

Supporting Information

For

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

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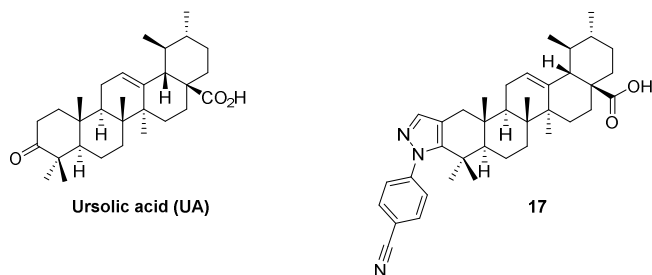
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1. Structure of ursolic acid (UA) and compound 17



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 μ M) with mobile phase gradient of 50-99% CH₃CN in H₂O (Table 1), with a flow rate of 1.0 mL/min.

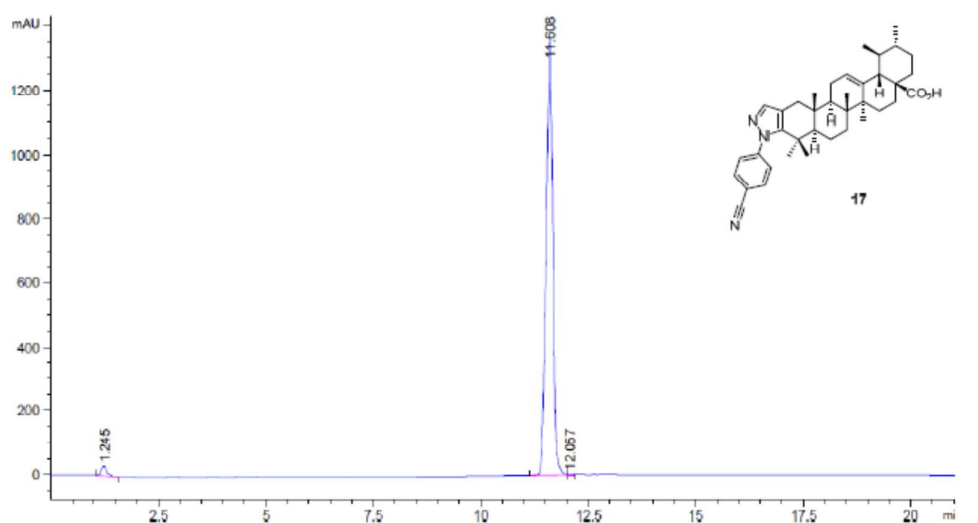
Table 1. HPLC conditions

Time (min)	H ₂ O (V%)	CH ₃ CN (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**



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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 μ M UA derivatives for 24 h, followed by imaging with phase-contrast microscopy.

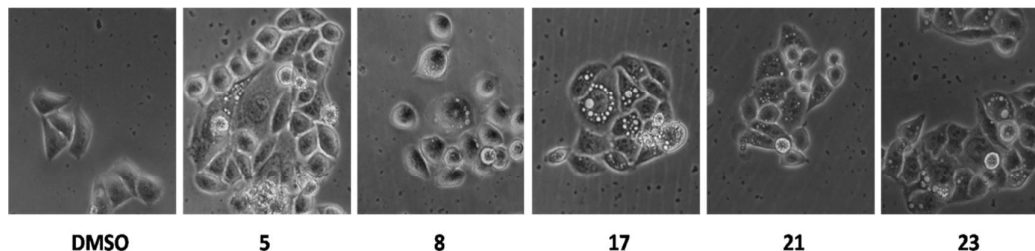


Figure S2. Cancer cells were treated with **17** at 20 μ M for 24 h, 48 h and 72 h, followed by CCK-8 assay.

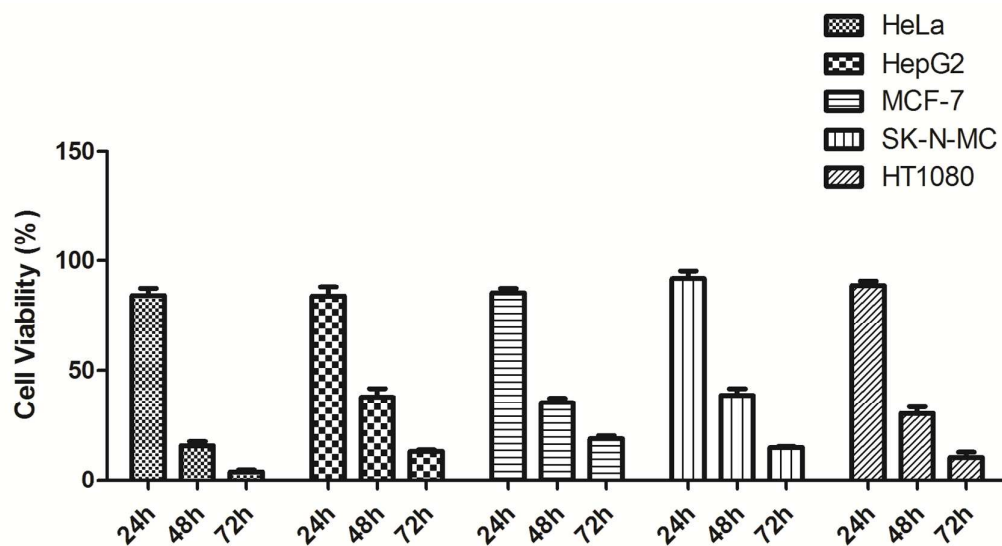


Figure S3. HeLa cells were treated with different concentrations of **17** or 0.1% DMSO for 2 days and colony forming assays were performed.

