Supplementary data for article:

Trmčić, M.; Matović, R. V.; Tovilović, G.; Ristic, B. Z.; Trajković, V. S.; Ferjančić, Z.; Saičić, R. A Novel C,D-Spirolactone Analogue of Paclitaxel: Autophagy Instead of Apoptosis as a Previously Unknown Mechanism of Cytotoxic Action for Taxoids. *Organic and Biomolecular Chemistry* **2012**, *10* (25), 4933–4942.
https://doi.org/10.1039/c2ob25514f

Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Electronic Supplementary Information

A Novel C,D-Spirolactone Analogue of Paclitaxel: Autophagy Instead of Apoptosis as a Previously Unknown Mechanism of Cytotoxic Action for Taxoids

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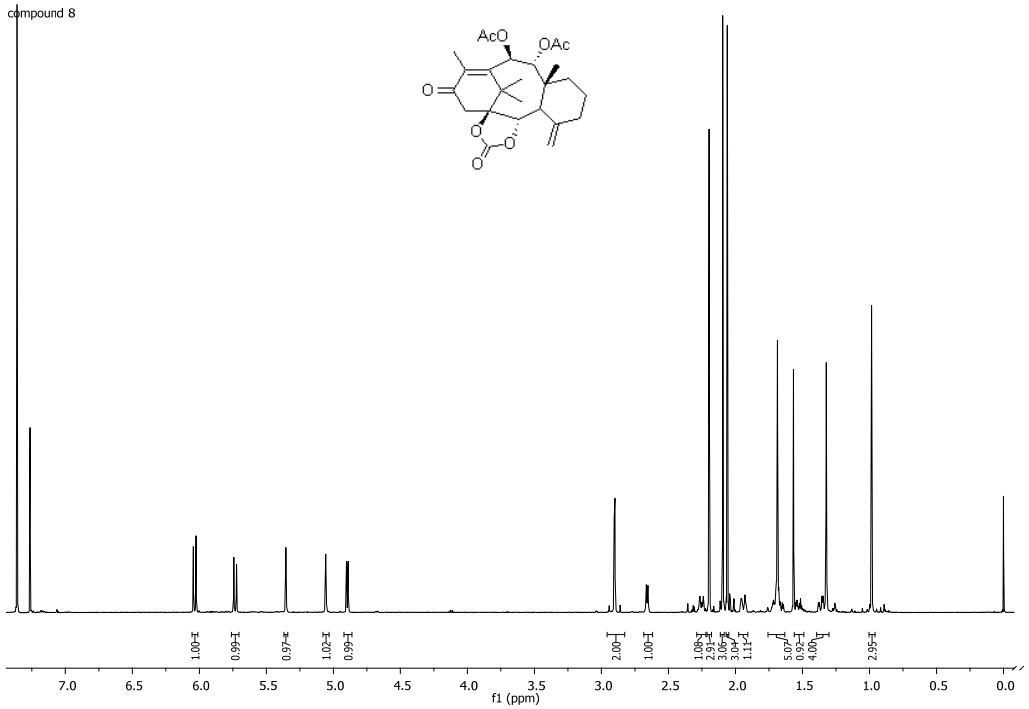

