Supporting Information

Contribution of Cage-shaped Structure of Physalins to their Mode of Action in Inhibition of NF-κB Activation

Masaaki Ozawa,[†,‡] Masaki Morita,[†,§] Go Hirai,[†,‡] Satoru Tamura,[†,ǁ] Masao Kawai,[⊥,#] Ayako Tsuchiya,[†] Kana Oonuma,[†,‡] Keiji Maruoka[§] and Mikiko Sodeoka[*,†,‡,ǁ]

[†]Synthetic Organic Chemistry Laboratory, RIKEN, 2-1 Hirosawa, Wako, Saitama, 351-0198, Japan

[‡]RIKEN Center for Sustainable Resource Science, 2-1 Hirosawa, Wako, Saitama, 351-0198, Japan

[§]Department of Chemistry, Graduate School of Science, Kyoto University, Sakyo, Kyoto 606-8502, Japan

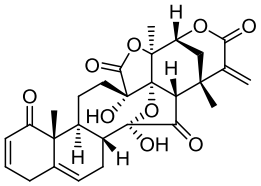
[ǁ]ERATO-JST, 2-1 Hirosawa, Wako, Saitama, 351-0198, Japan

[⊥]Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya, Aichi, 466-8555, Japan

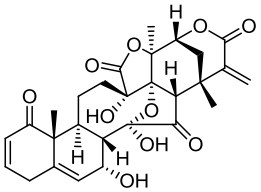
[#]Nakanoshima Science Laboratory, Osaka Science Museum, 4-2-1 Nakanoshima, Kita-ku, Osaka, 530-0005, Japan

Contents

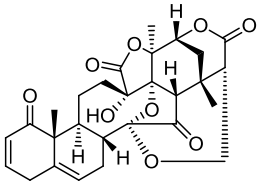
Compound List of Oxygenated Steroids



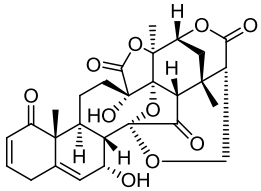
PA-1 (Physalin C)



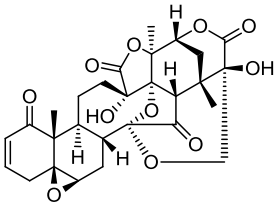
PA-2 (Physalin A)



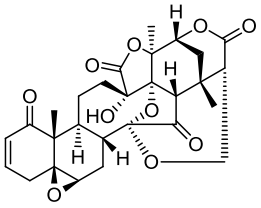
PB-1 (Physalin B)



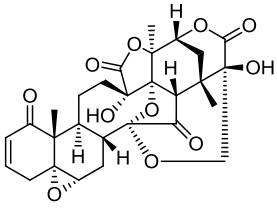
PB-2 (Physalin N)



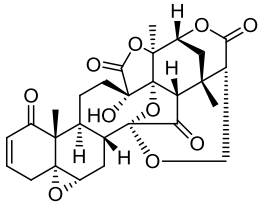
PB-3 (25-OH-physalin F)



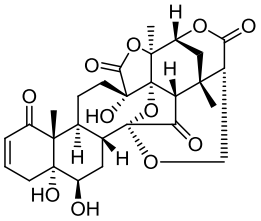
PB-4 (Physalin F)



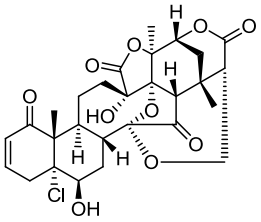
PB-5 (25-OH-physalin J)



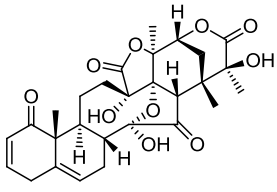
PB-6 (Physalin J)



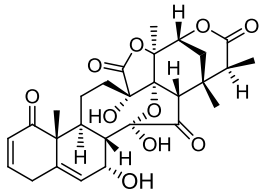
PB-S1 (Physalin D)