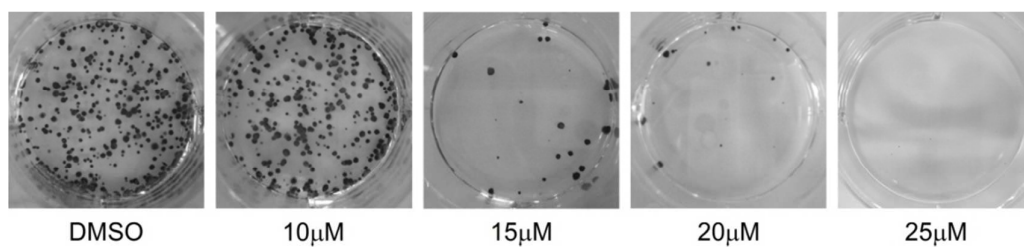


Figure S4. HeLa cells were incubated with indicated concentrations of **17** for 24 h or 48 h. The cytotoxicity of **17** was assessed with Alexa Fluor® 488 annexin V/Dead Cell Apoptosis Kit by flow cytometry.

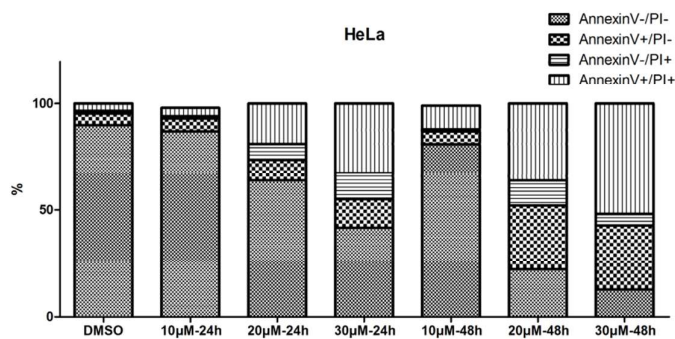
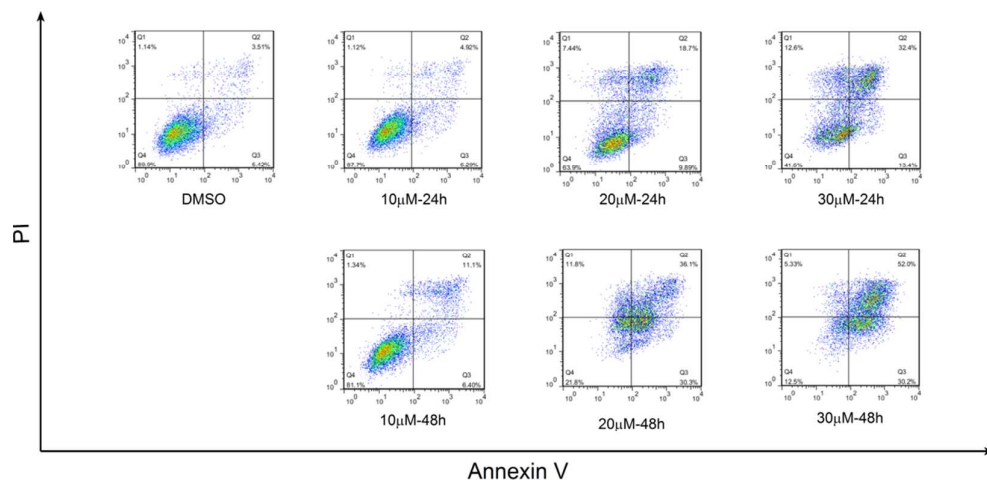


Figure S5. Effect of **17** on caspase activation and PARP cleavage. HeLa cells were treated for 24 h or 48 h with the indicated concentration of **17**. The cells were harvested and caspase 3 and PARP expression were determined by western blot.

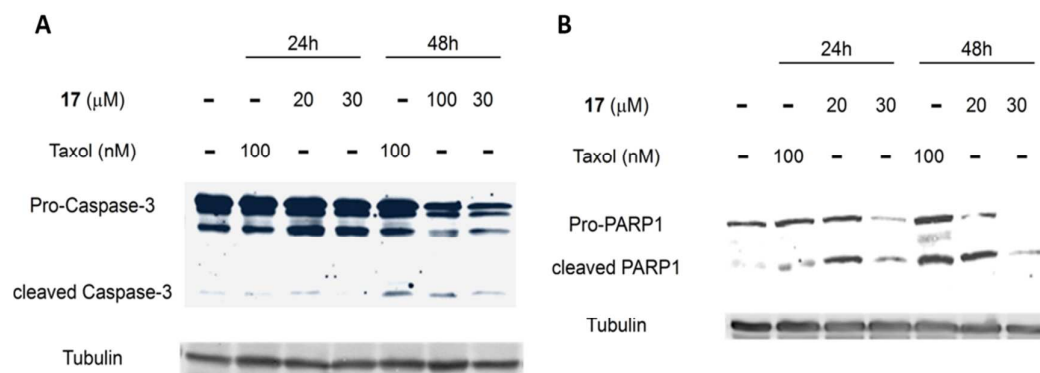


Figure S6. HeLa cells were pretreated for 1 h in the presence or absence of indicated inhibitors prior to addition of **17** or DMSO. Phase-contrast images were taken 24 h after addition of **17**.