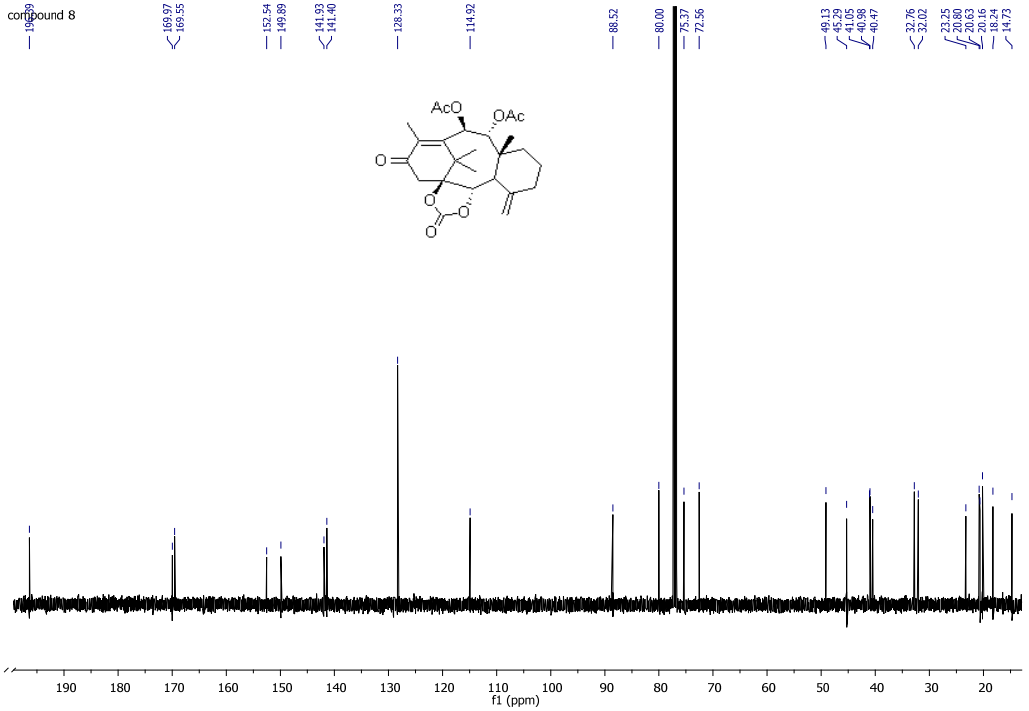

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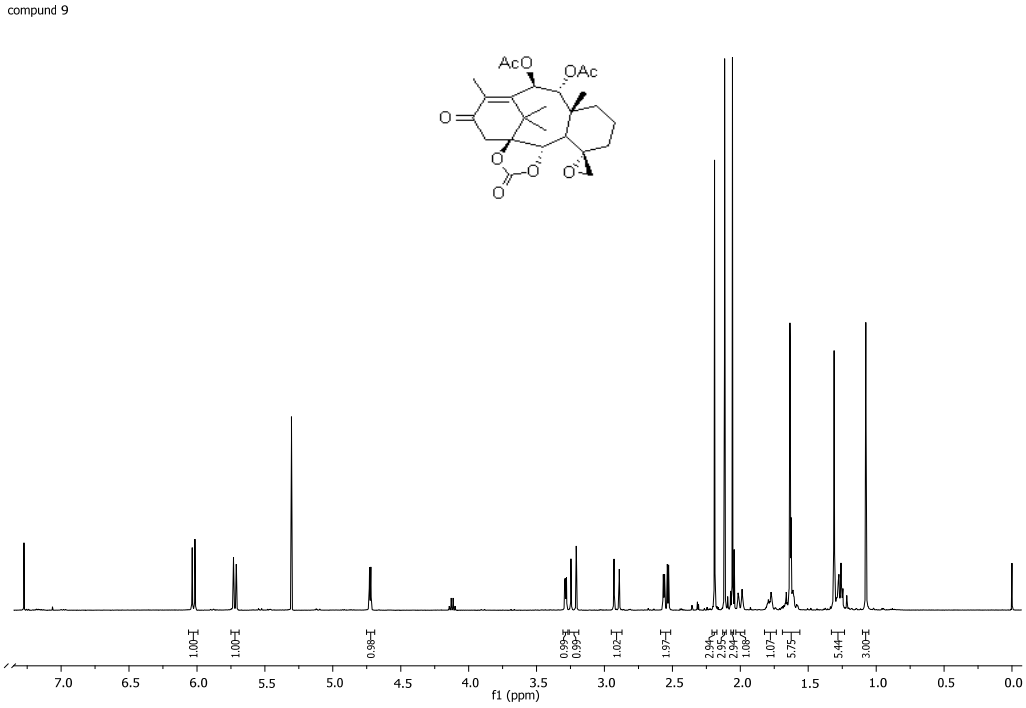

