

## Supplementary materials

# The Influence of Betulin and Its Derivatives on Selected Colorectal Cancer Cell Lines' Viability and Their Antioxidant Systems

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## Experimental

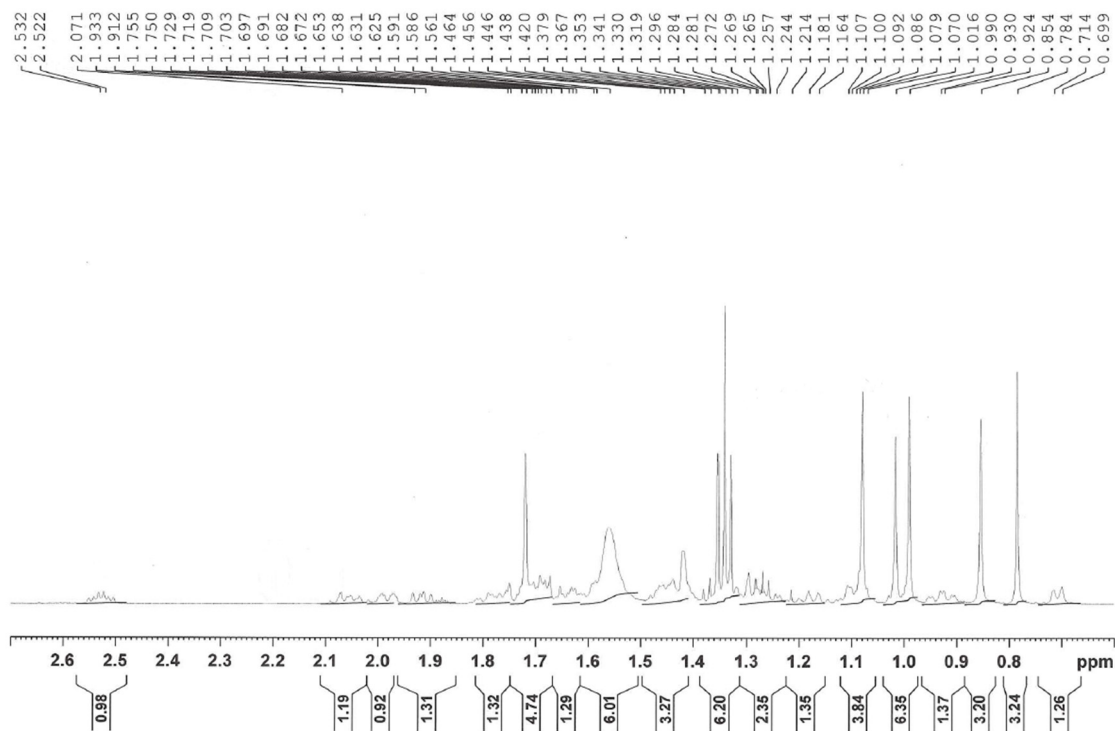
### Synthesis of compound TR50

28-O-Propynoylbetulin (EB5) (0.1 g, 0.203 mmol), copper(I) iodide (0.004 g, 0.020 mmol) and 3.5 ml of dry toluene were placed in a round-bottom flask. Then 0.213 mmol of diethyl (azidomethyl)phosphonate was added. The reaction mixture was stirred at 50°C for 72 hours. The course of the reaction was monitored using TLC chromatography. The spots in the chromatogram were visualized by spraying with a 10% solution of sulfuric acid in ethanol and then heated at 110°C for 3 minutes. After evaporating the solvent from the reaction mixture, the obtained crude product was purified by column chromatography using a mixture of chloroform : ethanol (15:1 v/v).