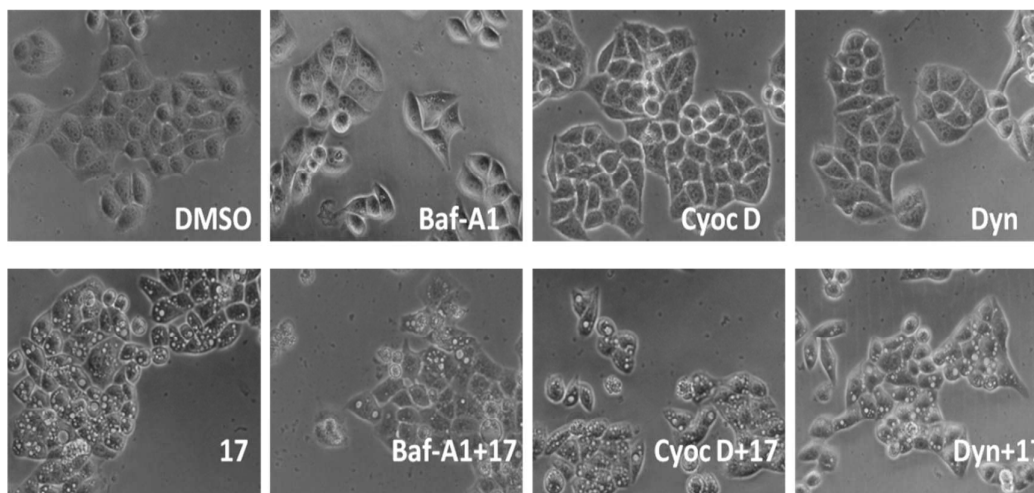
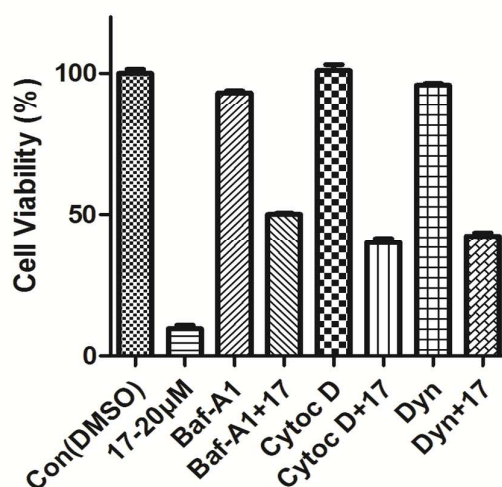
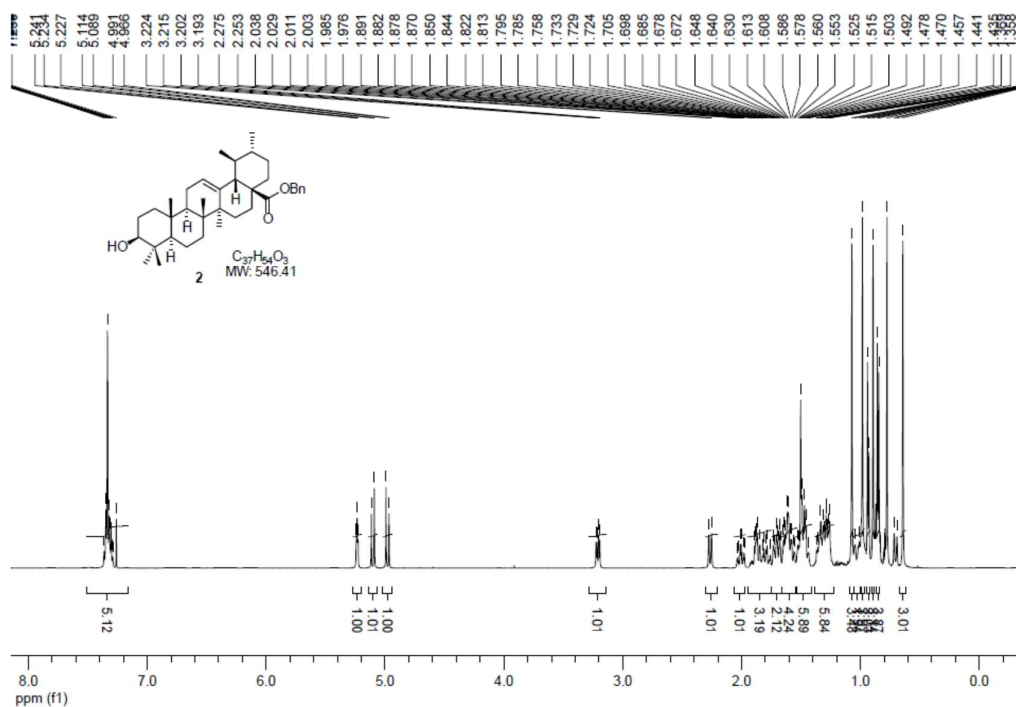


Figure S7. HeLa cells were pretreated for 1 h in the presence or absence of indicated inhibitors prior to addition of **17** or DMSO. CCK8 assay was performed 48 h after addition of **17**.

