SUPPLEMENTARY MATERIAL

Synthesis and antitumor activity of camptothecin-4β-triazolopodophyllotoxin conjugates

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Abstract: Two compounds (**9** and **10**) having a camptothecin (CPT) analog conjugated to the 4β-azido-4-deoxypodophyllotixin analog by untilizing the copper-catalyzed azide-alkyne cycloadditon (CuAAC) reaction, and were evaluated for their cytotoxicity against a panel of five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7 and SW480) using the MTT (3-(4,5-dimethyl-thiahiazo-2-yl)-2,5-diphenyltetrazolium bromide) assay. Two novel conjugates shown weak cytotoxicity, compound **10** showed highly potent against HL-60 cell line tested, with IC_{50} value 17.69±0.19 μM. This compound suggested its potential as anticancer agents for further development.

Keywords: antitumor activity; CuAAC reaction; camptothecin; podophyllotixin

1. Experimental

1.1. General information

Melting points were measured by an X-4 melting point apparatus and were uncorrected. MS data were obtained in the ESI mode on API Qstar Pulsar instrument; HRMS data were obtained in the ESI mode on LCMS-IT-TOF (Shimadzu, Kyoto, Japan); NMR spectra were acquired on Bruker AV-400 (Bruker BioSpin GmbH, Rheinstetten, Germany) instruments, using tetramethylsilane (TMS) as an internal standard: chemical shifts (δ) are given in ppm, coupling constants (J) in Hz, the solvent signals were used as references ($CDCl_3$: δ_C = 77.2 ppm; residual $CHCl_3$ in $CDCl_3$: δ_H = 7.26 ppm; CD_3OD: δ_C = 49.0 ppm; residual CH_3OH in CD_3OD: δ_H = 4.78 ppm). Column chromatography (CC): silica gel (200 – 300 mesh; Qingdao Makall Group CO., LTD; Qingdao; China). All reaction was monitored using thin-layer chromatography (TLC) on silica gel plates.

1.2. General procedure for the synthesis of 4β-azido-podophyllotoxins 7 and 8

To a solution of podophyllotoxin **1** (5 mmol) in dry dichloromethane (CH_2Cl_2, 50 mL), sodium iodide (NaI, 15 mmol) was added and stirred for 5 min. To this stirred suspension $MeSO_3H$ (15 mmol) was added dropwise with syringe at 0 ºC and the stirring was continued for another 5 h at room temperature. Nitrogen was bubbled through the solution to drive off the excess hydrogen iodide. This solution was then evaporated *in vacuo* and used for the next reaction without further purification. To the above crude product a mixture of H_2O-acetone (50 mL, 1:1) and anhydrous barium carbonate ($BaCO_3$, 10 mmol) were added successively. After 30 min at 40 ºC, the resultant mixture was diluted with CH_2Cl_2 (100 mL), then poured into 10% Sodium thiosulfate (NaS_2O_4) solution (500 mL). The organic layer over sodium sulfate

(Na_2SO_4) and the solvent removed *in vacuo*. The residue was dried *in vacuo* (Kanal et al. 2003; Hansen et al. 1993). To a solution of residue (1.0 mmol) and sodium azide (5.0 mmol)) in trichloromethane (4 mL) was added trifluoroacetic acid (TFA, 13.2 mmol) dropwise. The reaction mixture was stirred for 1 h, but in order to avoid gel formation during the reaction it was necessary to add further TFA (52.8 mmol). The solution was neutralized with aqueous saturated sodium bicarbonate ($NaHCO_3$). The phases were separated. The aqueous phase was extracted twice with $CHCl_3$ (20 mL). The combined organic phases were washed with water and dried over Na_2SO_4. Then the solvent was evaporation and the reaction mixture was chromatographed on silica gel to afford the product (Kuhn et al. 1969; Hansen et al. 1993).