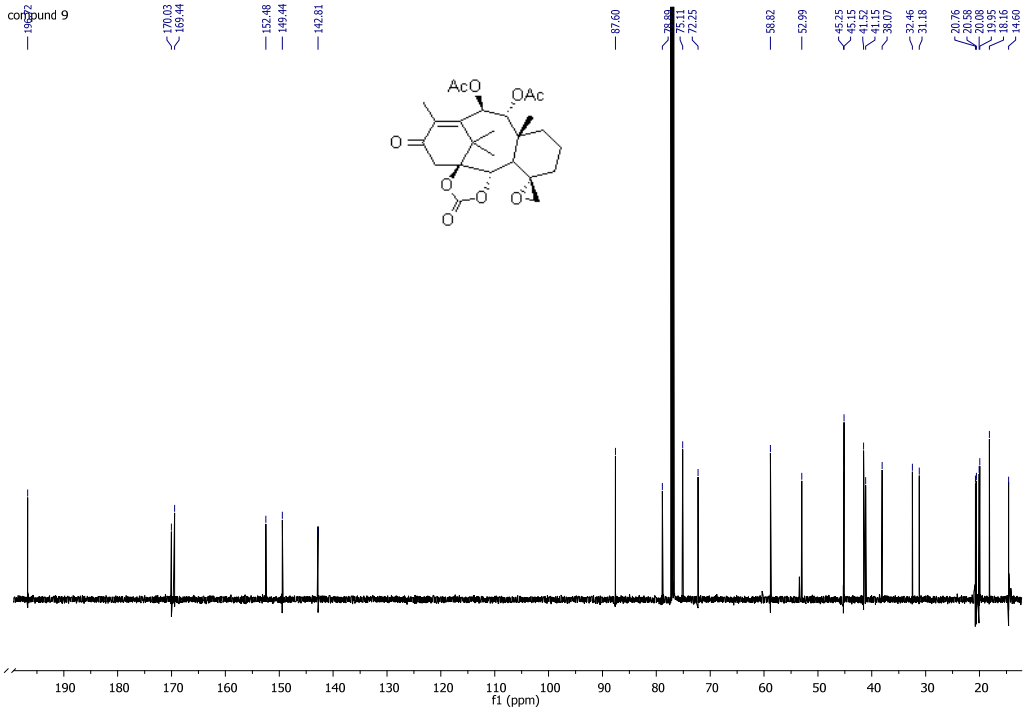

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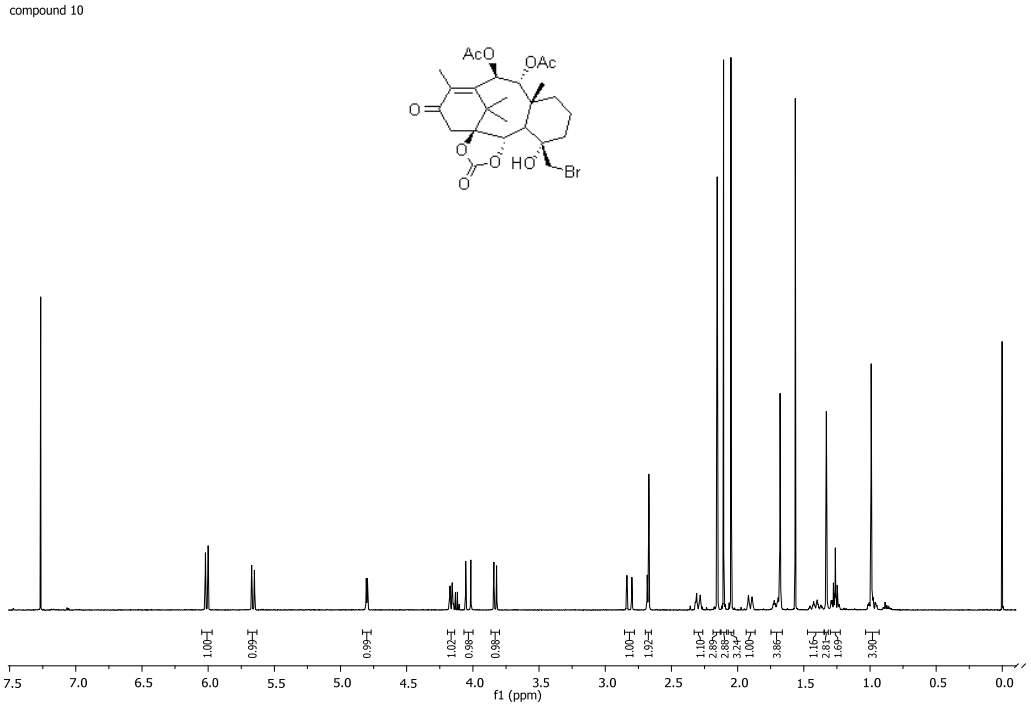
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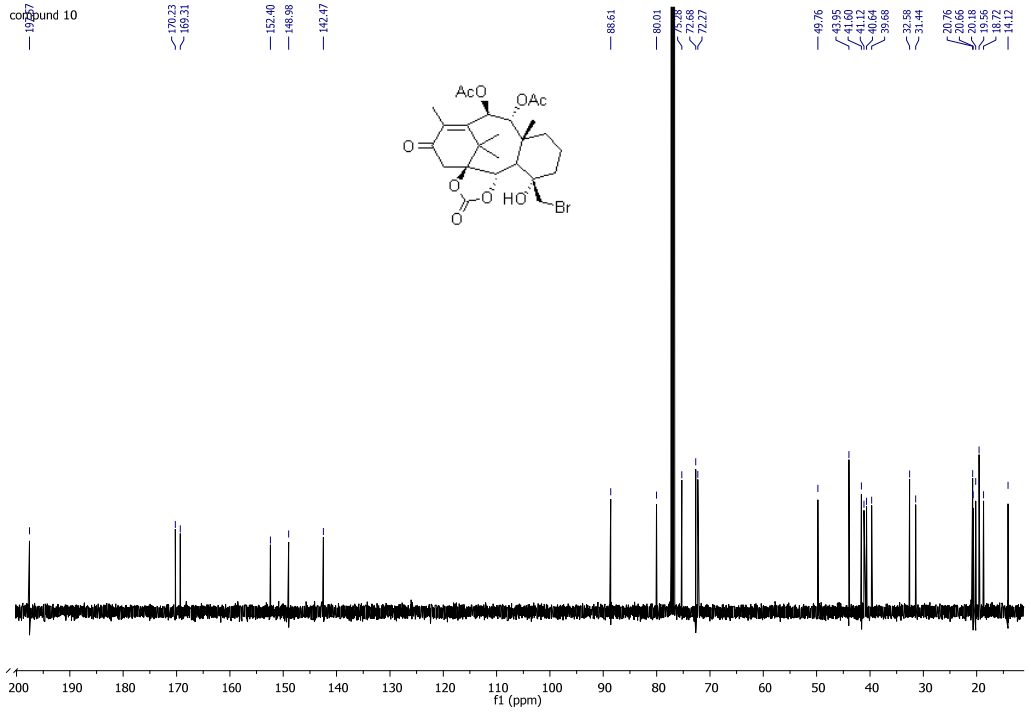
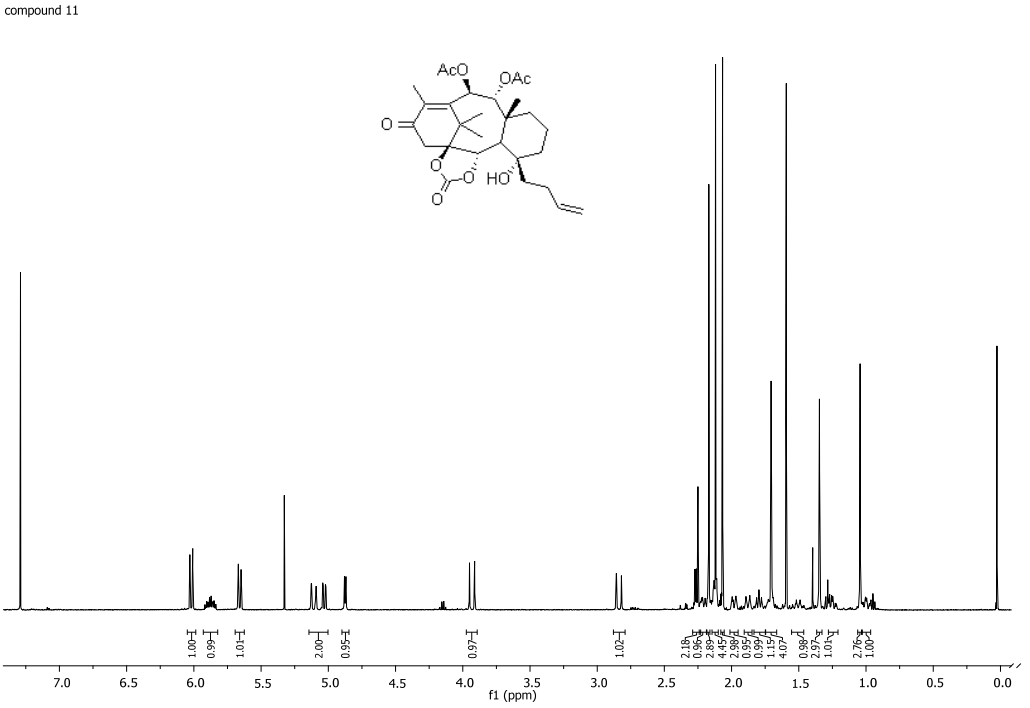
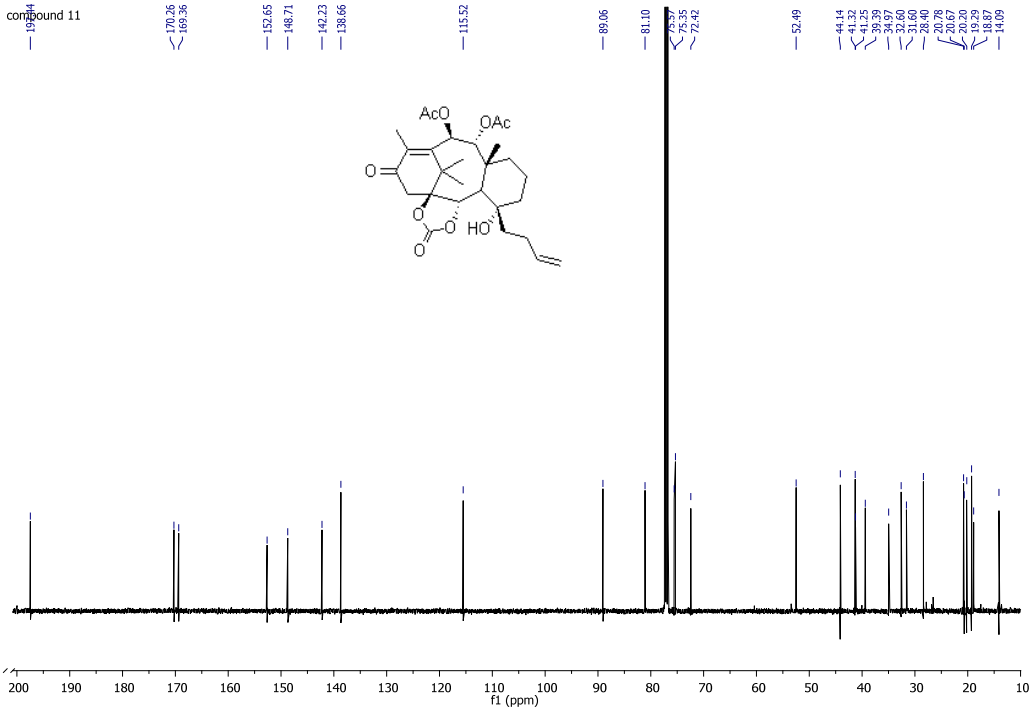
AcO
OAc
O
O
O
HO
Br
O
1.00
0.99
0.99
1.02
0.98
0.98
1.00
1.92
1.10
2.88
2.94
3.24
1.00
3.86
1.16
2.81
1.69
3.90
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


compound 10
170.23
169.31
152.40
148.98
142.47
88.61
80.01
75.28
72.68
72.27
49.76
43.95
41.60
41.12
40.64
39.68
32.58
31.44
20.76
20.66
20.18
19.56
18.72
14.12
AcO
OAc
O
O
O
HO
Br
O
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