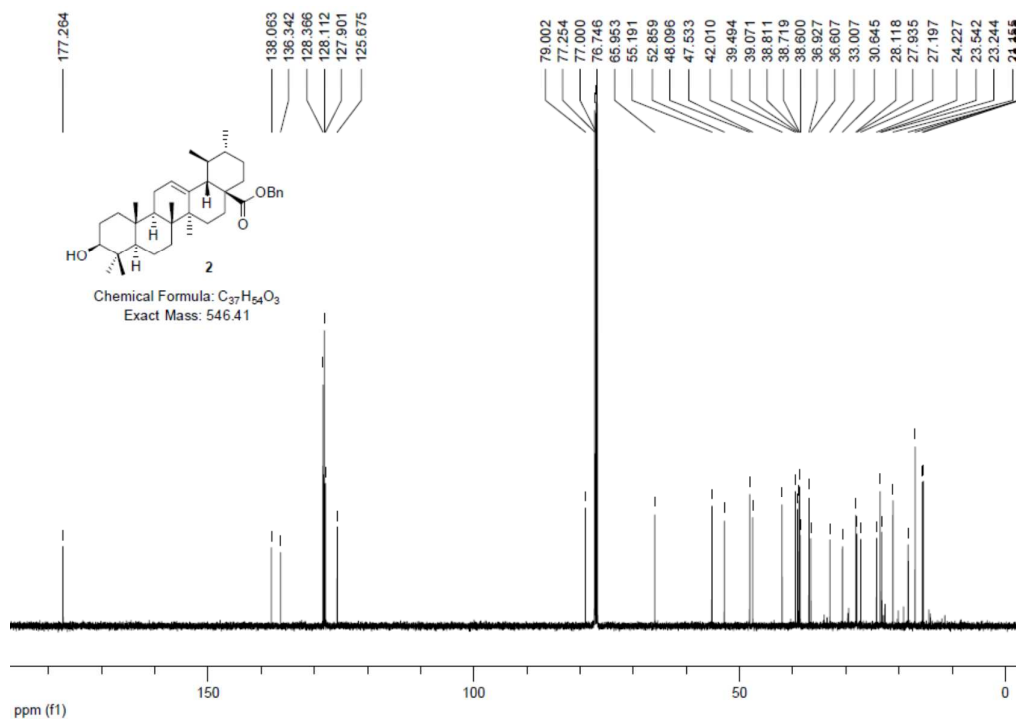


3. Copies of NMR Data (*selected examples*)

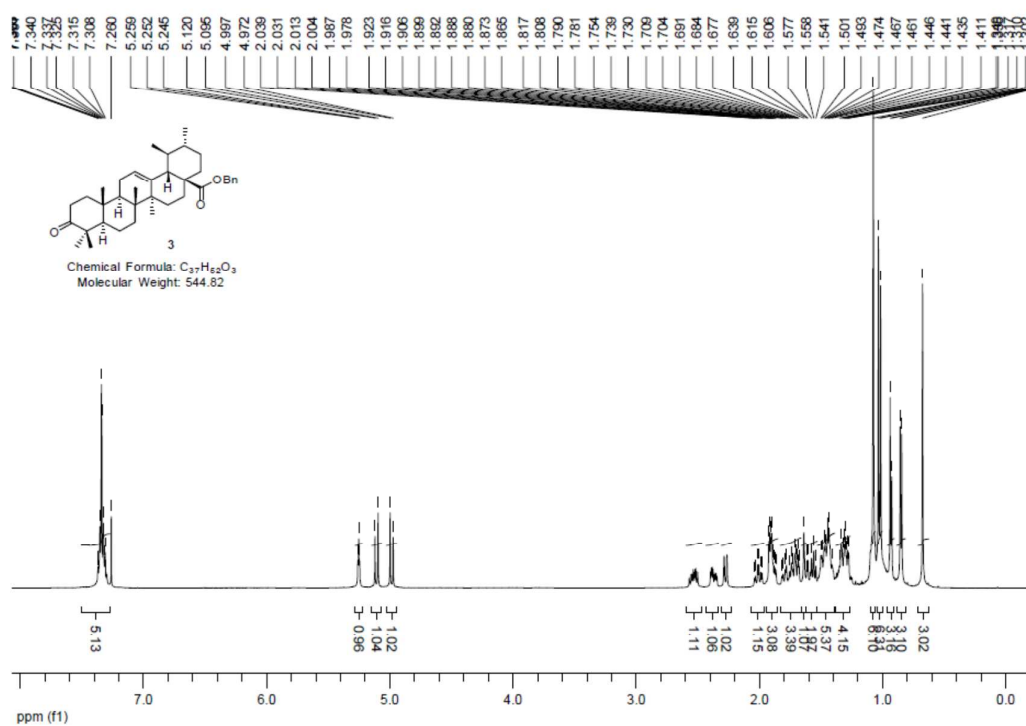
^1H NMR Spectrum of **Compound 2** (500 MHz, CDCl_3 , 25 °C)