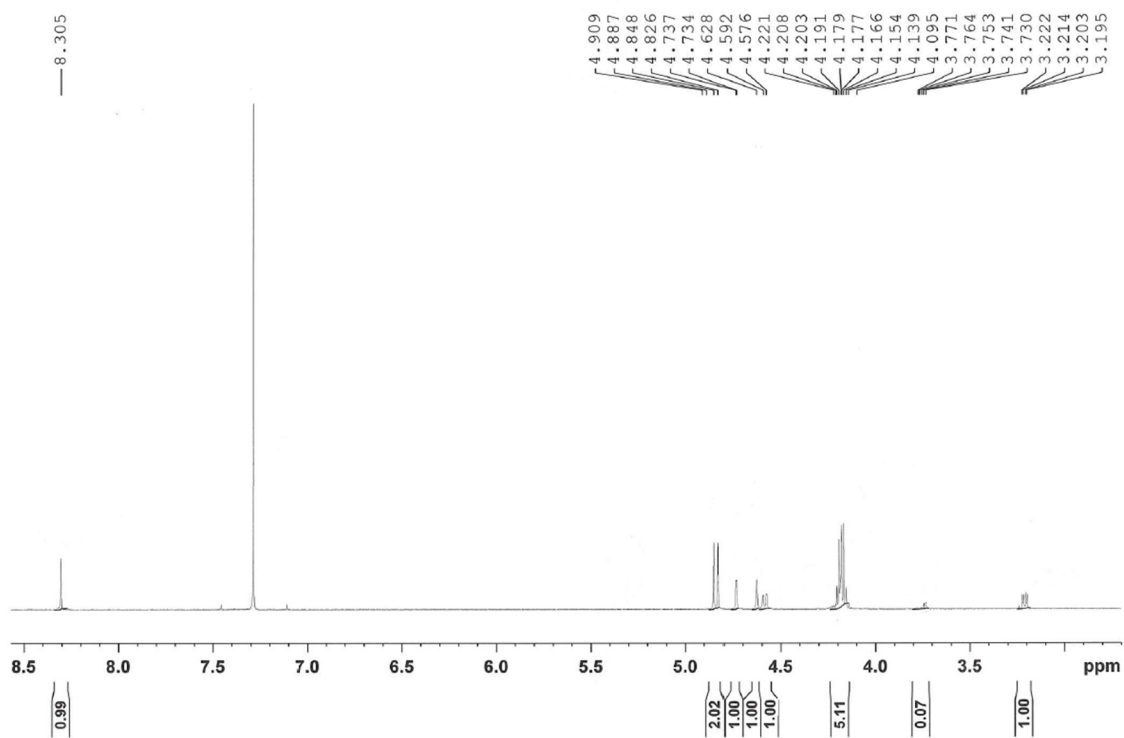
### Compound TR50

Yield 75%; mp 115-117°C;  $R_f$  0.43 (chloroform/ethanol, 15:1, v/v);