1.2.1. 4β-Azido-4-deoxypodophyllotoxin (7)

Yield 60 %. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.05 (s, 1H, C^8-H), 6.63 (s, 1H, C^5-H), 6.35 (s, 2H, $C^{2'}$, $C^{6'}$-H), 6.05 (d, 2H, J = 3.6 Hz, OCH_2O), 5.11 (d, 1H, J = 3.5 Hz, C^4-H), 4.65 (d, 1H, J = 5.3 Hz, C^1-H), 4.36 (dd, 1H, J = 8.3 Hz, 7.4 Hz, C^{11}-CH_β), 4.22 (dd, 1H, J = 8.6 Hz, 10.2 Hz, C^{11}-CH_α), 3.67 (s, 6H, 3', 5'-OCH_3), 3.66 (s, 3H, 4'-OCH_3), 3.21 (dd, 1H, J = 5.3 Hz, 14.0 Hz, C^2-H), 3.12 (m, 1H, C^3-H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 174.5 (C-12), 153.5 (C-3'), 153.5 (C-5'), 149.6 (C-7), 148.0 (C-6), 136.6 (C-1'), 136.6 (C-4'), 133.5 (C-9), 128.5 (C-10), 111.4 (C-5), 109.7 (C-8), 109.4 (C-2'), 109.4 (C-6'), 102.7 (OCH_2O), 68.2 (C-11), 66.2 (C-4), 56.3 (3', 5'-OCH_3), 44.5 (C-1), 41.6 (C-2), 37.8 (C-3); MS-ESI *m/z* (%): 462 ($[M+Na]^+$, 100).

1.2.2. 4β-Azido-4-deoxy-4'-demethypodophyllotoxin (8)

Yield 40%. ^{1}H-NMR ($CDCl_3$, 400MHz) δ 7.07 (s, 1H, C^5-H), 6.60 (s, 1H, C^8-H), 6.38

(s, 2H, $C^{2'}$, $C^{6'}$-H), 6.05 (d, 2H, *J* = 0.6 Hz, OCH_2O), 4.61 (q, 2H, *J* = 3.7 Hz, 5.3 Hz, C^4-H, C^1-H), 4.36 (dd, 2H, *J* = 8.5 Hz, 10.3 Hz, C^{11}-CH_2), 3.66 (s, 6H, $C^{3'}$, $C^{5'}$-OCH_3), 3.11 (dd, 1H, *J* = 4.7 Hz, 14.1 Hz, C^2-H), 2.96 (m, 1H, C^3-H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 174.3 (C-12), 148.9 (C-3'), 148.6 (C-5'), 148.0 (C-7), 148.0 (C-6), 136.0 (C-1'), 133.9 (C-4'), 131.2 (C-9), 129.4 (C-10), 110.8 (C-5), 109.2 (C-2'), 109.2 (C-6'), 107.5 (C-8), 102.6 (OCH_2O), 70.9 (C-11), 63.9 (C-4), 56.4 ($C^{3'}$, $C^{5'}$-OCH_3), 45.7 (C-1), 44.4 (C-2), 38.6 (C-3); MS-ESI m/z (%): 448 ($[M+Na]^+$, 100).

