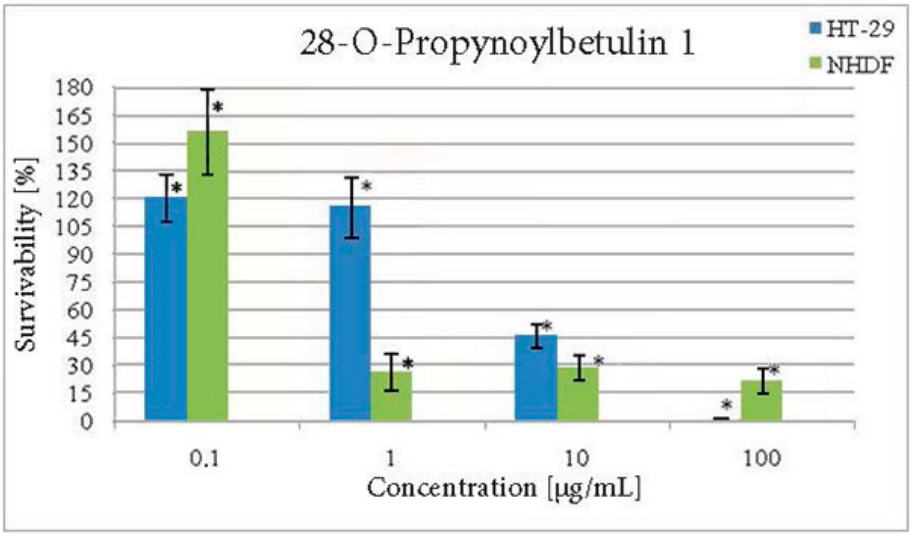

Figure S2. Viability of HT-29, NHDF after exposure to 2-O-Propynoylbetulin **1** at concentrations of 0.1 µg/mL; 1 µg/mL; 10 µg/mL; 100 µg/mL compared to control cells (0 µM). The absorbance value obtained in control cell cultures was taken as 100%. Cell survival is shown as a percentage of control values ± standard deviation of % cell growth. ANOVA, post hoc Tukey;

* - statistical significance, $p<0.05$

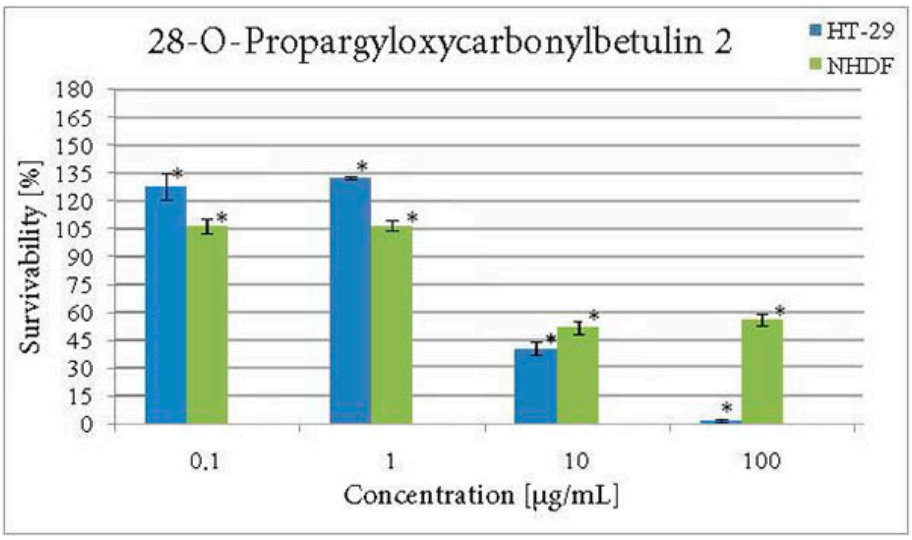

Figure S3. Viability of HT-29, NHDF after exposure to 28-O-propargyloxycarbonylbetulin **2** at concentrations of 0.1 µg/mL; 1 µg/mL; 10 µg/mL; 100 µg/mL compared to control cells (0 µM). The absorbance value obtained in control cell cultures was taken as 100%. Cell survival is shown as a percentage of control values ± standard deviation of % cell growth. ANOVA, post hoc Tukey;