Compound **11**





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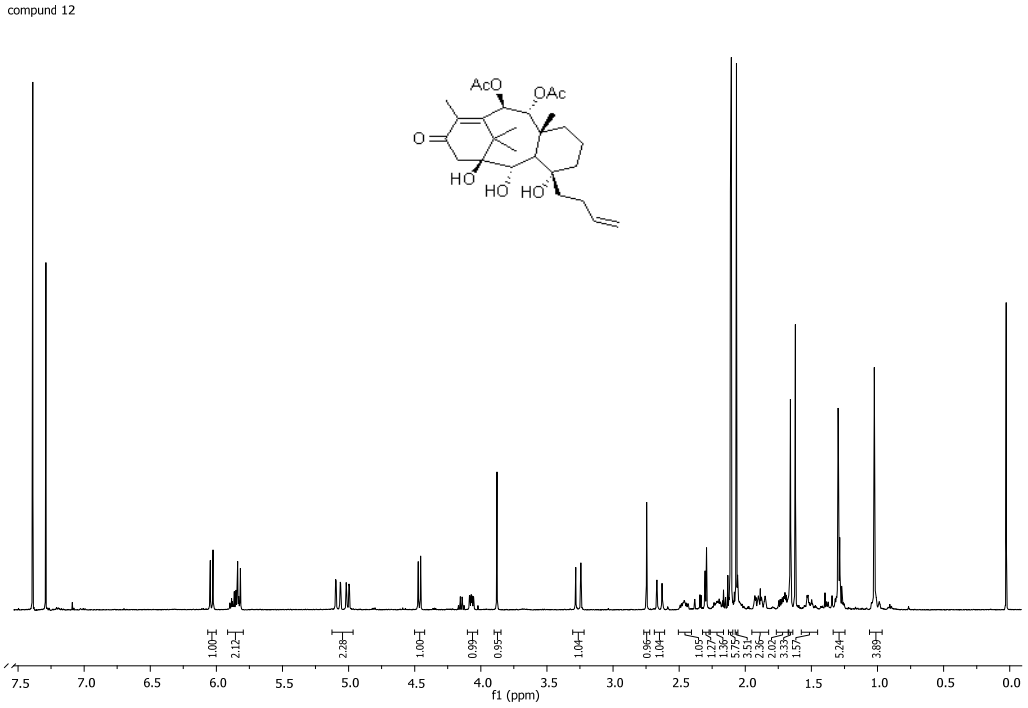

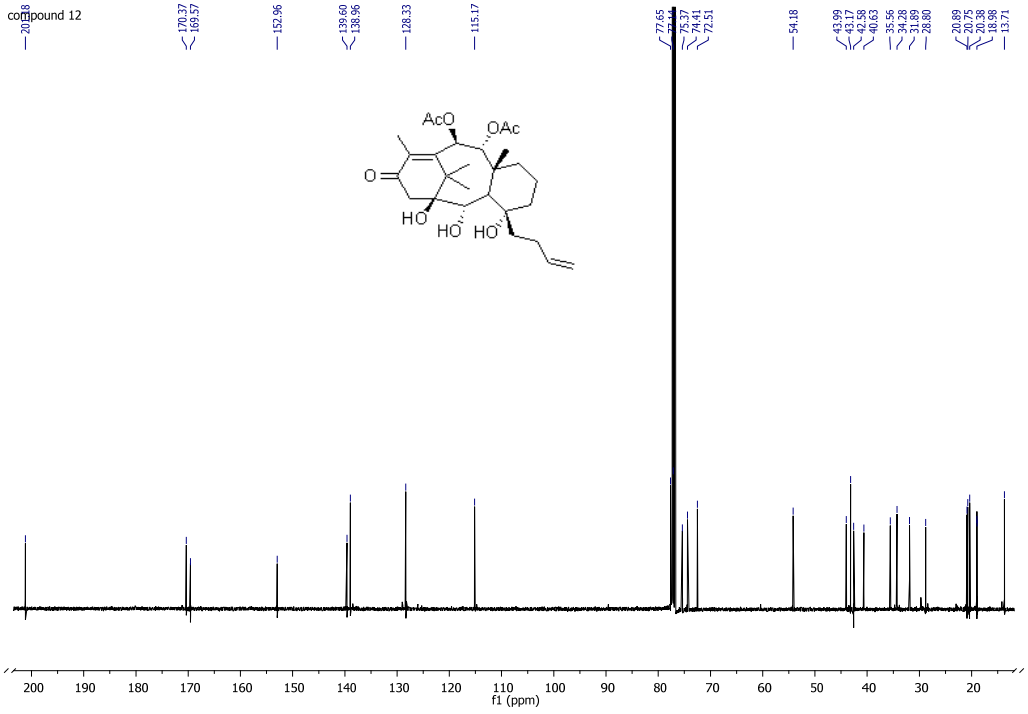

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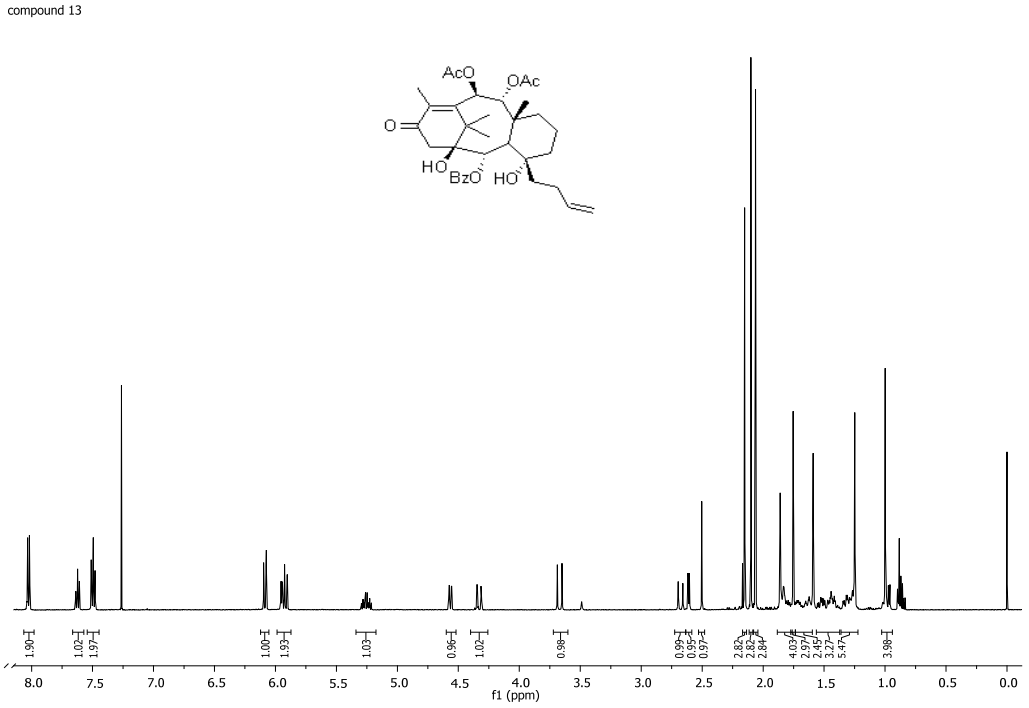