Figure S1. ^{1}H-NMR of compound 6

Figure S2. ^{13}C-NMR of compound 6

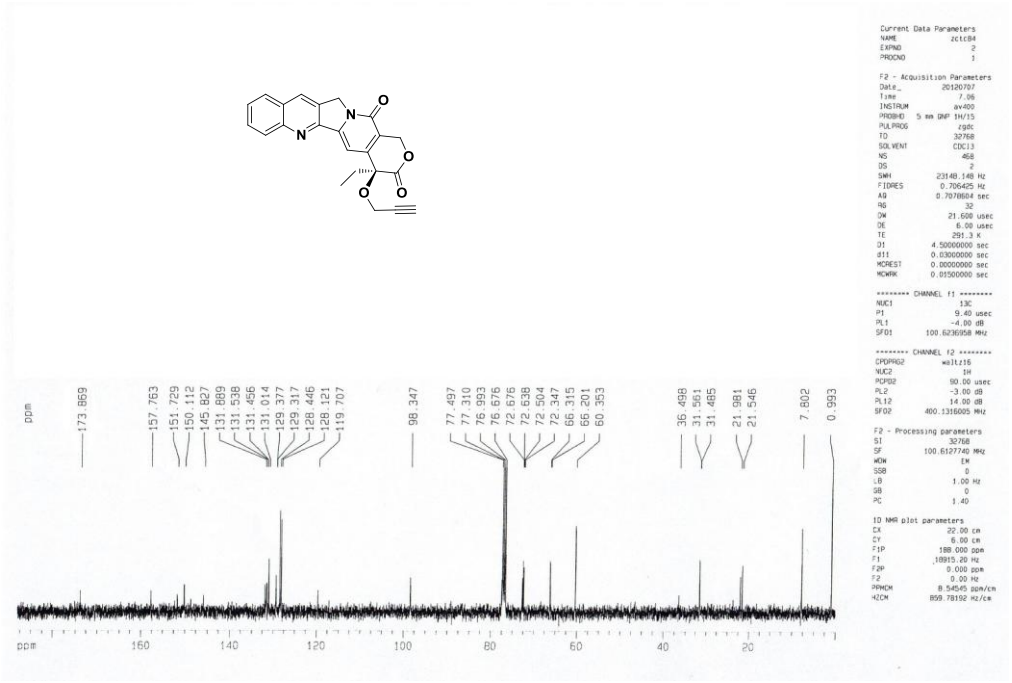


Figure S3. ^{1}H-NMR of compound 7

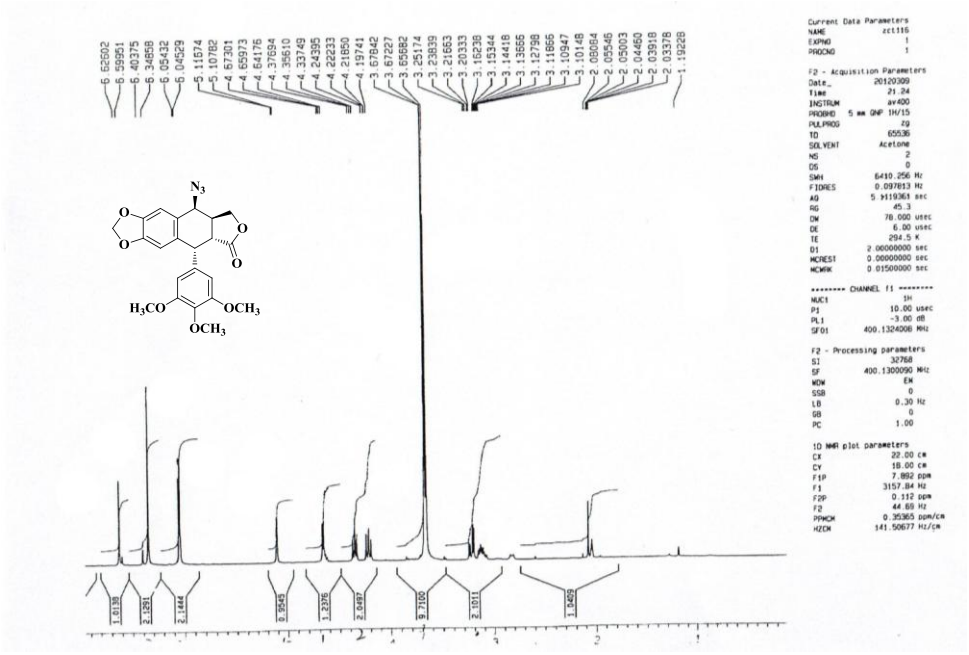


Figure S4. ^{13}C-NMR of compound 7

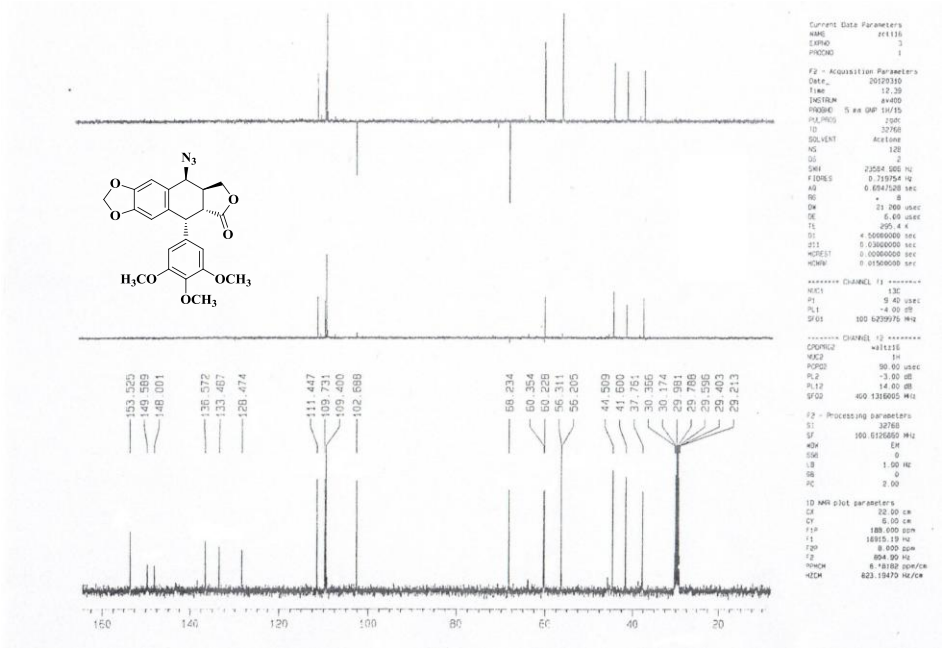


Figure S5. ^{1}H-NMR of compound 8