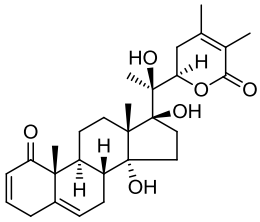
PB-S2 (Physalin H)



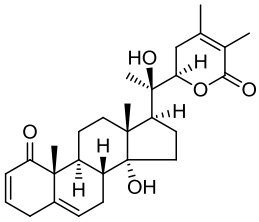
PC-1 (Physalin **III**)



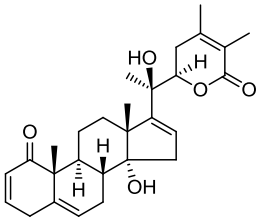
PC-2 (Physalin O)



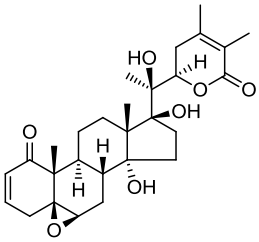
Wit-1 (Withanolide F)



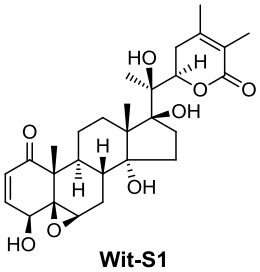
Wit-2 (Withanolide G)



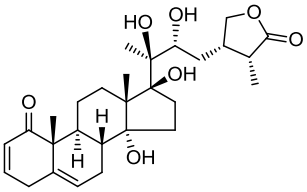
Wit-3 (Δ^{16}-withanolide)



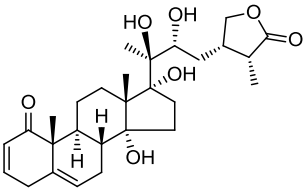
Wit-4 (Withanolide E)



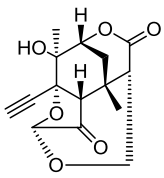
Wit-S1
(4β-hydroxywithanolide E)



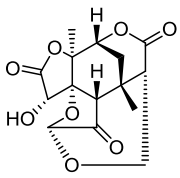
Per-1 (Perulactone B)



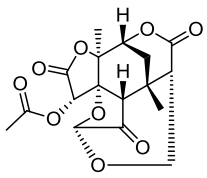
Per-2 (Perulactone H)



