Supporting Information
For

An Ursolic Acid-derived Small Molecule Triggers Cancer Cell Death through Hyperstimulation of Macropinocytosis

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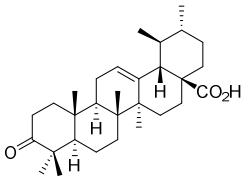
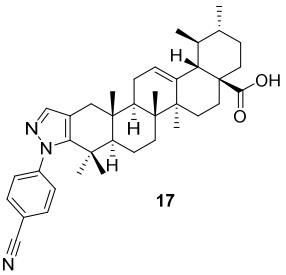
1. Structure of ursolic acid (UA) and compound 17



Ursolic acid (UA)



17

2. HPLC data on tested compounds and HPLC traces of compound 17

Analytical HPLC data on tested compounds was confirmed to be >95% (Table 2) as determined by HPLC analysis. HPLC analysis was conducted according to the following method with the retention time expressed in min at UV detection of 210 nm. For HPLC method, an Agilent 1260 series HPLC instrument was used, with chromatography performed on an agilent Eclipse Plus C18 column (100 x 4.6 mm, 3.5 μM) with mobile phase gradient of 50-99% CH_3CN in H_2O (Table 1), with a flow rate of 1.0 mL/min.

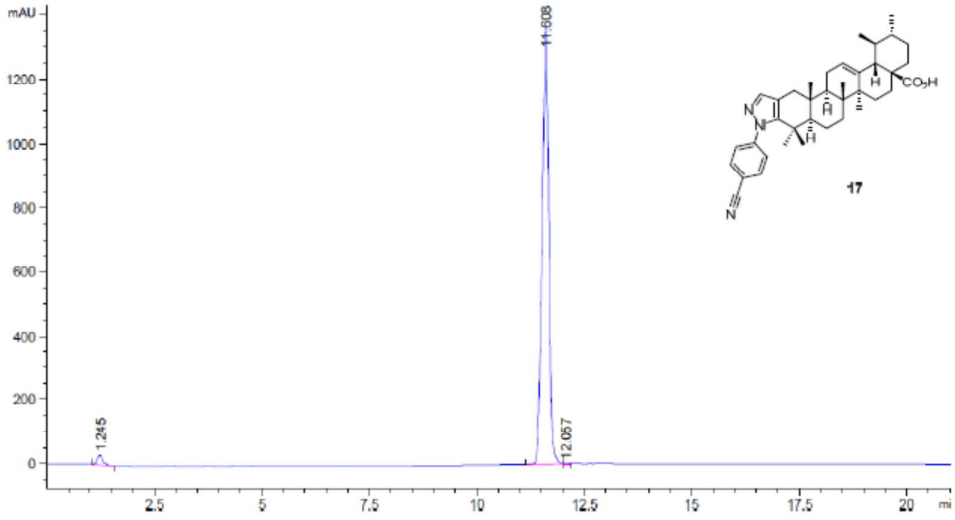
Table 1. HPLC conditions

Time (min)	H_2O (V%)	CH_3CN (V%)
0	50	50
25	1	99
30	1	99
32	50	50

Table 2. HPLC purity data on tested compounds

Compound	UV (nm)	RT (min)	Purity (%)	Compound	UV (nm)	RT (min)	Purity (%)
5	210	12.324	95.2%	**18**	210	17.272	95.6%
6	210	8.390	97.7%	**19**	210	20.865	98.8%
8	210	22.810	96.2%	**20**	210	20.729	98.4%
11	210	18.199	96.5%	**21**	210	20.918	97.4%
12	210	5.133	95.1%	**22**	210	21.732	96.2%
17	210	19.003	97.5	**23**	210	18.981	97.1%

Figure 1. HPLC traces of compound **17**



1	1.245	VB	0.1349	284.09592	29.87693	1.9013
2	11.608	BV	0.1632	1.46237e4	1362.42847	97.8678
3	12.057	VV	0.1343	34.50672	3.85880	0.2309
总量 :				1.49423e4	1396.16419	

3. Supplemental Figures

Figure S1. HeLa cells were treated with 20 μM UA derivatives for 24 h, followed by imaging with phase-contrast microscopy.

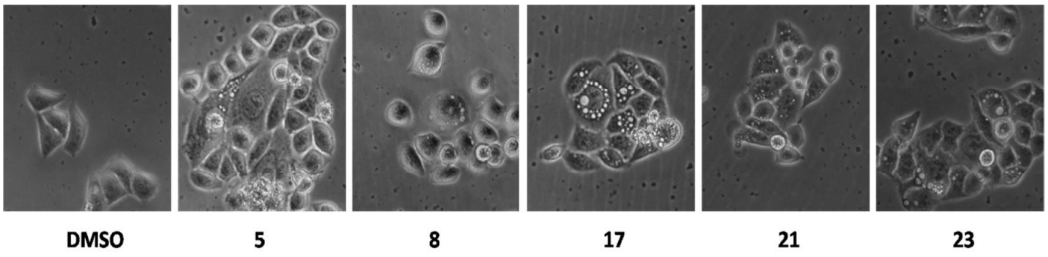


Figure S2. Cancer cells were treated with **17** at 20 μM for 24 h, 48 h and 72 h, followed by CCK-8 assay.