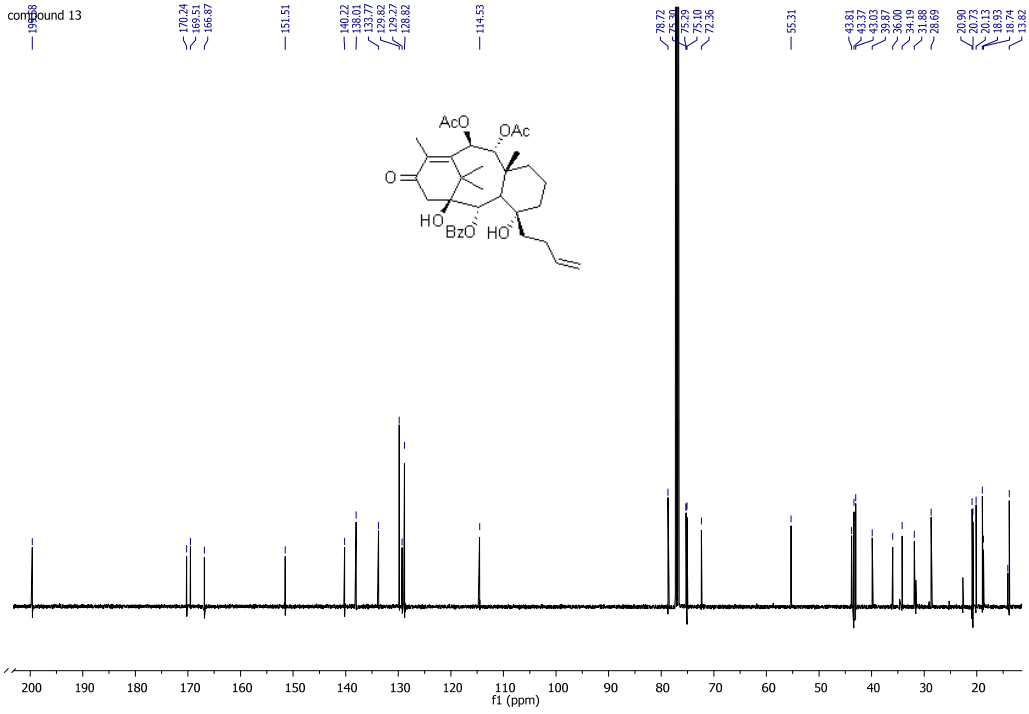

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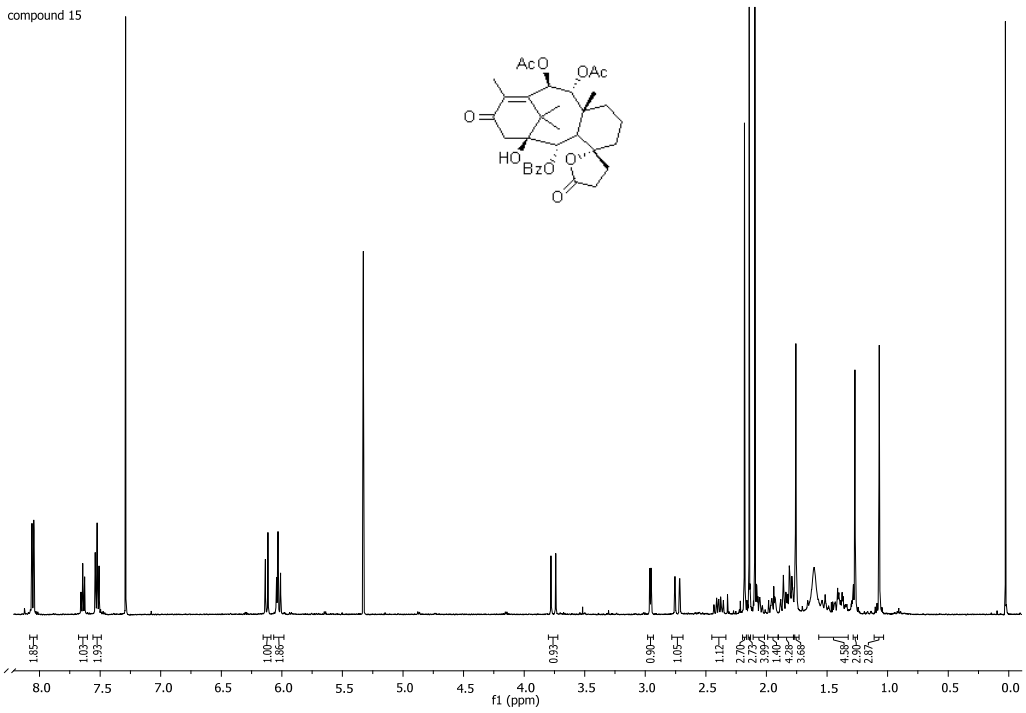