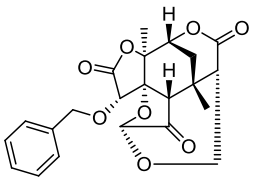
PBright-1



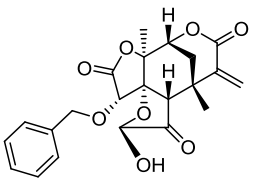
PBright-2



PBright-3



PBright-4

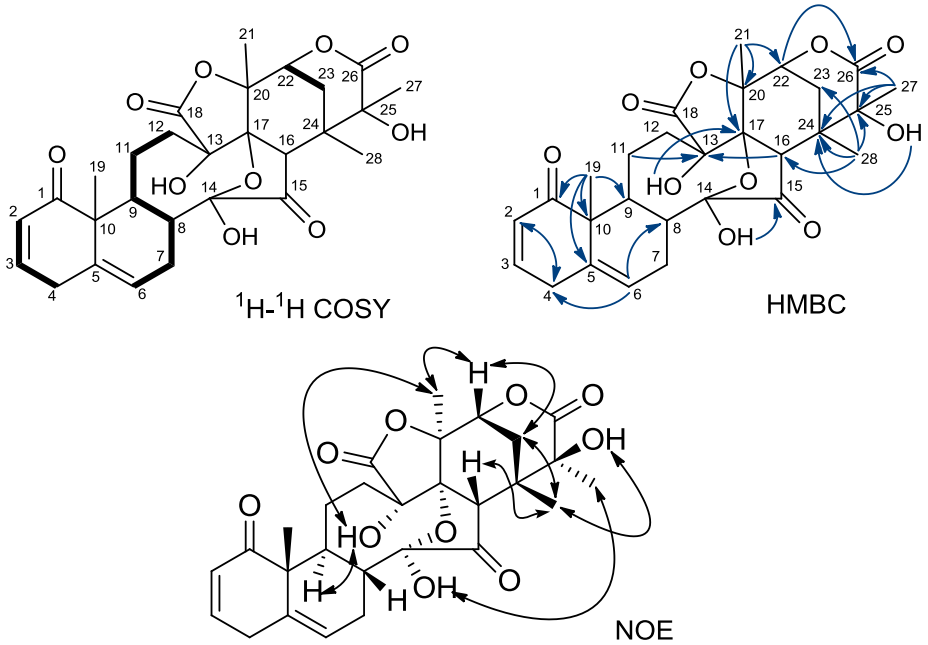


PAright-1

Structural Analysis for PC-1 (Physalin III)

Table S1. ^{1}H and ^{13}C NMR (in DMSO-d_6 at 500 MHz and 125 MHz) of PC-1.

position	δ_H (J in Hz)	δ_C	position	δ_H (J in Hz)	δ_C
1		202.6	15		215.3
2	5.79 dd (10.3, 2.3)	127.0	16	2.78 s	51.4
3	6.90 ddd (10.3, 4.6, 2.3)	146.5	17		82.2
4	3.26 m	32.2	18		172.4
	2.89 dd (21.8, 4.6)		19	1.05 s	16.2
5		135.8	20		82.8
6	5.61 br d (6.3)	124.4	21	1.74 s	20.7
7	2.31 m	25.9	22	4.42 d (4.6)	76.6
	2.01 m		23	2.71 dd (14.3, 4.6)	27.0
8	1.88 m	42.0		1.39 d (14.3)	
9	2.82 dd (11.5, 7.5)	34.5	24		41.1
10		52.6	25		72.6
11	1.95 m	23.2	26		169.9
	1.13 m		27	1.36 s	19.5
12	2.23 m	28.9	28	1.43 s	22.4
	1.87 m		13-OH	6.07 s	
13		79.2	14-OH	6.42 s	
14		100.9	25-OH	5.91 s	

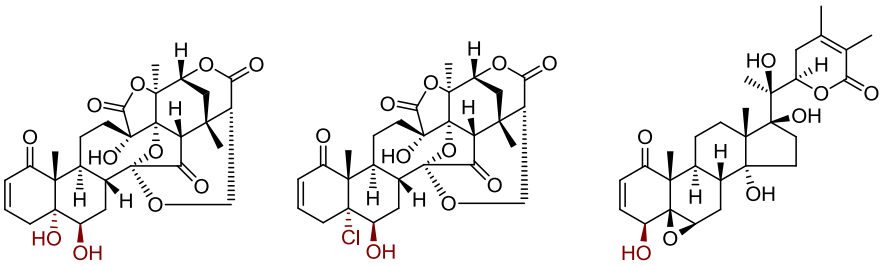


$[\alpha]_D^{27}$ –127.5 (*c* 0.04, $CHCl_3$-MeOH (1:1)); IR (cm^{-1}): 3554-3239(br), 2960, 2924, 2852, 1776, 1756, 1733, 1659, 1455, 1382, 1261, 1168, 1088, 1062, 1024, 803; HRMS-ESI (*m*/*z*): $[M+Na]^+$ calcd for $C_{28}H_{32}NaO_{10}$, 551.1893; found, 551.1888.

Figure S1. Structural Analysis of physalin III (PC-1).

Inhibition of NF-κB Activation and IκBα Phosphorylation by Oxygenated Steroids PB-S1, PB-S2, and Wit-S1

Figure S2. Inhibitory activities of PB-S1, PB-S2, and Wit-S1 for NF-κB Activation and IκBα Phosphorylation



IC_{50} values [μM]	**PB-S1** (Physalin D)	**PB-S2** (Physalin H)	**Wit-S1** (4β-hydroxywitanolide E)
Inhibition of NF-κB Activation[a]	> 50	15.3 (3.1)	7.3 (2.4)
Inhibition of IκBα Phosphorylation	> 50	> 50	> 50

[a]IC_{50} values were measns of three independent experiments. SD values are shown in parenthesis.