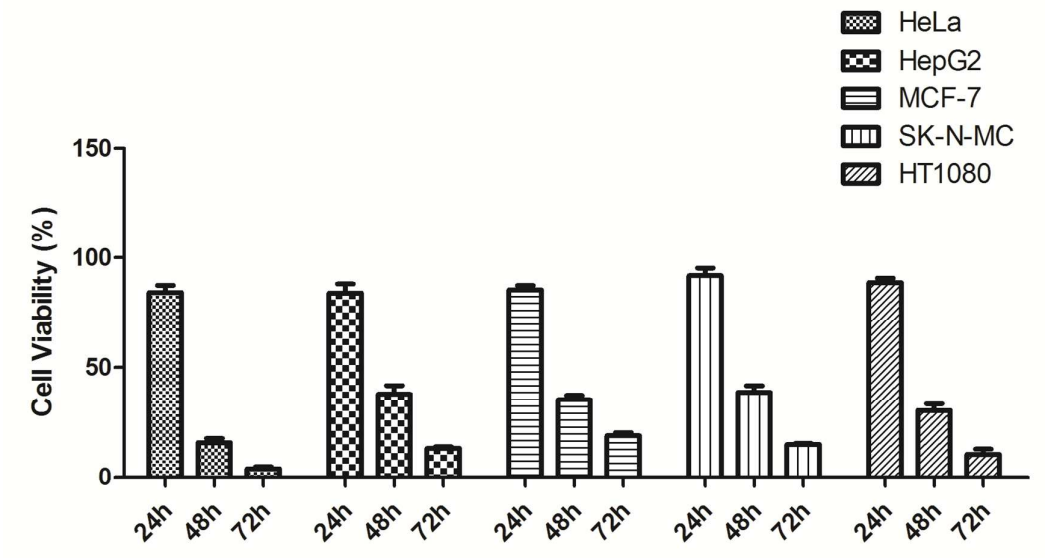


Figure S3. HeLa cells were treated with different concentrations of **17** or 0.1% DMSO for 2 days and colony forming assays were performed.

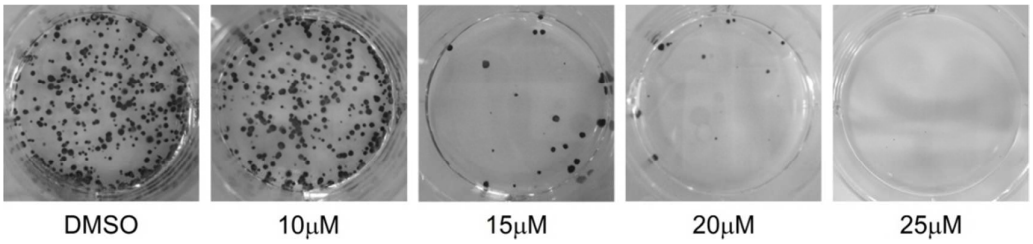


Figure S4. HeLa cells were incubated with indicated concentrations of **17** for 24 h or 48 h. The cytotoxicity of **17** was assessed with Alexa Fluor® 488 annexin V/Dead Cell Apoptosis Kit by flow cytometry.

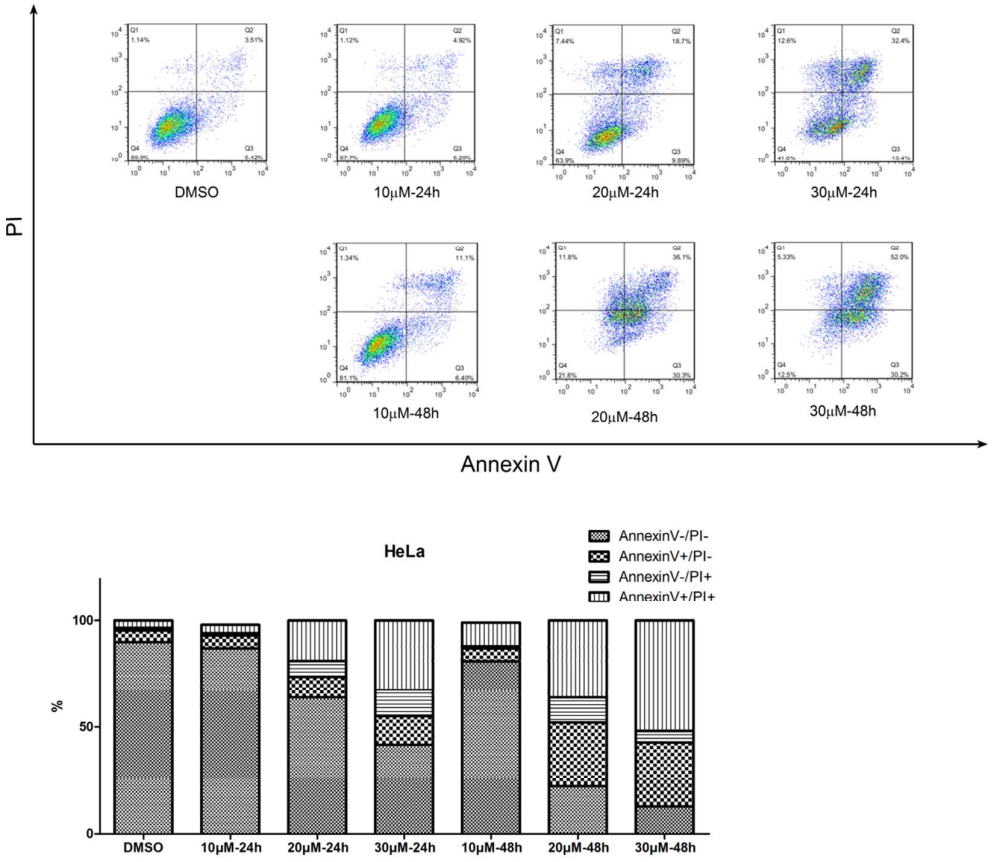


Figure S5. Effect of **17** on caspase activation and PARP cleavage. HeLa cells were treated for 24 h or 48 h with the indicated concentration of **17**. The cells were harvested and caspase 3 and PARP expression were determined by western blot.