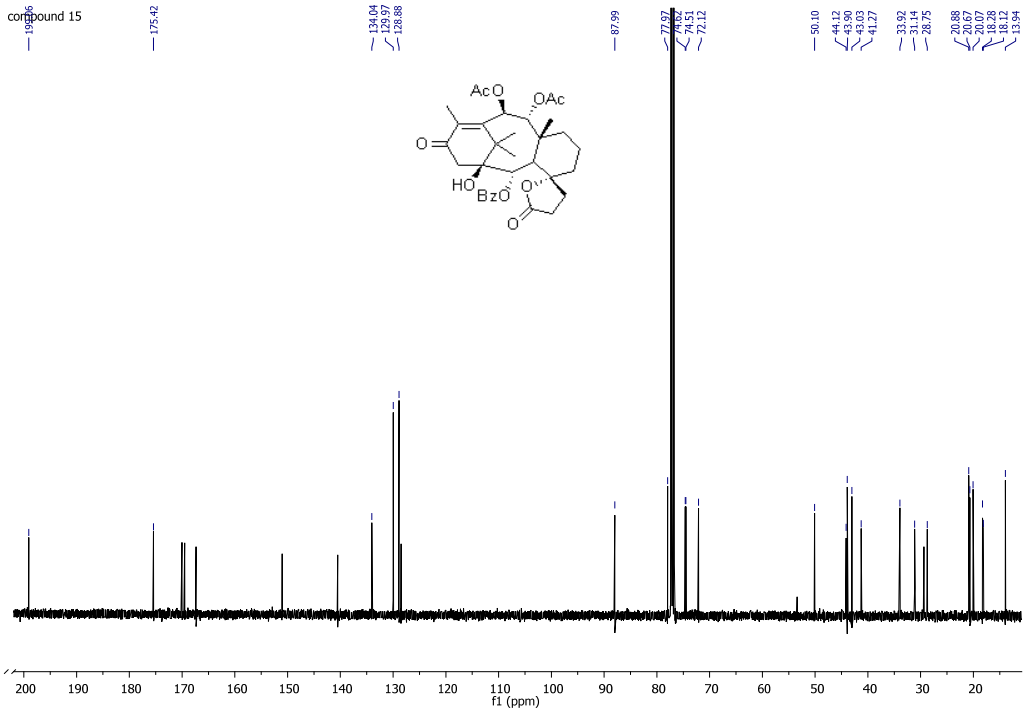
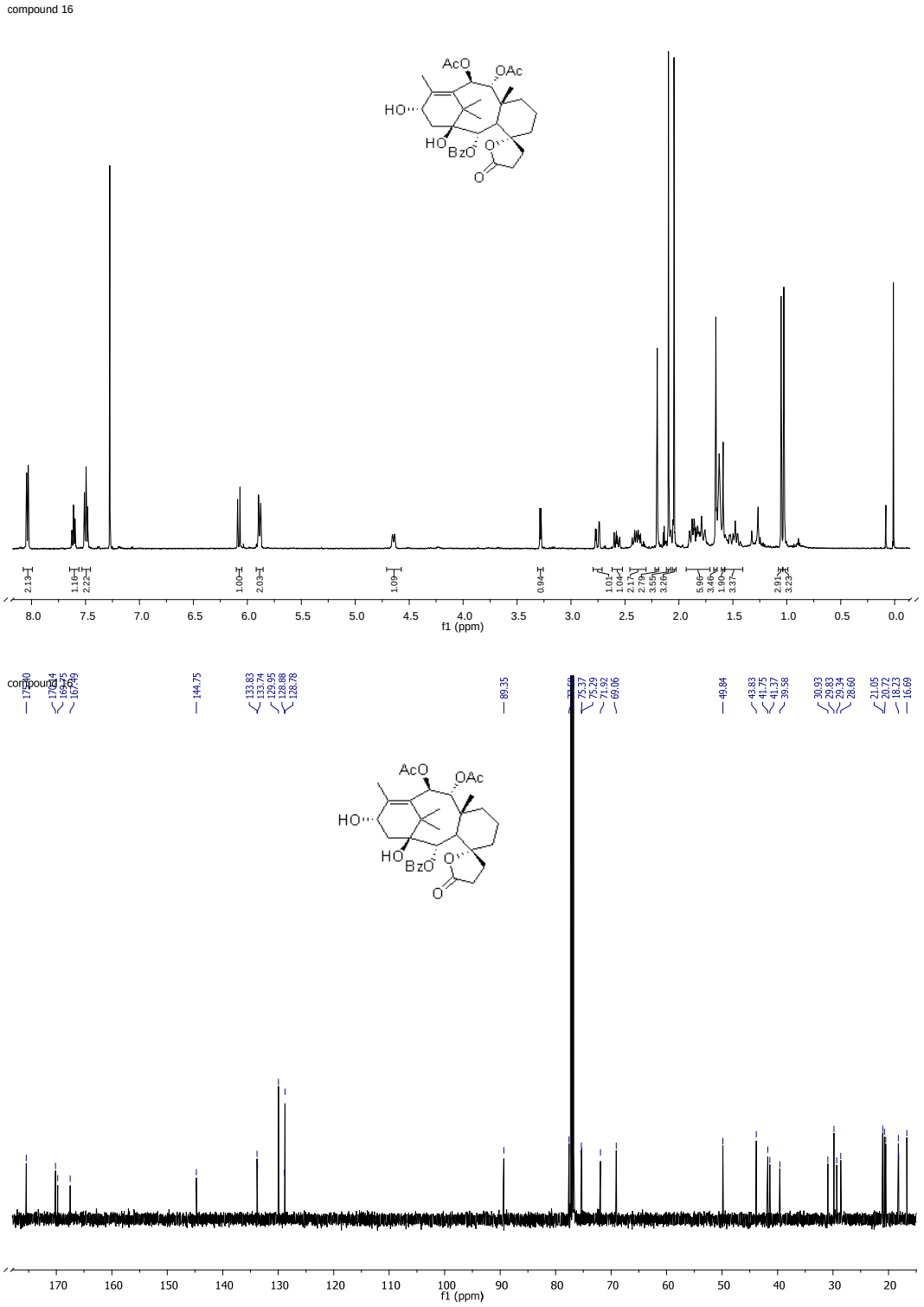
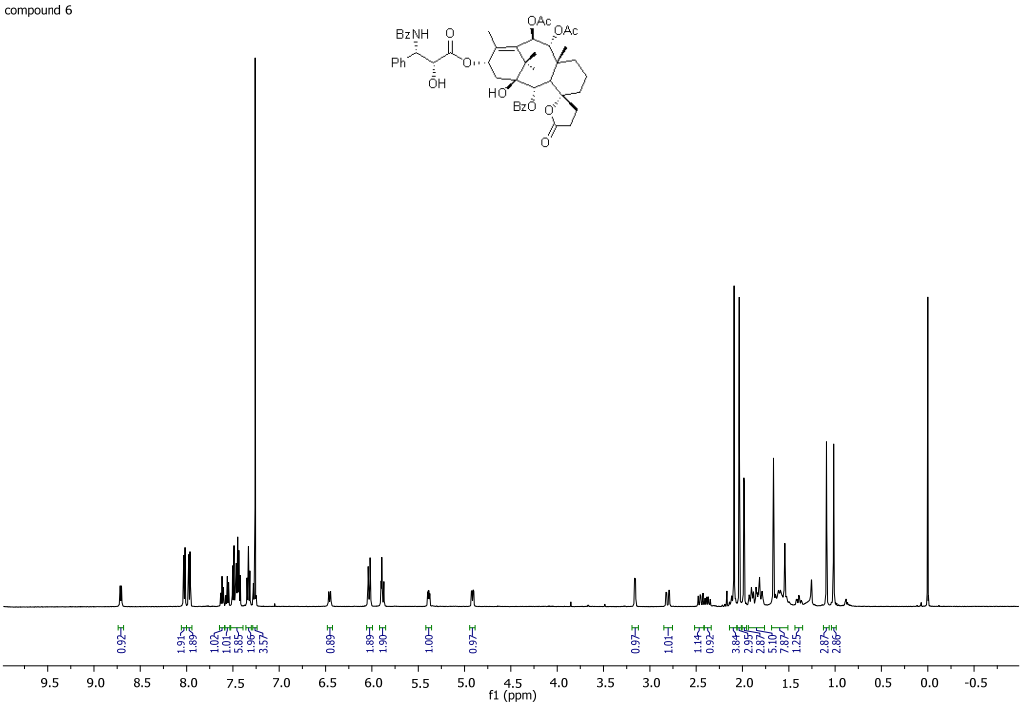