Structure of Withaferin A

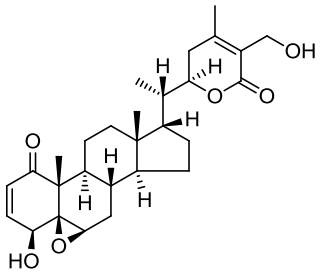


Figure S3. Structure of Withaferin A.

Inhibition of IκBα Degradation by 5β,6β-epoxy Derivatives

As shown in Figure 2A and S4-a, the 5β,6β-epoxy derivative potently inhibited IκBα degradation stimulated by TNF-α. The time course of total IκBα proteins was quantified using the DuoSet® ELISA method (the ELISA system had to be changed because production of the PathScan® ELISA system had been discontinued). Due to the difference in sensitivity of the DuoSet® ELISA, the experimental conditions were slightly changed. HeLa cells were stimulated with TNF-α (***250 ng/mL***) for various times (10, 20, 30, 60, and 120 min) after pretreatment either with test compounds (final 50 μM), MG-132 (proteasome inhibitor) or vehicle (DMSO) for 30 min. TNF-α alone induced IκBα degradation within 20 minutes, and the quantity of IκBα proteins returned to the initial level by 120 minutes after TNF-α stimulation. Pretreated MG-132 (final 10 μM) blocked the degradation of phosphorylated IκBα. **Wit-4** (withanolide E) having the 5β,6β-epoxide structure showed comparable inhibition to MG-132. The 5β,6β-epoxy derivative **PB-4** (physalin F) showed slightly weaker potency (Figure 2A and Figure S4-a), while the C5-C6 olefin derivatives **PA-1** (physalin C) and **PB-1** (physalin B) had little effect. All compounds having inhibitory activity for the degradation of IκB were 5,6-epoxy derivatives. These 5,6-epoxy derivatives have the potential to irreversibly inhibit the phosphorylation and degradation of IκB by forming covalent bond with their target proteins. In contrast, inhibition of the phosphorylation of IκB by **PA-1** or **PB-1** may be reversible. Therefore, these compounds could not inhibit the degradation of IκB proteins efficiently.

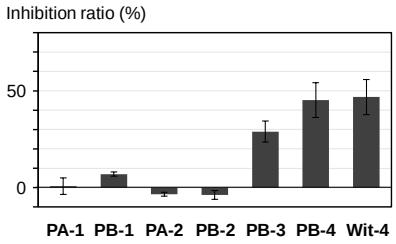


Figure S4-a. Inhibition of IκBα degradation (same results as in Figure 2A). HeLa cells were stimulated with **TNF-α (50 ng/mL)** for 12.5 min after pretreatment either with various concentrations of test compound (50 μM) or DMSO for 30 min. Total IκBα proteins were quantified using the PathScan® ELISA method.

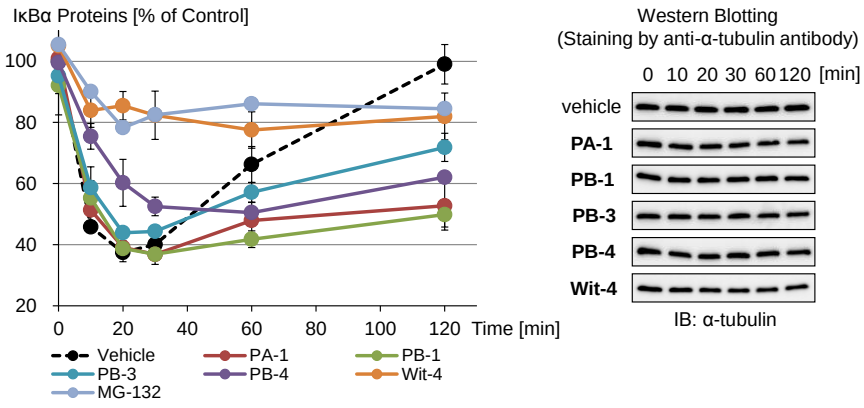


Figure S4-b. Time course of total IκBα. HeLa cells were stimulated with **TNF-α (250 ng/mL)** for various times after pretreatment either with test compound (50 μM), MG-132 (10 μM, proteasome inhibitor) or DMSO for 30 min. In each case, the control value was the value of "vehicle" at t = 0. Total IκBα proteins were quantified using the DuoSet® ELISA method. Western blotting analysis of whole-cell proteins with anti-α-tubulin antibody is shown on the right (loading control).