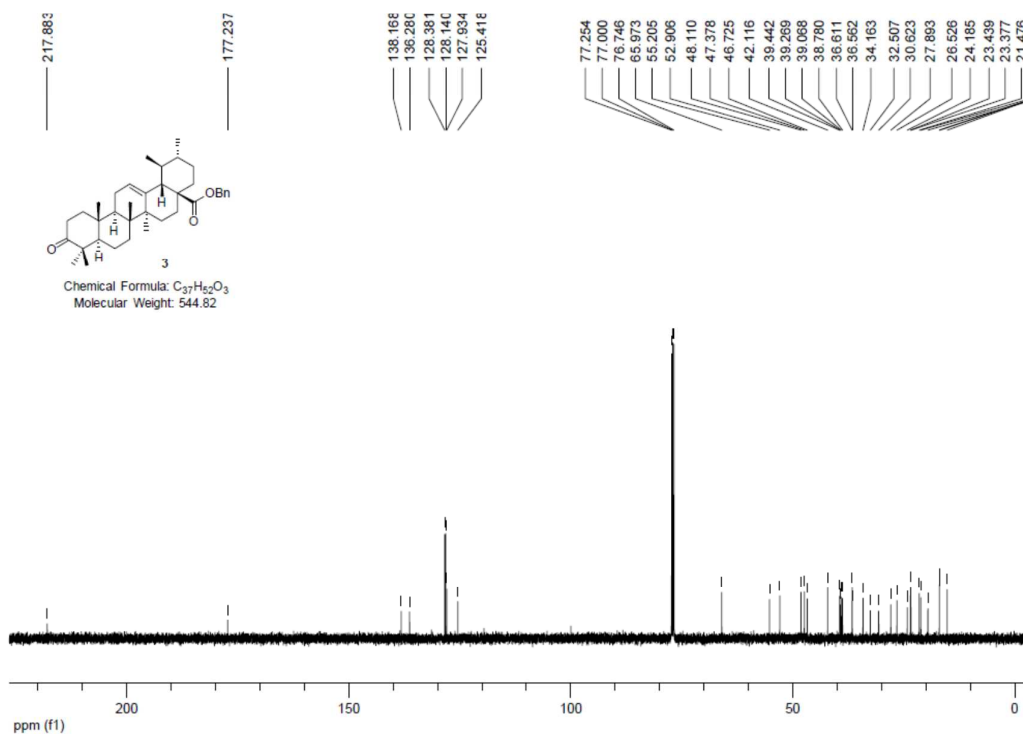
^{13}C NMR Spectrum of **Compound 2** (125 MHz, CDCl_3 , 25 °C)



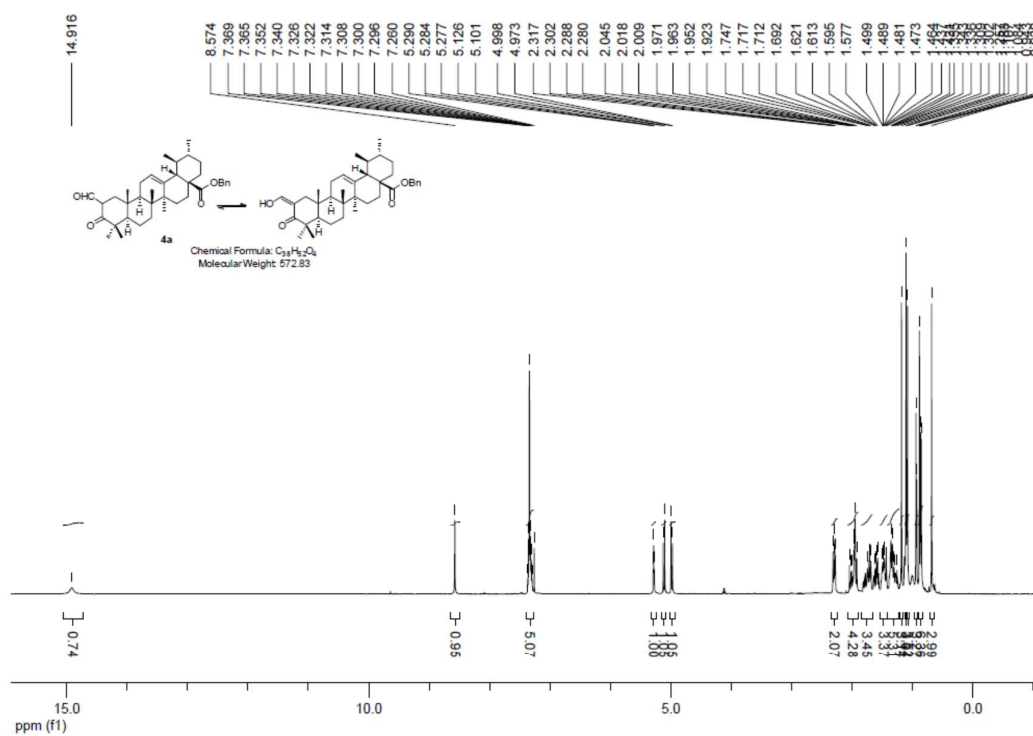
¹H NMR Spectrum of **Compound 3** (500 MHz, CDCl₃, 25 °C)