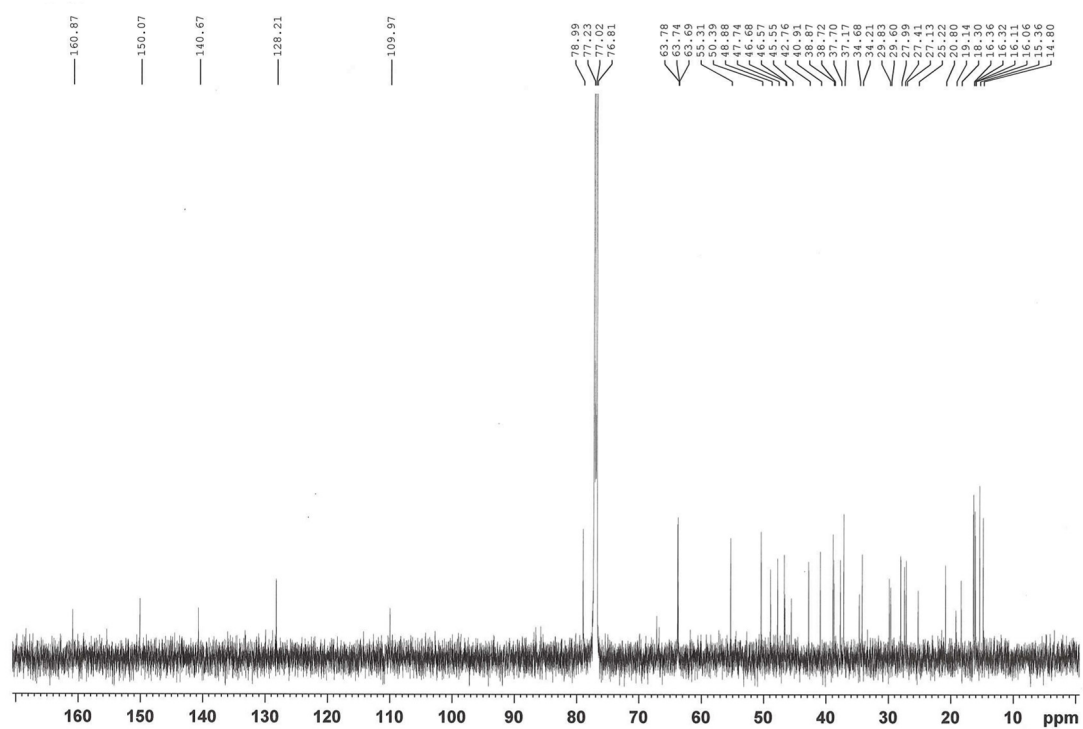
$^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$ : 0.78 (s, 3H,  $\text{CH}_3$ ), 0.85 (s, 3H,  $\text{CH}_3$ ), 0.99 (s, 3H,  $\text{CH}_3$ ), 1.02 (s, 3H,  $\text{CH}_3$ ), 1.08 (s, 3H,  $\text{CH}_3$ ), 1.34 (2 x t, 6H, 2 x  $\text{CH}_3$ ), 1.72 (s, 3H,  $\text{CH}_3$ ), 0.69-2.07 (m, 26H, CH,  $\text{CH}_2$  from the basic lupane system) 2.52 (m, 1H, H-19), 3.21 (m, 1H, H-3), 4.18 (m, 5H, 2x  $\text{OCH}_2$ , H-28), 4.59 (m, 1H, H-28), 4.73 (bs, 1H, H-29), 4.84 (bs, 1H, H-29), 4.90 (d,  $J$  = 13.2 Hz, 2H,  $\text{NCH}_2$ ), 8.30 (s, 1H, C-H triazol);  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$ : 14.8, 15.4, 16.06, 16.1, 16.3, 16.4, 18.3, 19.1, 20.8, 25.2, 27.1, 27.4, 28.0, 29.6, 29.8, 34.2, 34.7, 37.2, 37.7, 38.7, 38.9, 40.9, 42.8, 45.5, 46.6, 46.7, 47.7, 48.9, 50.4, 55.3, 63.6, 63.7, 63.8, 80.0, 110.0, 128.2, 140.67, 150.1, 160.9; IR ( $\nu_{\text{max}}$   $\text{cm}^{-1}$ , KBr): 1244-1201, 1456, 1544, 1724, 2945, 3442;  $^{31}\text{P}$ -NMR (243 MHz,  $\text{CDCl}_3$ )  $\delta$ : 14.97; HRMS (ESI)  $m/z$  (positive):  $\text{C}_{38}\text{H}_{63}\text{N}_3\text{O}_6\text{P}$   $[\text{M}+\text{H}]^+$  Calculated 688.4454, Found 688.4458;  $\text{C}_{38}\text{H}_{62}\text{N}_3\text{O}_6\text{PNa}$   $[\text{M}+\text{Na}]^+$  Calculated 710.4274, Found 710.4279;  $\text{C}_{38}\text{H}_{62}\text{N}_3\text{O}_6\text{PK}$   $[\text{M}+\text{K}]^+$  Calculated 726.4013, Found 726.4021.