compound 15
AcO
OAc
O
HO
BzO
O
O
1.85
1.03
1.93
1.00
1.86
0.93
0.90
1.05
1.12
2.70
2.57
2.07
1.40
4.28
3.68
4.58
2.90
2.87
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

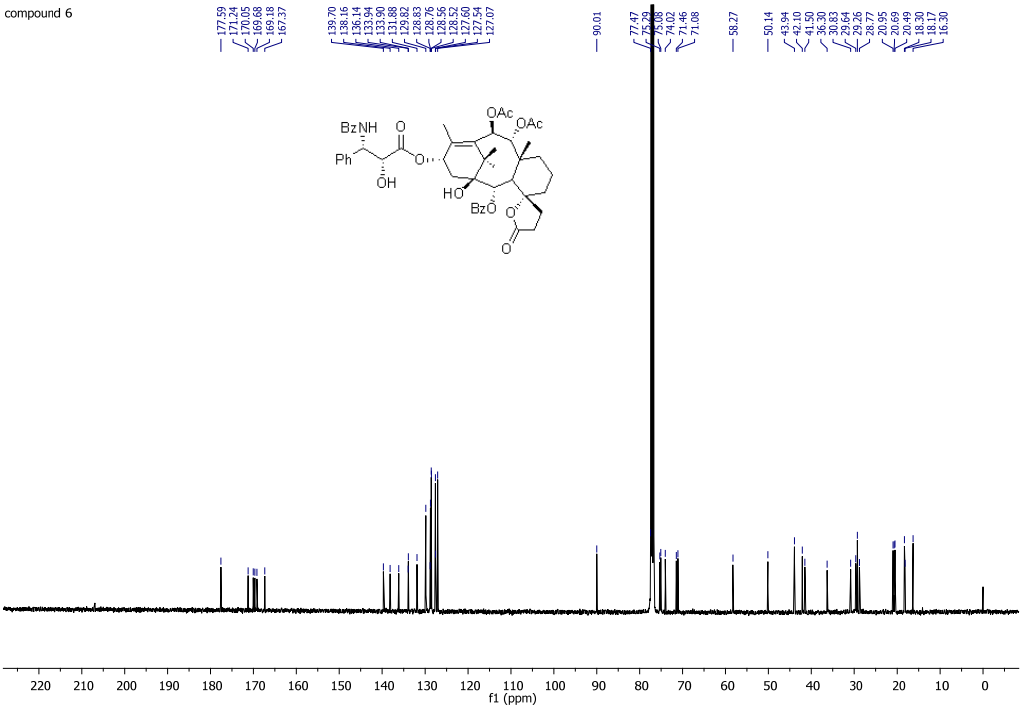

compound 15
196.06
175.42
134.04
129.97
128.88
87.99
77.97
74.62
74.51
72.12
50.10
44.12
43.90
43.03
41.27
33.92
31.14
28.75
20.88
20.67
20.07
18.28
18.12
13.94
AcO
OAc
O
HO
BzO
O
O
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

Compound **16**



Compound **6**


compound 6
BzNH
O
Ph
OH
OAc
OAc
HO
BzO
O
O
0.92
1.91
1.89
1.02
1.01
5.85
1.96
3.57
0.89
1.89
1.98
1.00
0.97
0.97
1.01
1.14
0.92
3.84
2.85
2.87
5.10
7.87
1.25
2.87
2.86
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


compound 6
177.59
171.24
170.05
169.68
169.18
167.37
139.70
138.16
135.14
133.94
133.90
131.88
129.82
128.83
128.76
128.56
128.52
127.60
127.54
127.07
90.01
77.47
75.29
75.08
74.02
71.46
71.08
58.27
50.14
43.94
42.10
41.50
36.30
30.83
29.64
29.26
28.77
20.95
20.69
20.49
18.30
18.17
16.30
BzNH
O
Ph
OH
OAc
OAc
HO
BzO
O
O
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

Tubulin polymerization assay

Effect of compound **6** on tubulin polymerization was determined using method of Gaskin, Cantor and Shelanski,[i] with some modifications. Tubulin was isolated from bovine brain as described previously.[ii] Freshly prepared tubulin solution at approximate concentration of 2.2 mg/ml and MES (2-(*N*-morpholino)ethanesulfonic acid) buffer containing GTP were kept on ice before the experiment. Stock solutions of paclitaxel and compound **6** were prepared in DMSO at concentration of 10^{-2} M, and afterwards diluted with DMSO/H_20 (1:1 *v/v*) to 10^{-3} M. From this solution, the desired concentrations (in range of 1-1000 μM) were prepared in H_2O. Solutions of various concentrations of paclitaxel (positive control) and examined compound **6** (40 μL) were added to a tubulin solution (360 μL) and incubated for 45 minutes at 37 ºC. Mixture of 40 μL MES buffer and 360 of μL tubulin solution was used as blank. After incubation, solutions were transferred to UV cuvettes and absorbance was measured at 350 nm continuously for 15 minutes on an instrument cooled to 4 ºC. Percentage of tubulin polymerization was determined as difference in absorbance at t=0 min (37 ºC) and t=15 min (4 ºC), comparing to corresponding difference for a blank. Effect of paclitaxel and investigated compound **6** on polymerization of purified tubulin was expressed as IC_{50}, i.e. concentration of agents producing 50% tubulin polymerization. UV absorption was measured on a GBC Cintra 40 UV-Visible spectrometer equipped with thermostatic circulator Petrotest 25-0395.

Different concentrations of compound **6** (0.1, 1, 5, 10, and 50 μM) were incubated with tubulin solution and microtubule disassembly was followed turbidimetrically. The results are presented in Figure 1. The IC_{50} value, determined by graphical method, was ~ 9 μM. Paclitaxel, which was used as positive control, showed IC_{50} value of ~ 0.7 μM (Figure 2).