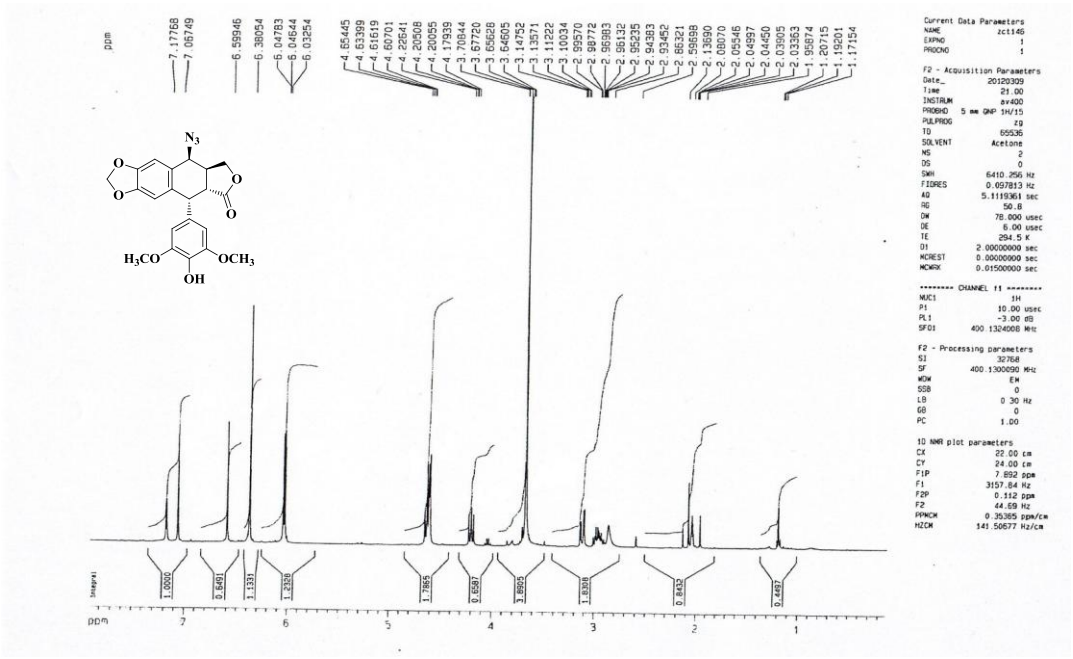


Figure S6. ^{13}C-NMR of compound 8

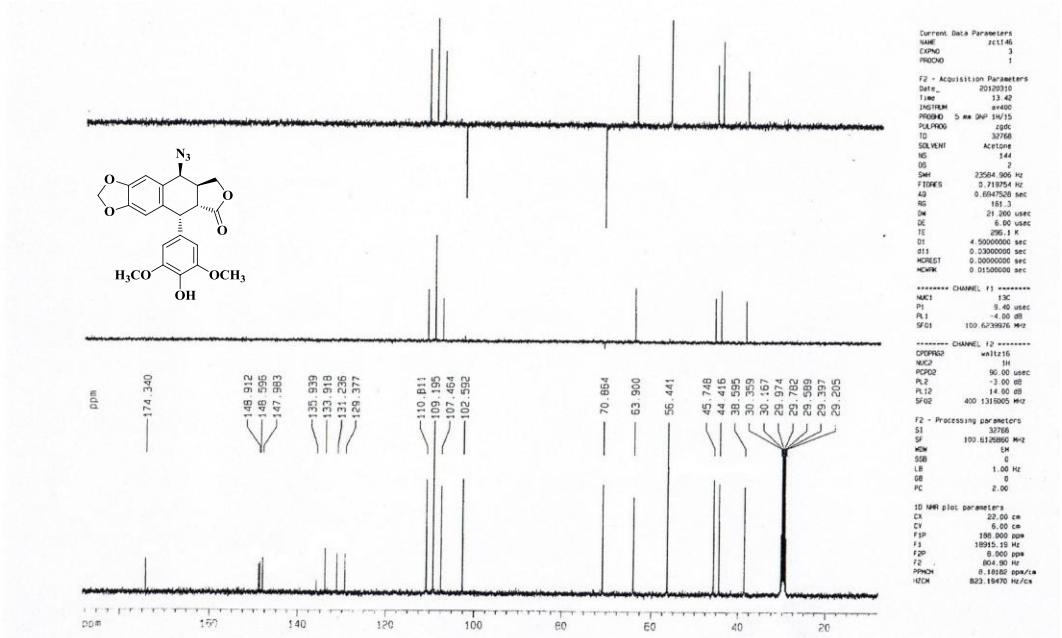
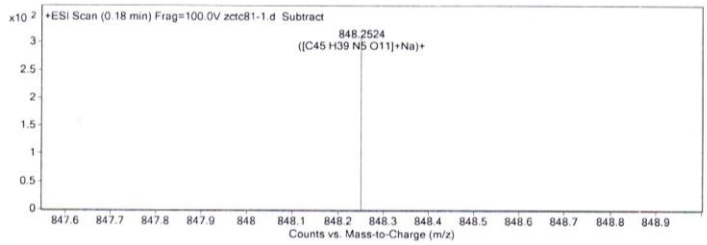


Figure S7. HRESIMS of compound 9

Sample Group	**Info.**
Acquisition SW	6200 series TOF/6500 series
Version	Q-TOF B.05.01 (B5125.2)

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
100	0	ESI



Peak List

m/z	z	Abund
74.0968	1	43194.46
102.128	1	1944371.38
103.1314	1	127977.78
104.1071	1	63046.23
118.1226	1	25823.19
239.2249	1	38085.93
274.2744	1	82975.98
318.3006	1	76268.47
362.3267	1	27639.64
384.3089	1	23752.78