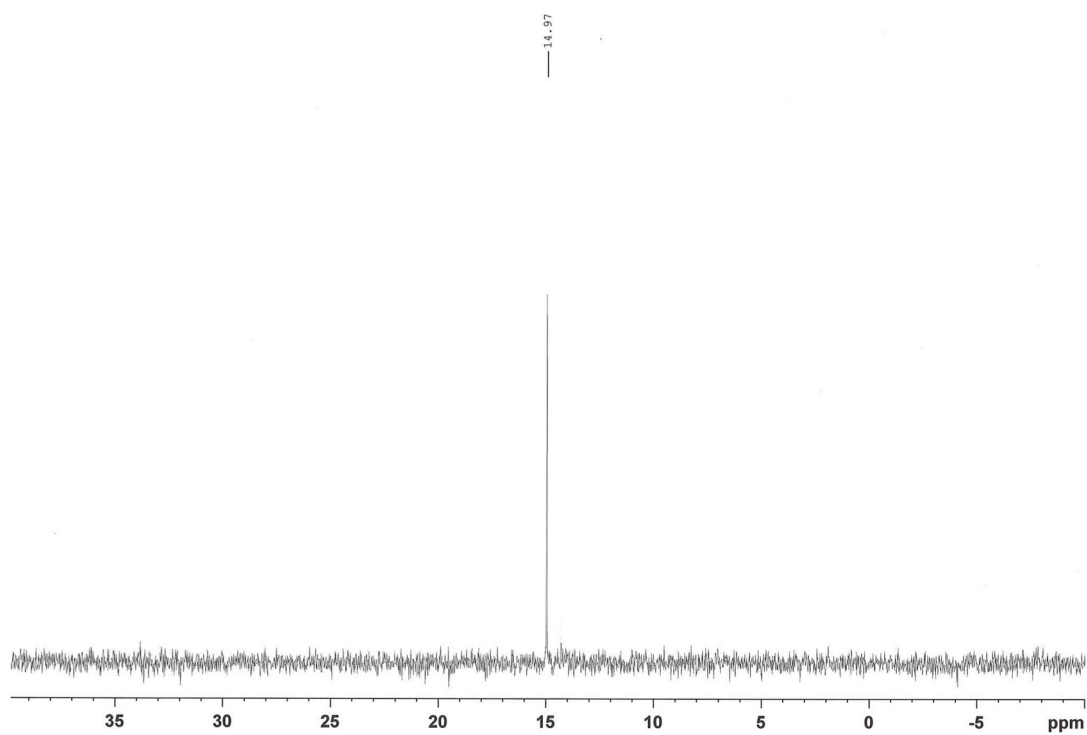
**Figure S1A.**  $^1\text{H}$  NMR spectrum of compound TR50 in the range of 0.7-2.53 ppm.