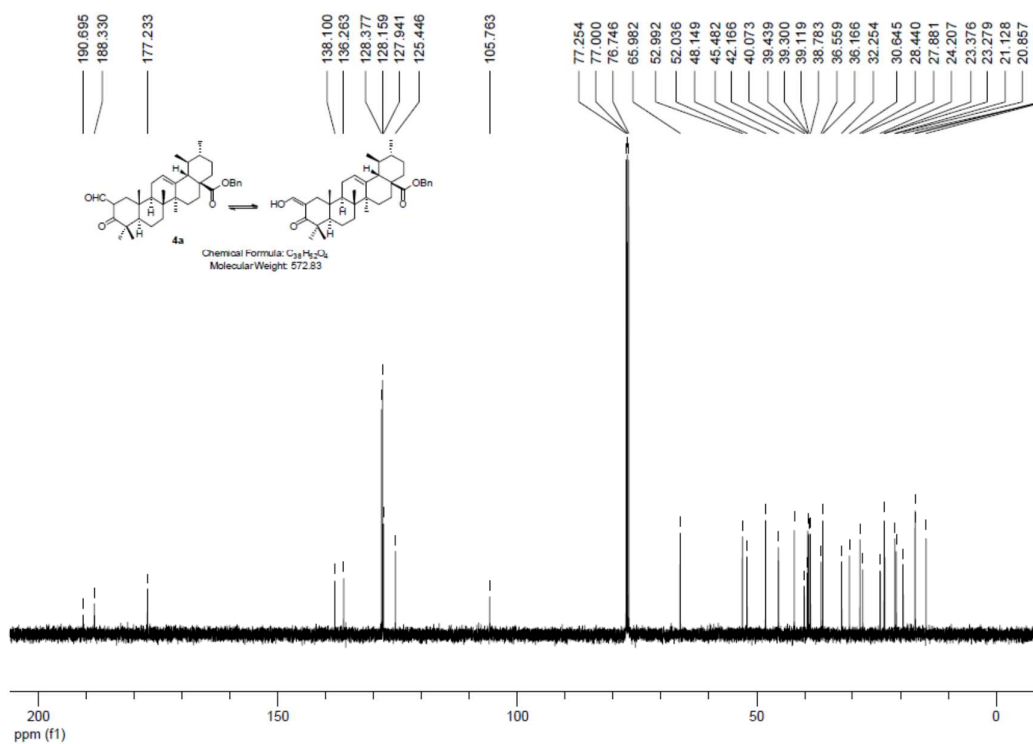
¹³C NMR Spectrum of **Compound 3** (125 MHz, CDCl₃, 25 °C)



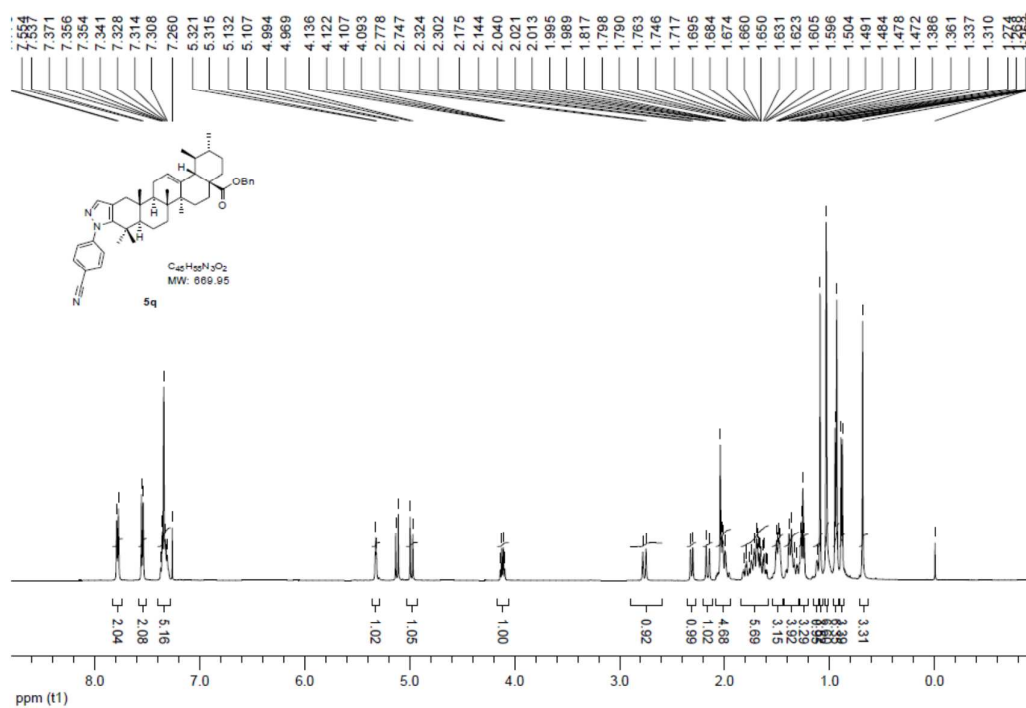
¹H NMR Spectrum of **Compound 4a** (500 MHz, CDCl₃, 25 °C)