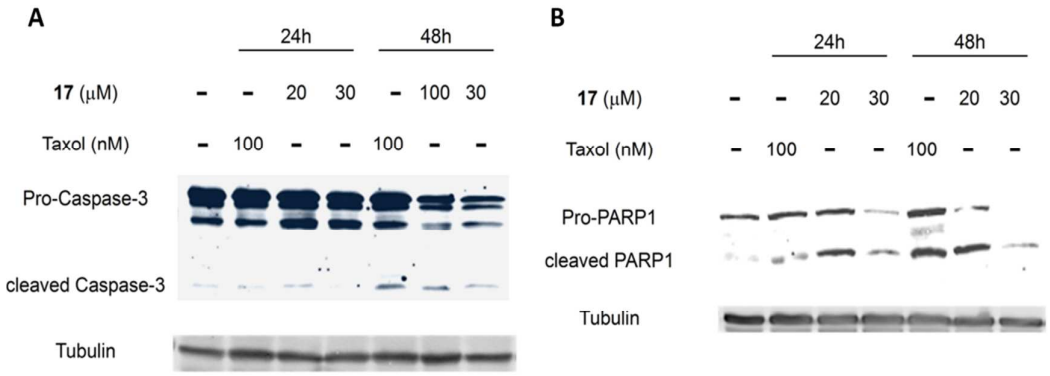


Figure S6. HeLa cells were pretreated for 1 h in the presence or absence of indicated inhibitors prior to addition of **17** or DMSO. Phase-contrast images were taken 24 h after addition of **17**.

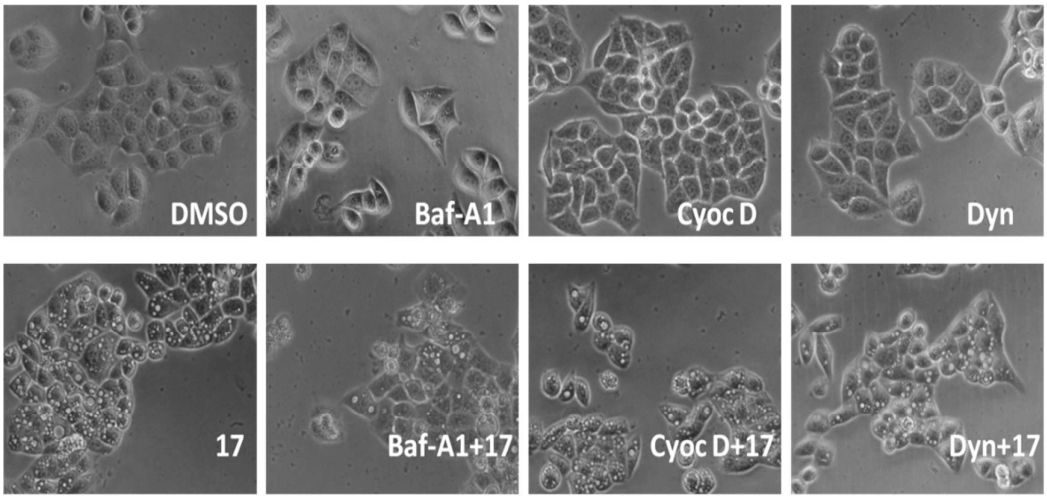
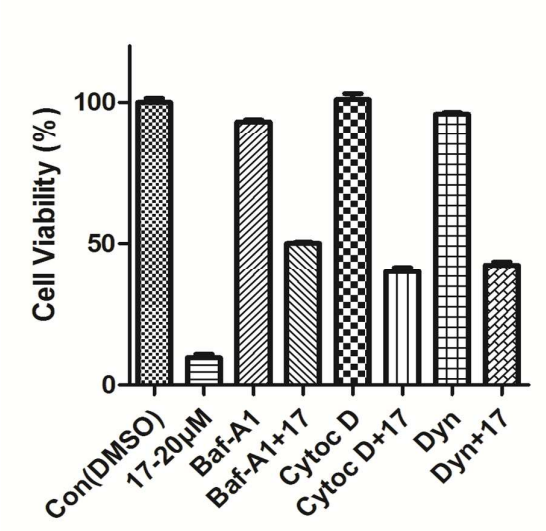
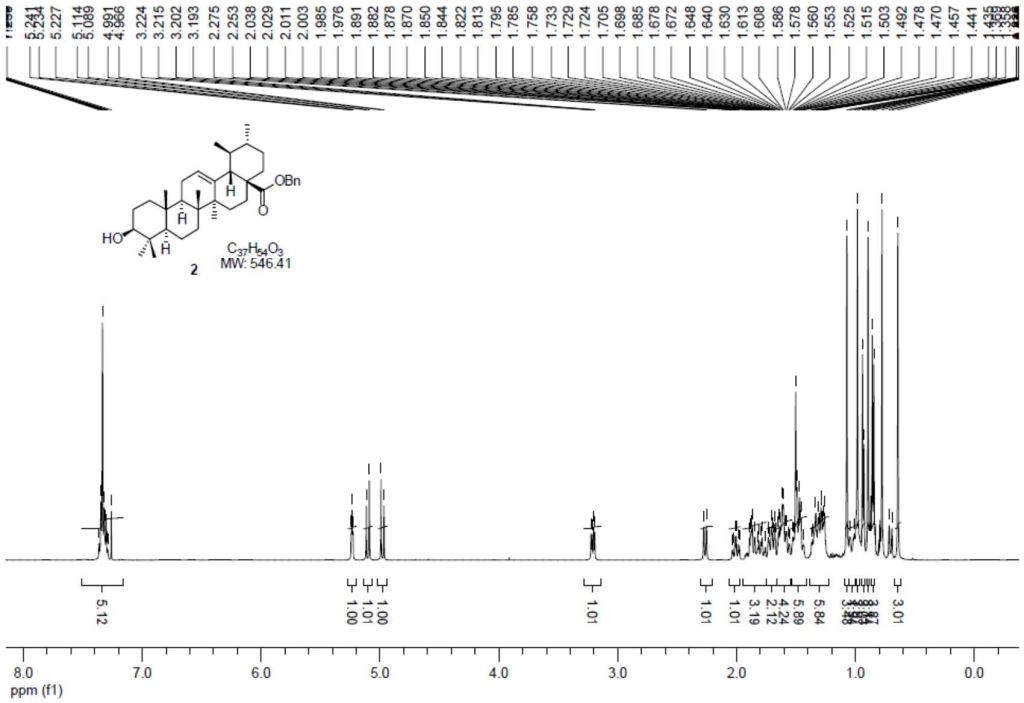