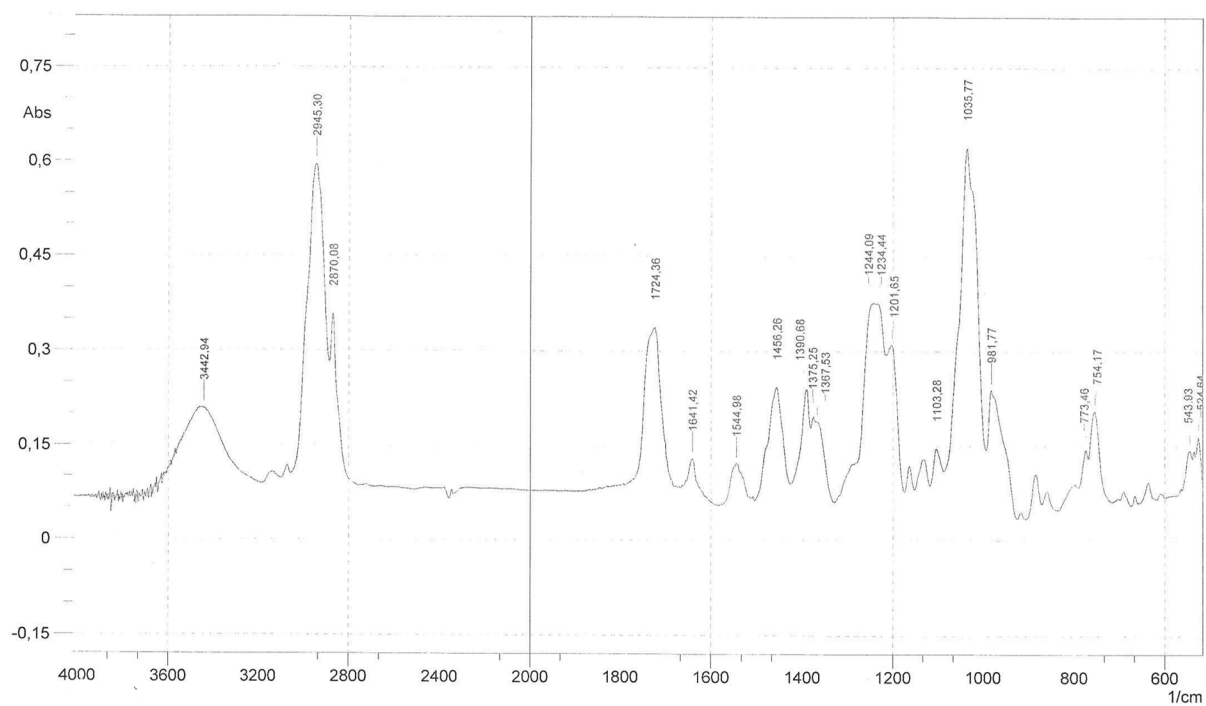
**Figure S1B.**  $^1\text{H}$  NMR spectrum of compound TR50 in the range of 3.19-8.30 ppm.



**Figure S2.**  $^{13}\text{C}$  NMR, compound TR50.



**Figure S3.**  $^{31}\text{P}$  NMR, compound TR50.



**Figure S4.** IR, compound TR50.

## Mass Spectrum List Report

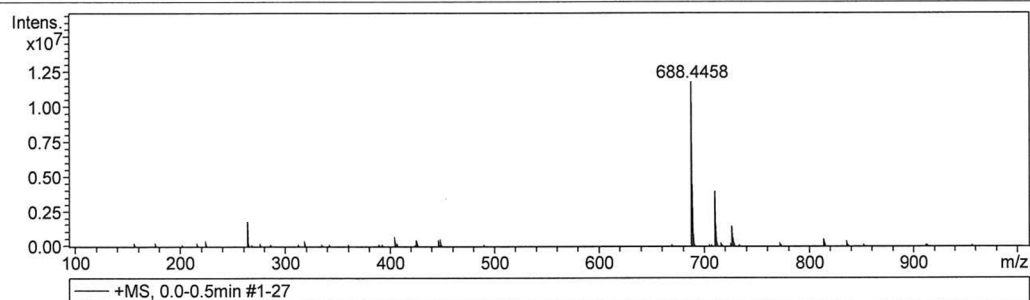
### Analysis Info

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Method low\_mass\_positive.m  
Sample Name cf3  
Comment

Acquisition Date 12/8/2023 12:57:46 PM  
Operator KM  
Instrument impact II 1825265.10082

### Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.3 Bar
Focus	Active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	3.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C



#	m/z	Res.	S/N	I	I %	FWHM
1	688.4458	53202	24553.9	11720679	100.0	0.0129
2	710.4279	45292	8262.0	4041695	34.5	0.0157
3	726.4021	35844	2950.8	1462067	12.5	0.0203

Figure S5. HRMS (ESI), compound TR50.