* - statistical significance, $p<0.05$

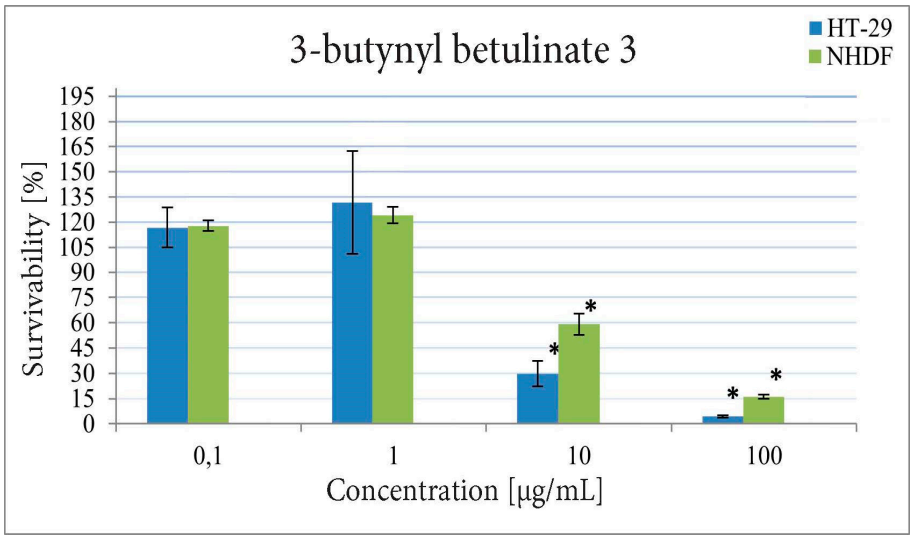

Figure S4. Viability of HT-29, NHDF after exposure to betulinic acid 3-butynyl ester **3** at concentrations of 0.1 µg/mL; 1 µg/mL; 10 µg/mL; 100 µg/mL compared to control cells (0 µM). The absorbance value obtained in control cell cultures was taken as 100%. Cell survival is shown as a percentage of control values ± standard deviation of % cell growth. ANOVA, post hoc Tukey;

* - statistical significance, $p<0.05$

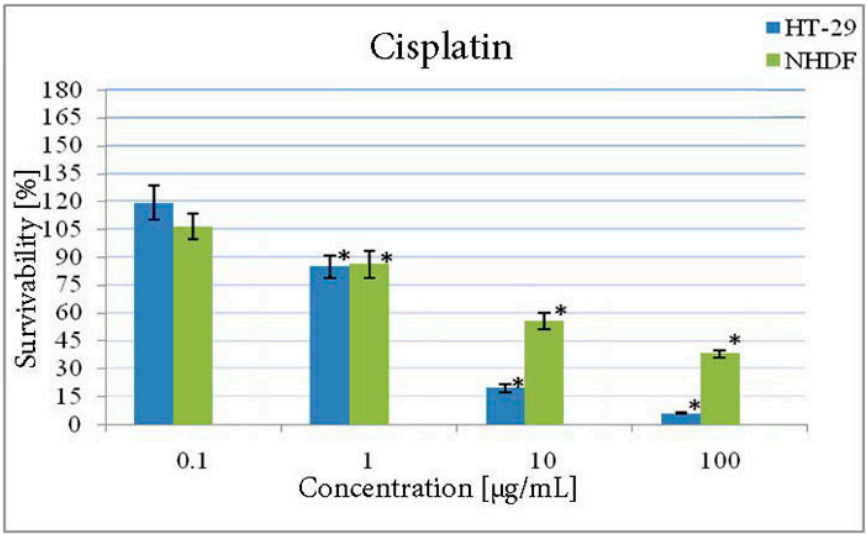

Figure S5. Viability of HT-29, NHDF after exposure to cisplatin at concentrations of 0.1 µg/mL; 1 µg/mL; 10 µg/mL; 100 µg/mL compared to control cells (0 µM). The absorbance value obtained in control cell cultures was taken as 100%. Cell survival is shown as a percentage of control values ± standard deviation of % cell growth. ANOVA, post hoc Tukey;

* - statistical significance, $p<0.05$