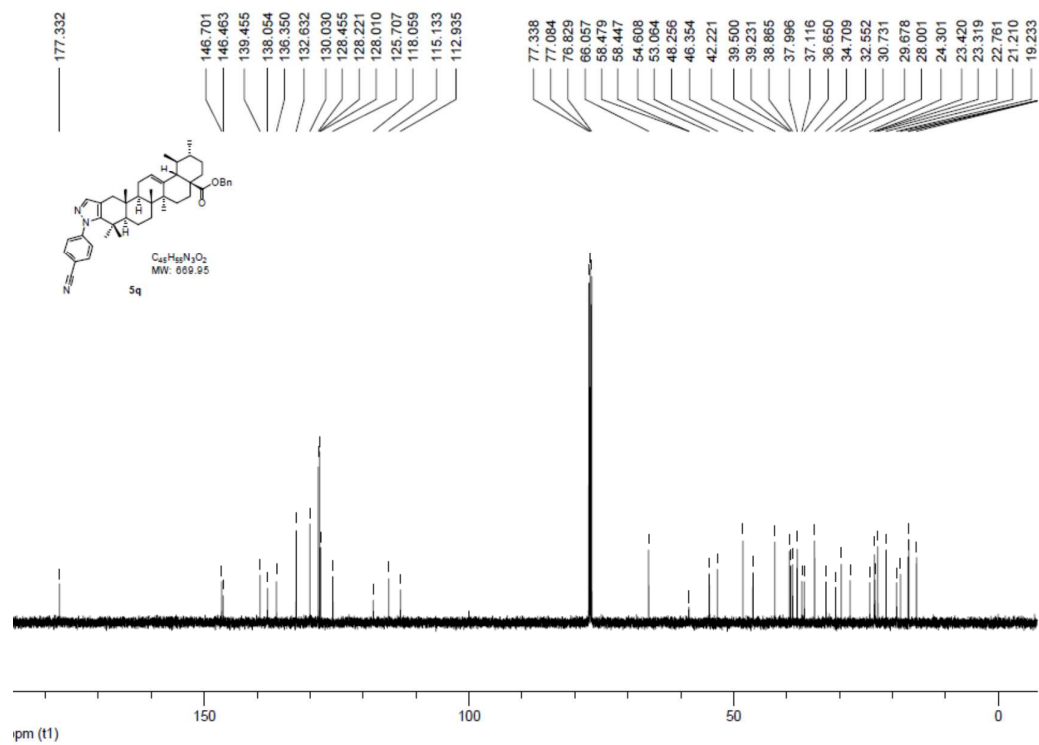
¹³C NMR Spectrum of **Compound 4a** (125 MHz, CDCl₃, 25 °C)



¹H NMR Spectrum of **Compound 5q** (500 MHz, CDCl₃, 25 °C)



¹³C NMR Spectrum of **Compound 5q** (125 MHz, CDCl₃, 25 °C)



Chemical structure of compound 17: Cc1ccc(C#N)cc1[C@H]2[C@H]3[C@H]4[C@@H]5[C@H]2CC[C@@H]3C[C@H]4[C@@H](C)[C@H]5C(=O)O

$C_{22}H_{29}N_3O_2$
MW: 387.50

17

1H NMR spectrum (CDCl₃) of compound 17. The x-axis represents chemical shift in ppm (t1) from 0.0 to 8.0. The spectrum shows several peaks with integration values below the baseline.

Chemical shift values (ppm): 7.765, 7.748, 7.530, 7.513, 7.372, 7.280, 5.330, 5.323, 5.316, 2.656, 2.626, 2.240, 2.217, 2.156, 2.125, 2.039, 1.904, 1.896, 1.876, 1.868, 1.849, 1.841, 1.743, 1.717, 1.700, 1.695, 1.691, 1.681, 1.673, 1.664, 1.647, 1.530, 1.526, 1.506, 1.484, 1.421, 1.400, 1.395, 1.381, 1.375, 1.350, 1.327, 1.283, 1.269, 1.260, 1.252, 1.244, 1.236, 1.228, 1.220, 1.212, 1.204, 1.196, 1.188, 1.180, 1.172, 1.164, 1.156, 1.148, 1.140, 1.132, 1.124, 1.116, 1.108, 1.100, 1.092, 1.084, 1.076, 1.068, 1.060, 1.052, 1.044, 1.036, 1.028, 1.020, 1.012, 1.004, 0.996, 0.988, 0.980, 0.972, 0.964, 0.956, 0.948, 0.940, 0.932, 0.924, 0.916, 0.908, 0.900, 0.892, 0.884, 0.876, 0.868, 0.860, 0.852, 0.844, 0.836, 0.828, 0.820, 0.812, 0.804, 0.796, 0.788, 0.780, 0.772, 0.764, 0.756, 0.748, 0.740, 0.732, 0.724, 0.716, 0.708, 0.700, 0.692, 0.684, 0.676, 0.668, 0.660, 0.652, 0.644, 0.636, 0.628, 0.620, 0.612, 0.604, 0.596, 0.588, 0.580, 0.572, 0.564, 0.556, 0.548, 0.540, 0.532, 0.524, 0.516, 0.508, 0.500, 0.492, 0.484, 0.476, 0.468, 0.460, 0.452, 0.444, 0.436, 0.428, 0.420, 0.412, 0.404, 0.396, 0.388, 0.380, 0.372, 0.364, 0.356, 0.348, 0.340, 0.332, 0.324, 0.316, 0.308, 0.300, 0.292, 0.284, 0.276, 0.268, 0.260, 0.252, 0.244, 0.236, 0.228, 0.220, 0.212, 0.204, 0.196, 0.188, 0.180, 0.172, 0.164, 0.156, 0.148, 0.140, 0.132, 0.124, 0.116, 0.108, 0.100, 0.092, 0.084, 0.076, 0.068, 0.060, 0.052, 0.044, 0.036, 0.028, 0.020, 0.012, 0.004, 0.000.