Figure S7. HeLa cells were pretreated for 1 h in the presence or absence of indicated inhibitors prior to addition of **17** or DMSO. CCK8 assay was performed 48 h after addition of **17**.

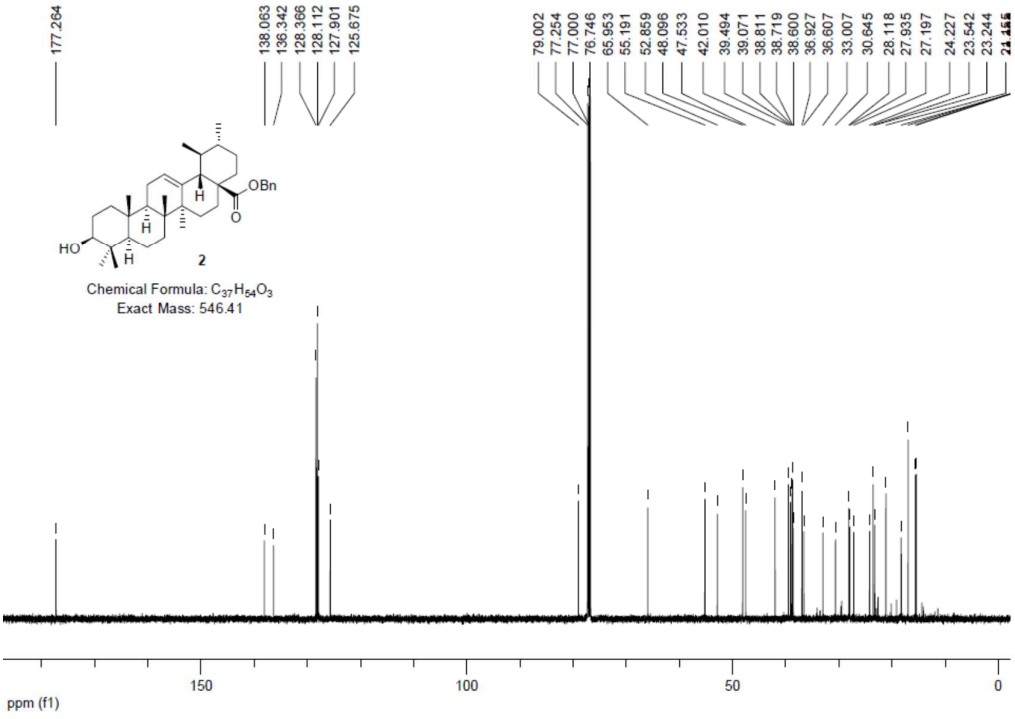


3. Copies of NMR Data (*selected examples*)

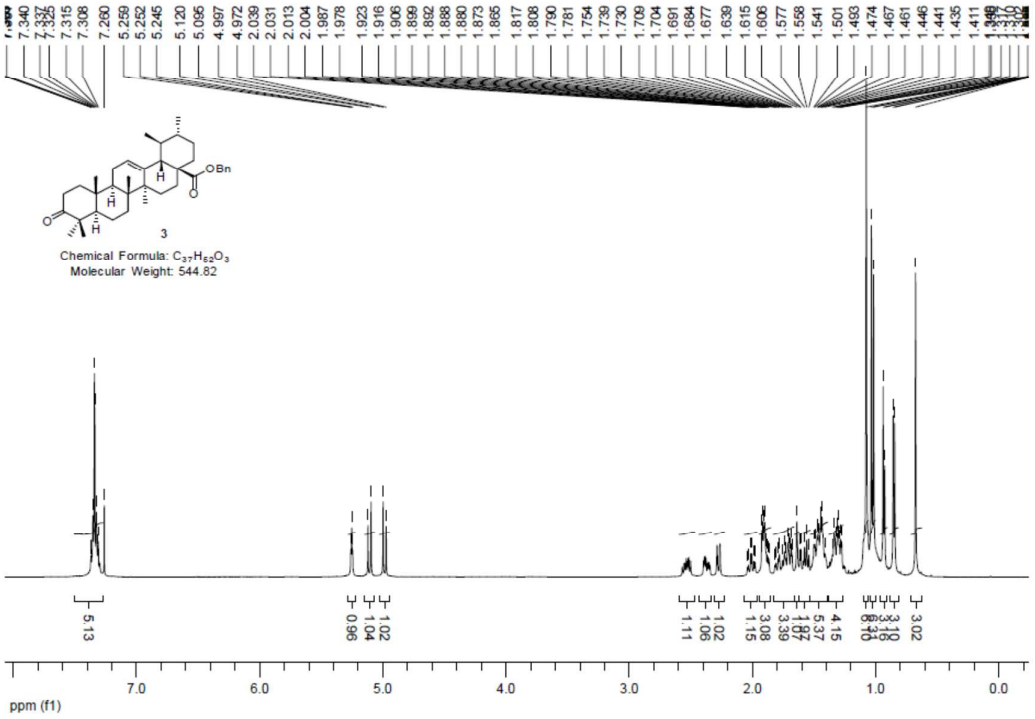
^{1}H NMR Spectrum of **Compound 2** (500 MHz, $CDCl_3$, 25 ^{o}C)