Compound **6**

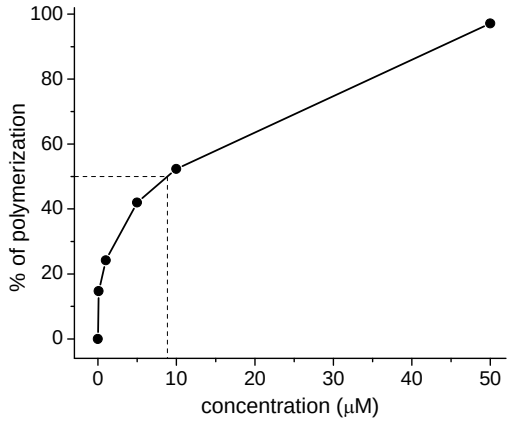


Figure 1. The effect of compound **6** on tubulin polymerization

conc. (μM)	% of polimerization
0.1	14.73
1	24.19
5	41.99
10	52.32
50	97.16

Paclitaxel

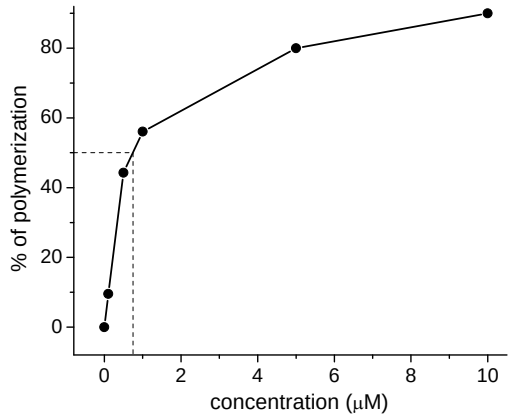


Figure 2. The effect of paclitaxel on tubulin polymerization

conc. (μM)	% of polimerization
0.1	9.5
0.5	44.25
1	56.07
5	79.97
10	89.99

Cytotoxicity of paclitaxel and compound **6** in L929 mouse fibrosarcoma cells

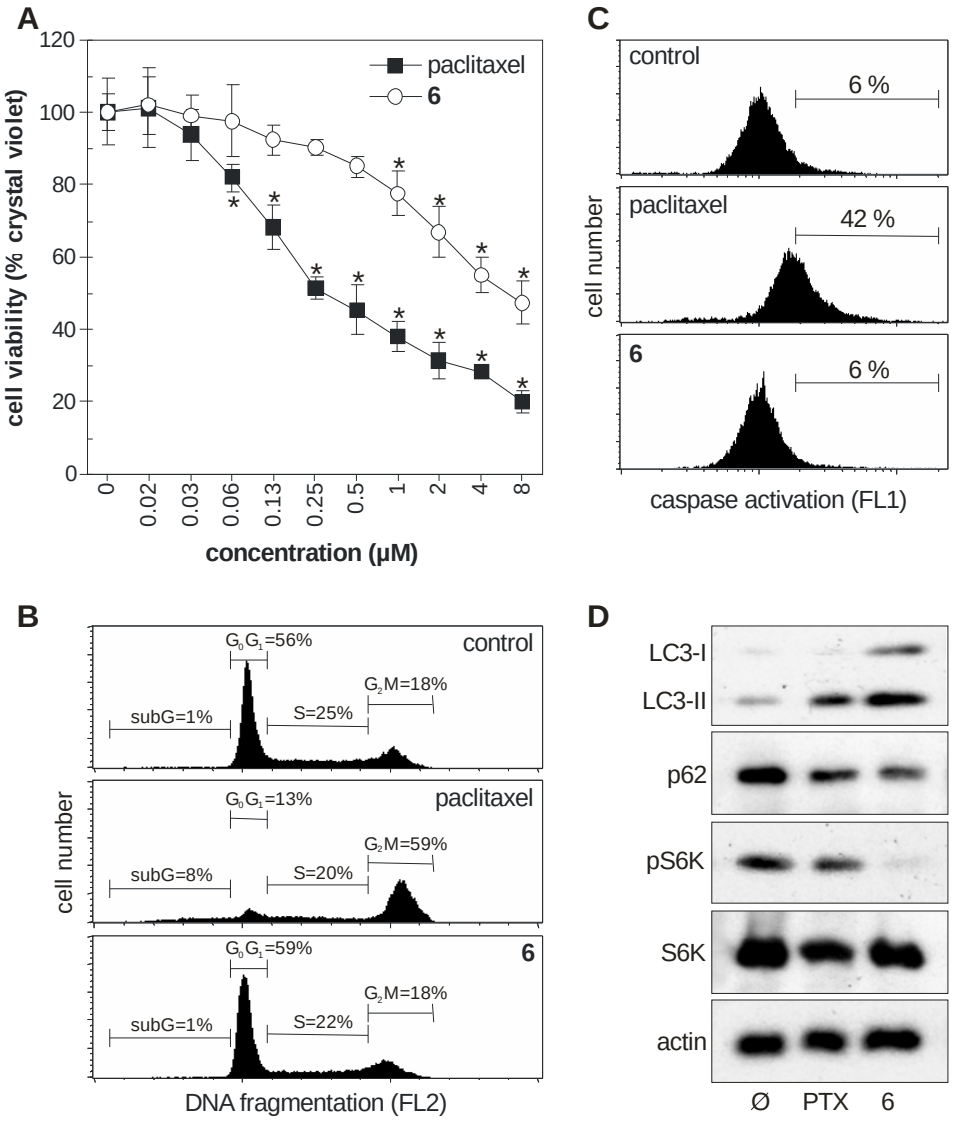


Figure 3. Cytotoxicity of paclitaxel and compound **6** in L929 mouse fibrosarcoma cells. (A) L929 cells were incubated with different concentrations of paclitaxel or compound **6** for 24 h and cell viability was assessed by crystal violet staining. Data are mean ± SD of triplicate measurements (*p < 0.05). (B-D) L929 cells were treated with paclitaxel (0.5 µM) or compound **6** (4 µM). Cell cycle distribution (B) and caspase activation (C) were determined by flow cytometry after 24 h, while immunoblot analysis of LC3 conversion, p62 and phospho-S6K (pS6K) levels was performed after 16 h of incubation (D).

[i]Gaskin, F.; Cantor, C. R.; Shelanski, M. L. Turbidimetric studies of the *in vitro* assembly and disassembly of porcine neurotubules. *J. Mol. Biol* **1974,** *89*, 737–755.
[ii]Shelanski, M. L.; Gaskin, F.; Cantor, C. R. Microtubule Assembly in the Absence of Added Nucleotides. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 765–768.