Integration values: 2.08, 2.00, 1.01, 1.11, 1.23, 1.38, 1.36, 1.30, 4.74, 3.46, 6.67, 2.68, 2.60, 2.52, 2.44, 2.36, 2.28, 2.20, 2.12, 2.04, 1.96, 1.88, 1.80, 1.72, 1.64, 1.56, 1.48, 1.40, 1.32, 1.24, 1.16, 1.08, 1.00, 0.92, 0.84, 0.76, 0.68, 0.60, 0.52, 0.44, 0.36, 0.28, 0.20, 0.12, 0.04, 0.00.

Chemical structure of compound 17 is shown, with its molecular formula $C_{26}H_{35}N_3O_2$ and molecular weight (MW): 439.55. The structure is a complex polycyclic molecule featuring a nitrile group, a pyrazole ring, and a ketone group.

The ^{13}C NMR spectrum (CDCl₃) of compound 17 is displayed below the structure. The spectrum shows peaks corresponding to the carbon atoms in the molecule, with the following chemical shifts (ppm):

Chemical Shift (ppm)
183.613
146.630
146.197
139.321
137.789
132.523
129.927
125.601
117.908
115.024
112.917
77.254
77.000
76.746
54.447
52.582
47.984
46.288
42.056
39.374
39.054
38.745
37.886
36.971
36.624
34.593
32.342
30.563
29.514
27.912
24.014
23.364
23.235
22.669
21.126
19.106

Chemical structure of compound **23** is shown, with molecular formula $C_{38}H_{49}N_3O_2$ and molecular weight 579.83.

1H NMR spectrum (CDCl₃) of compound **23** is displayed, showing peaks from 0.0 to 8.0 ppm. The spectrum includes integration values below the baseline and a list of peak chemical shifts (δ) on the right side.

Chemical structure of compound 23: C38H48N2O2 (MW: 579.83). The structure is a complex polycyclic molecule with a nitrile group and a carboxylic acid group.

¹³C NMR spectrum (ppm (t1)) showing peaks at the following chemical shifts (ppm):

- 181.551
- 148.681
- 143.923
- 139.972
- 139.599
- 135.339
- 134.544
- 134.034
- 131.405
- 126.893
- 118.500
- 116.522
- 114.191
- 55.964
- 54.525
- 49.561
- 49.391
- 49.221
- 49.140
- 49.050
- 48.880
- 48.710
- 48.539
- 47.701
- 43.458
- 40.762
- 40.560
- 40.450
- 39.178
- 38.126
- 38.028
- 35.830
- 33.813
- 33.149
- 31.876
- 30.890
- 30.850
- 30.563
- 30.175
- 29.302
- 25.417
- 24.427
- 24.004
- 23.854
- 23.684
- 23.514
- 23.344
- 23.174
- 23.004
- 22.834
- 22.664
- 22.494
- 22.324
- 22.154
- 21.984
- 21.814
- 21.644
- 21.474
- 21.304
- 21.134
- 20.964
- 20.794
- 20.624
- 20.454
- 20.284
- 20.114
- 19.944
- 19.774
- 19.604
- 19.434
- 19.264
- 19.094
- 18.924
- 18.754
- 18.584
- 18.414
- 18.244
- 18.074
- 17.904
- 17.734
- 17.564
- 17.394
- 17.224
- 17.054
- 16.884
- 16.714
- 16.544
- 16.374
- 16.204
- 16.034
- 15.864
- 15.694
- 15.524
- 15.354
- 15.184
- 15.014
- 14.844
- 14.674
- 14.504
- 14.334
- 14.164
- 13.994
- 13.824
- 13.654
- 13.484
- 13.314
- 13.144
- 12.974
- 12.804
- 12.634
- 12.464
- 12.294
- 12.124
- 11.954
- 11.784
- 11.614
- 11.444
- 11.274
- 11.104
- 10.934
- 10.764
- 10.594
- 10.424
- 10.254
- 10.084
- 9.914
- 9.744
- 9.574
- 9.404
- 9.234
- 9.064
- 8.894
- 8.724
- 8.554
- 8.384
- 8.214
- 8.044
- 7.874
- 7.704
- 7.534
- 7.364
- 7.194
- 7.024
- 6.854
- 6.684
- 6.514
- 6.344
- 6.174
- 6.004
- 5.834
- 5.664
- 5.494
- 5.324
- 5.154
- 4.984
- 4.814
- 4.644
- 4.474
- 4.304
- 4.134
- 3.964
- 3.794
- 3.624
- 3.454
- 3.284
- 3.114
- 2.944
- 2.774
- 2.604
- 2.434
- 2.264
- 2.094
- 1.924
- 1.754
- 1.584
- 1.414
- 1.244
- 1.074
- 1.004
- 0.834
- 0.664
- 0.494
- 0.324
- 0.154
- 0.004