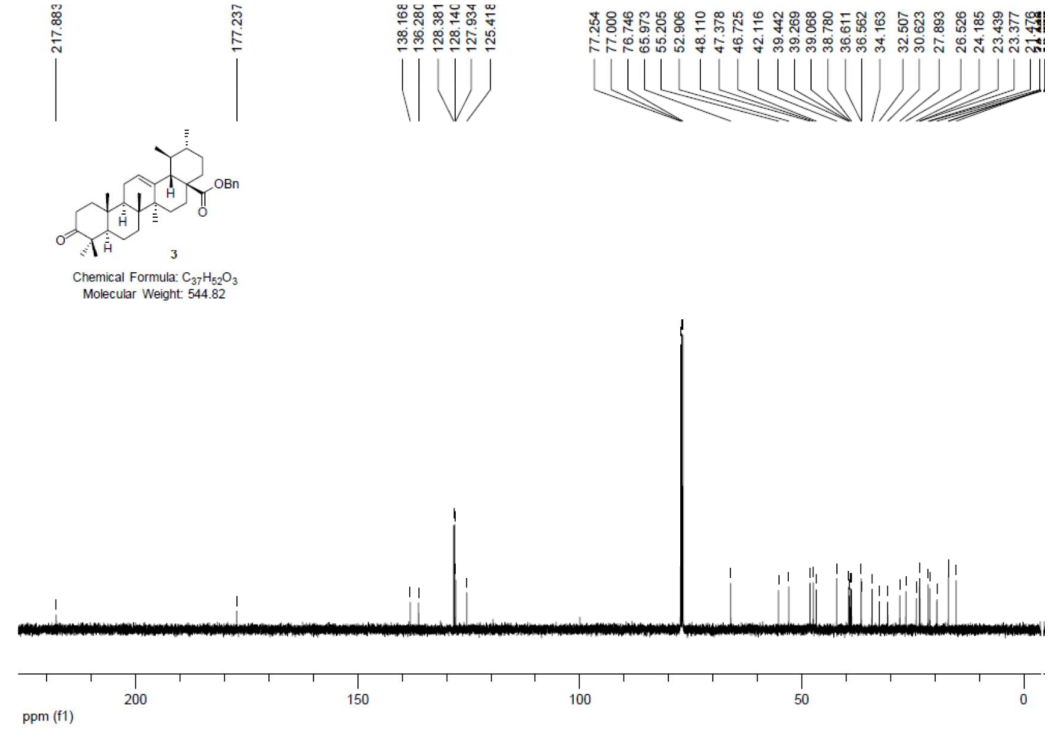
^{13}C NMR Spectrum of **Compound 2** (125 MHz, $CDCl_3$, 25 ^{o}C)



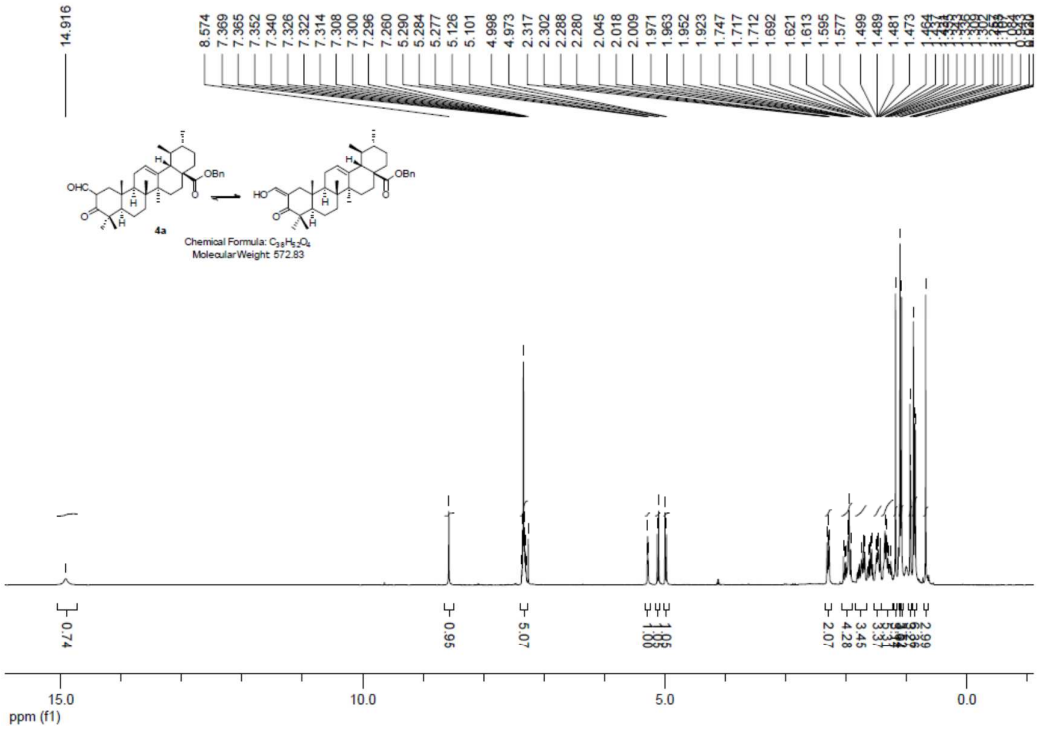
^{1}H NMR Spectrum of **Compound 3** (500 MHz, $CDCl_3$, 25 ^{o}C)