Formula Calculator Element Limits

Element	Min	Max
C	3	120
H	0	240
O	0	30
N	0	10

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C45 H39 N5 O11	825.2646	848.2538	848.2524	1.40	1.65	29.0000

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Figure S8. ^{1}H-NMR of compound 9

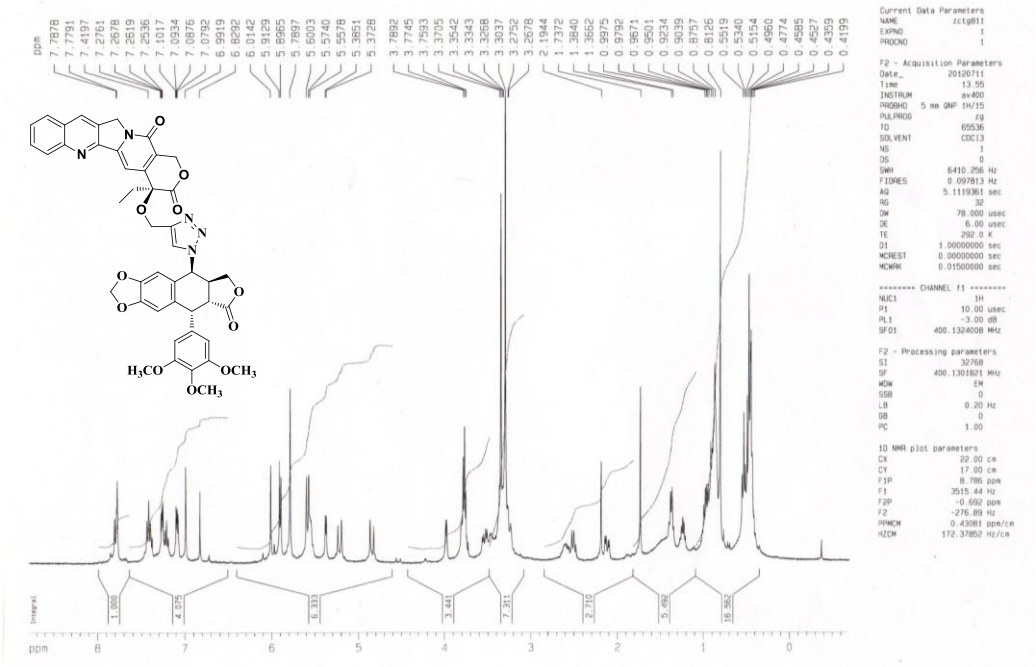


Figure S9. ^{13}C-NMR of compound 9

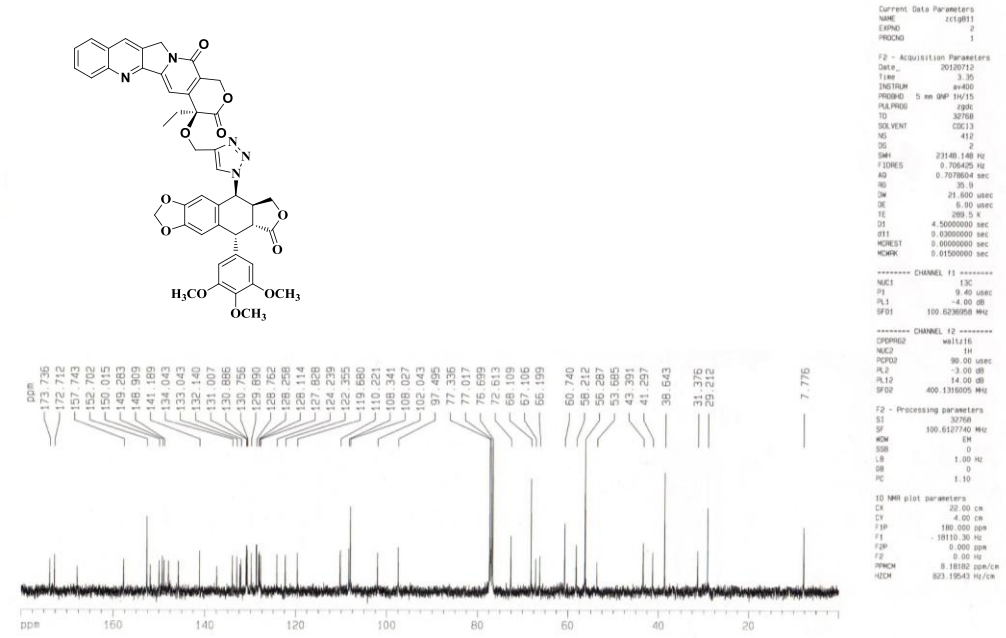
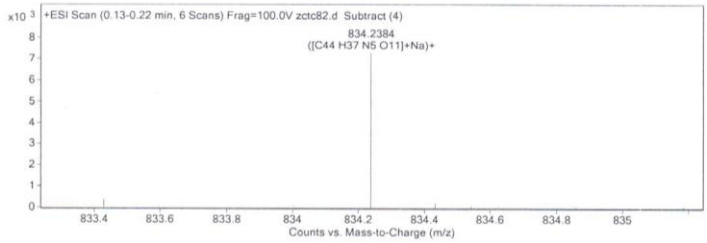


Figure S10. HRESIMS of compound 10

Sample Group	Info.
Acquisition SW	6200 series TOF/6500 series
Version	Q-TOF B.05.01 (B5125.2)

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
100	0	ESI



Peak List

m/z	z	Abund	Formula	Ion
102.128	1	28188.65		
104.1071	1	22662.71		
219.6485	2	6946.79		
381.2982	1	27493.08		
382.3014	1	6709.22		
397.272	1	22828.44		
398.2752	1	5794.45		
812.2562	1	4919.41		
834.2384	1	7335.27	C44 H37 N5 O11	(M+Na)+
850.2148	1	4312.62		

Formula Calculator Element Limits

Element	Min	Max
C	3	120
H	0	240
O	0	30
N	0	10

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C44 H37 N5 O11	811.2490	834.2382	834.2384	-0.20	-0.24	29.0000