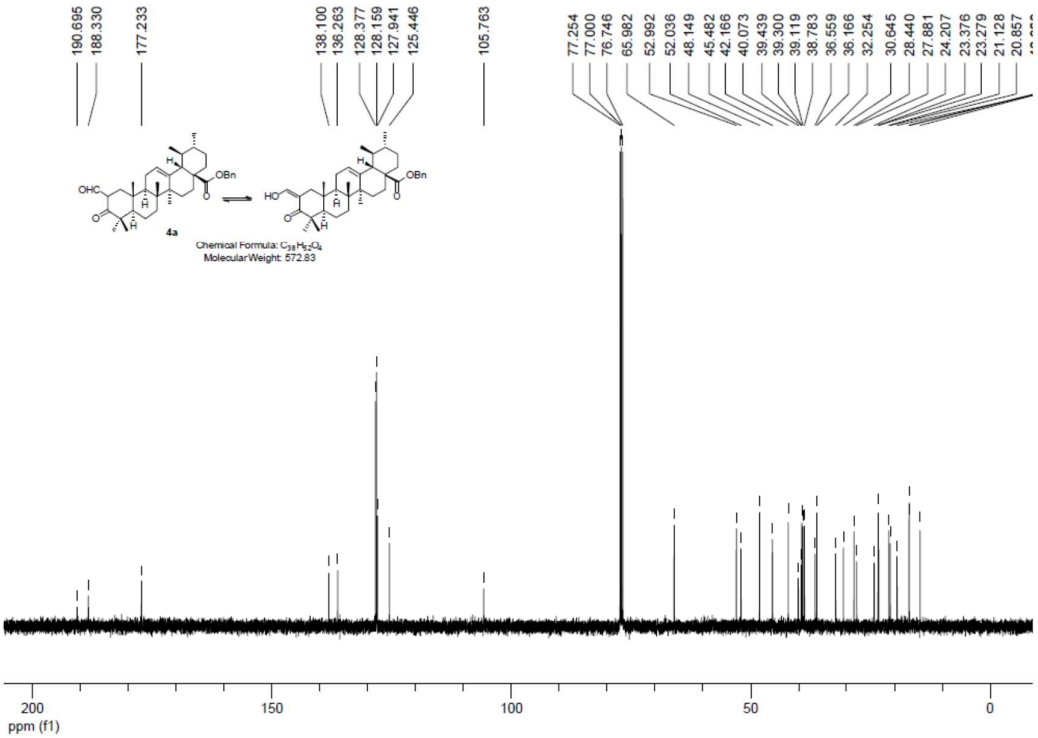
^{13}C NMR Spectrum of **Compound 3** (125 MHz, $CDCl_3$, 25 ^{o}C)



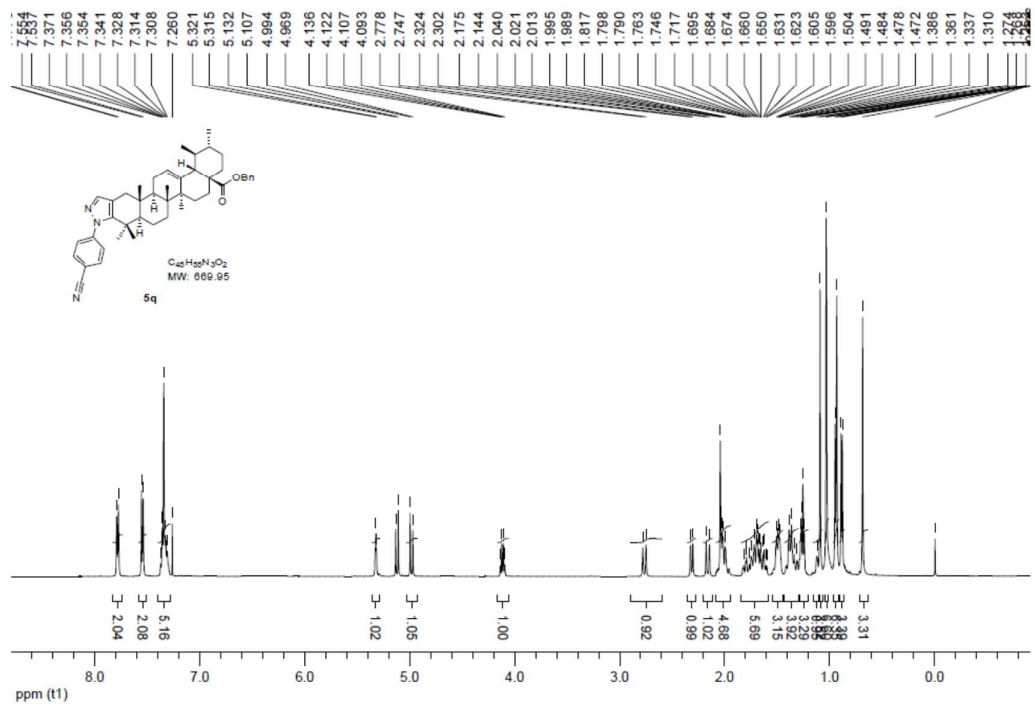
^{1}H NMR Spectrum of **Compound 4a** (500 MHz, $CDCl_3$, 25 ^{o}C)



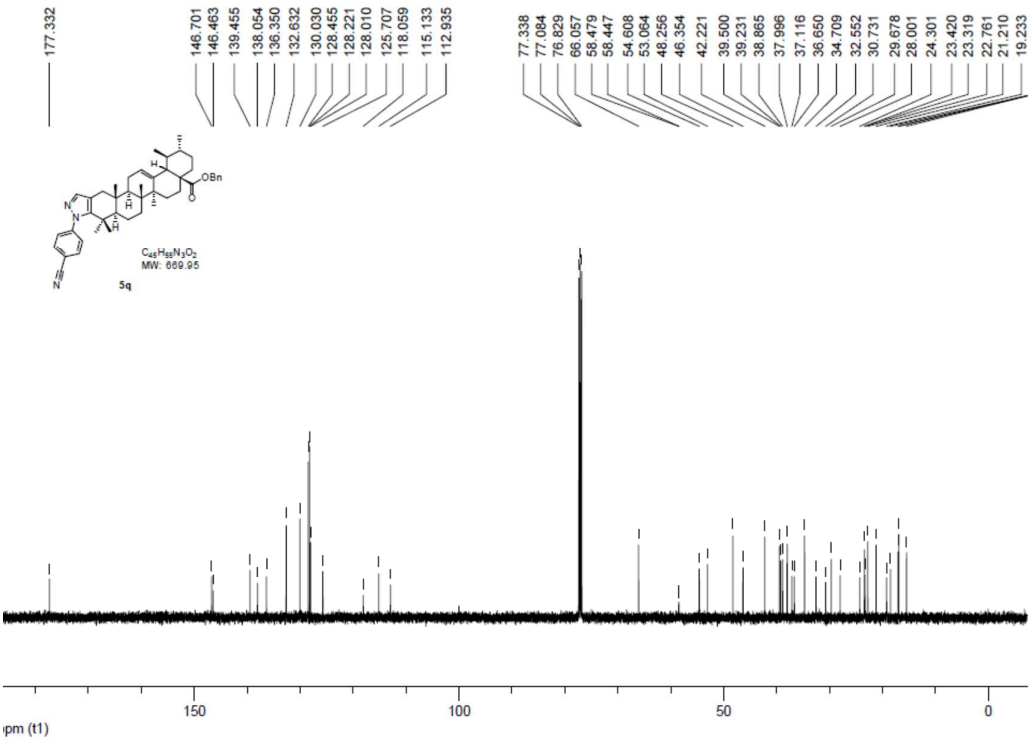
^{13}C NMR Spectrum of **Compound 4a** (125 MHz, $CDCl_3$, 25 ^{o}C)



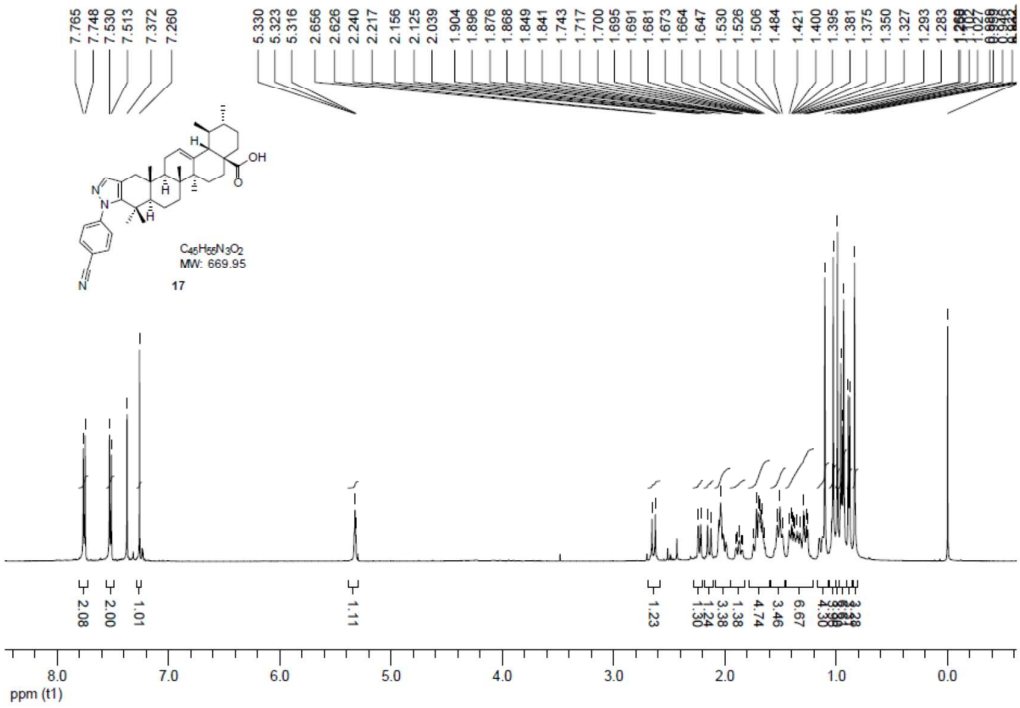
^{1}H NMR Spectrum of **Compound 5q** (500 MHz, $CDCl_3$, 25 ^{o}C)