--- End Of Report ---

Figure S11. ^{1}H-NMR of compound 10

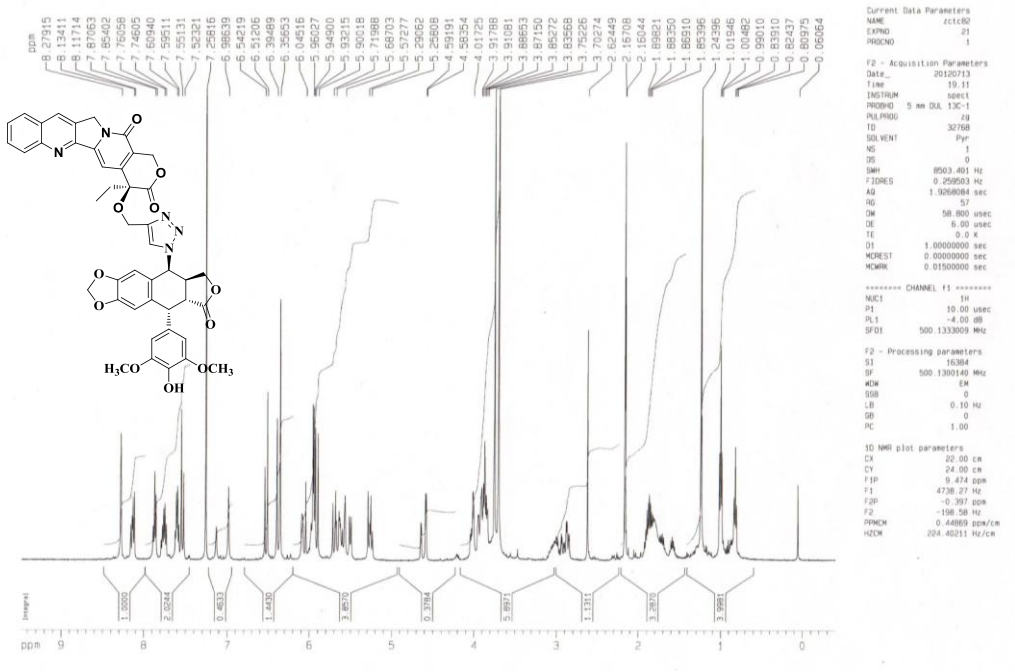
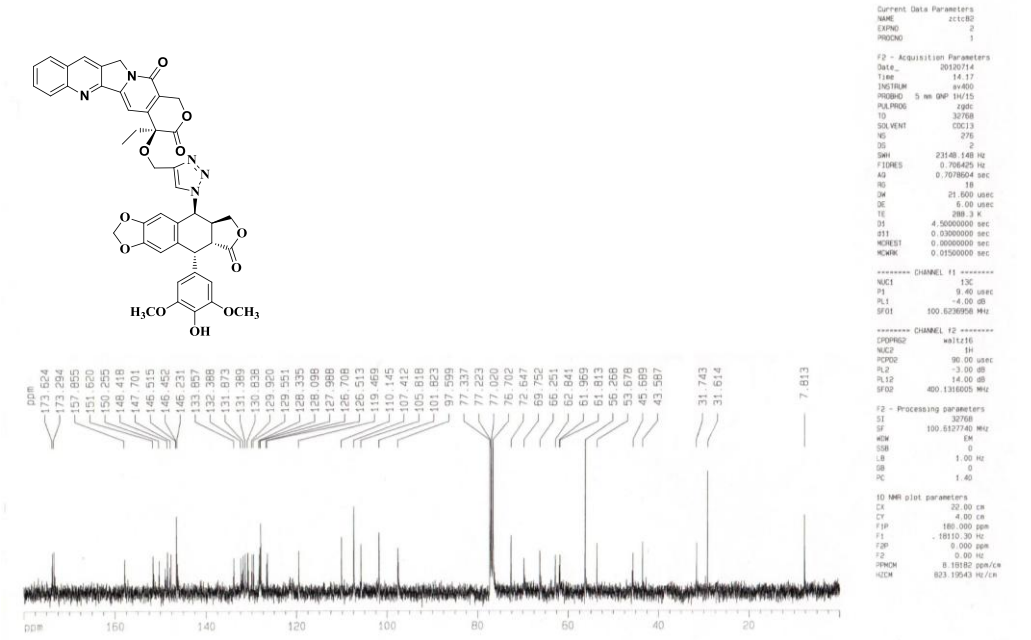


Figure S12. ^{13}C-NMR of compound 10



References

Hansen HF, Jesen RB, Willumsen AM, Norsko-Lauritsen N, Ebbesen P, Nielen PE, Buchardt O. 1993. New compounds related to podophyllotoxin and congeners: Synthesis, structure elucidation and biological testing. Acta. Chem. Scand. 47:1190–1200.

Kamal A, Kumar BA, Arifuddin M. 2003. A one-pot, efficient and facile synthesis of 4-arylaminopodophyllotoxins: synthesis of NPF and GL-331 as DNA topoisomerase II inhibitors. Tetrahedron Lett. 44(46):8457–8459.

Kuhn M, Kellero-Juslén C, von Wartburg A. 1969. Partialsynthese von 4′-Demethylepipodophyllotoxin. 22. Mitteilung über mitosehemmende Naturstoffe [1]. Helv. Chim. Acta 52(4):944–947.