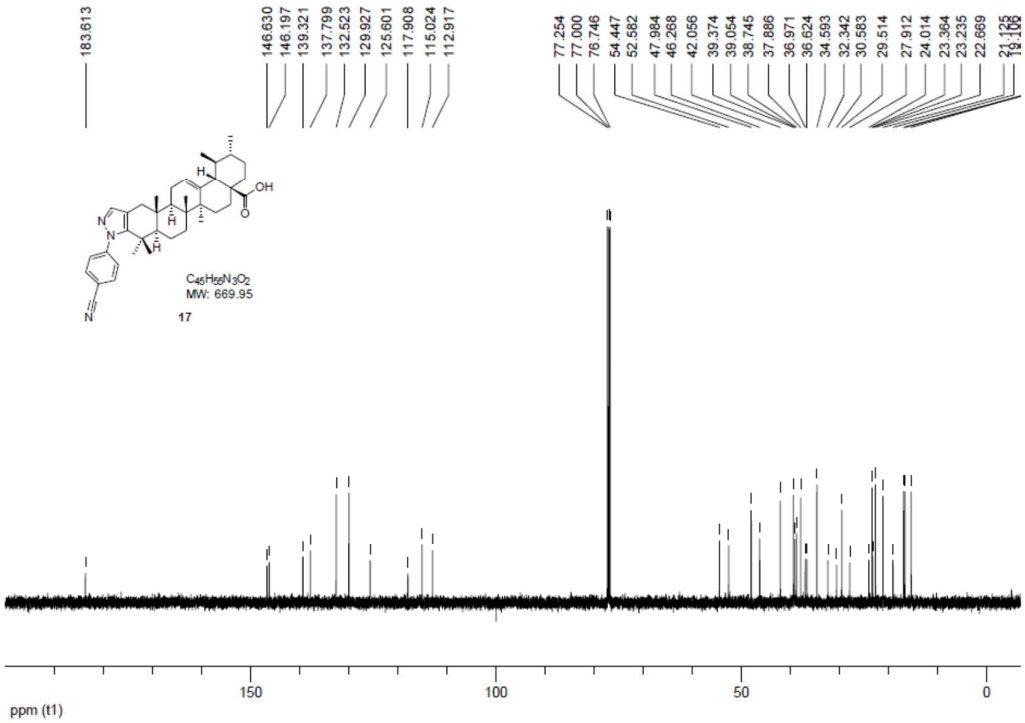
^{13}C NMR Spectrum of **Compound 5q** (125 MHz, $CDCl_3$, 25 ^{o}C)



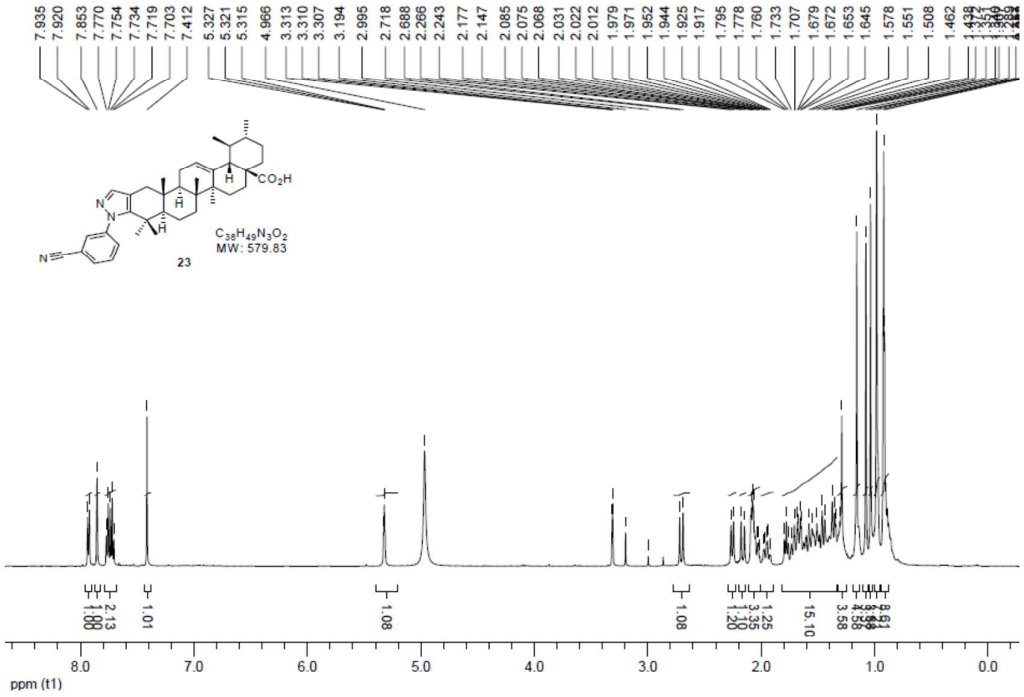
^{1}H NMR Spectrum of compound **17** (500 MHz, $CDCl_3$, 25 ^{o}C)



^{13}C NMR Spectrum of compound **17** (125 MHz, $CDCl_3$, 25 ^{o}C)



^{1}H NMR Spectrum of compound **23** (500 MHz, CD_3OD, 25 ^{o}C)



^{13}C NMR Spectrum of compound **23** (125 MHz, CD_3OD, 25 ^{o}C)