Effect of PB-1 (physalin B), Wit-1 (withanolide F) and PBright-4 on DNA binding of RelA/p50 proteins

When DIG-labeled NF-κB oligonucleotide was incubated with nuclear extracts of TNF-α-stimulated HeLa cells, NF-κB proteins bound with the DNA-probe, as shown in Lane 2. Further treatment with anti-RelA antibody resulted in a supershift of the NF-κB/DNA-probe complex (Lane 7). This characteristic band completely disappeared when a 100-fold excess of unlabeled NF-κB oligonucleotide was used as a competitor (Lane 3). Strong band of the protein dimer-DNA complex was observed in the presence of 50 μM of **PB-1** (physalin B), **Wit-1** (withanolide F), and **PBright-4** (Lane 4-6). Test compounds had no effect on the chemiluminescence in the absence of nuclear protein (Lanes 8-10).

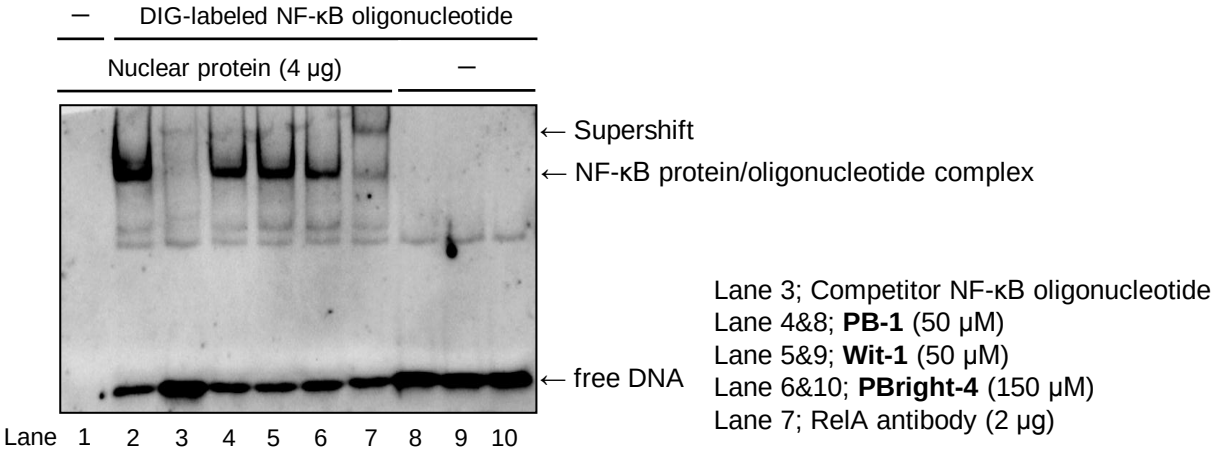


Figure S5. HeLa cells were stimulated with TNF-α (50 ng/mL) for 30 min, and nuclear proteins were extracted. Before the addition of labeled probe, the nuclear proteins were incubated with each test compound (Lanes 4-6, 50 μM or 150 μM), unlabeled NF-κB oligonucleotide (lane 3, 100-fold excess over labeled oligonucleotide), or antibody against NF-κB subunit, RelA (Lane 7, 2 μg) for 30 min at room temperature.

The Effect of PBright-4 on NF-κB Activation

PBright-4 showed a weak inhibitory effect on IκBα phosphorylation (Figure S6A). It had no effect on IκBα degradation (Figure S6B). But, it inhibited nuclear translocation (Figure S6C) and DNA binding (Figure S6D) of RelA/p50 dimer at high concentration (150 μM).

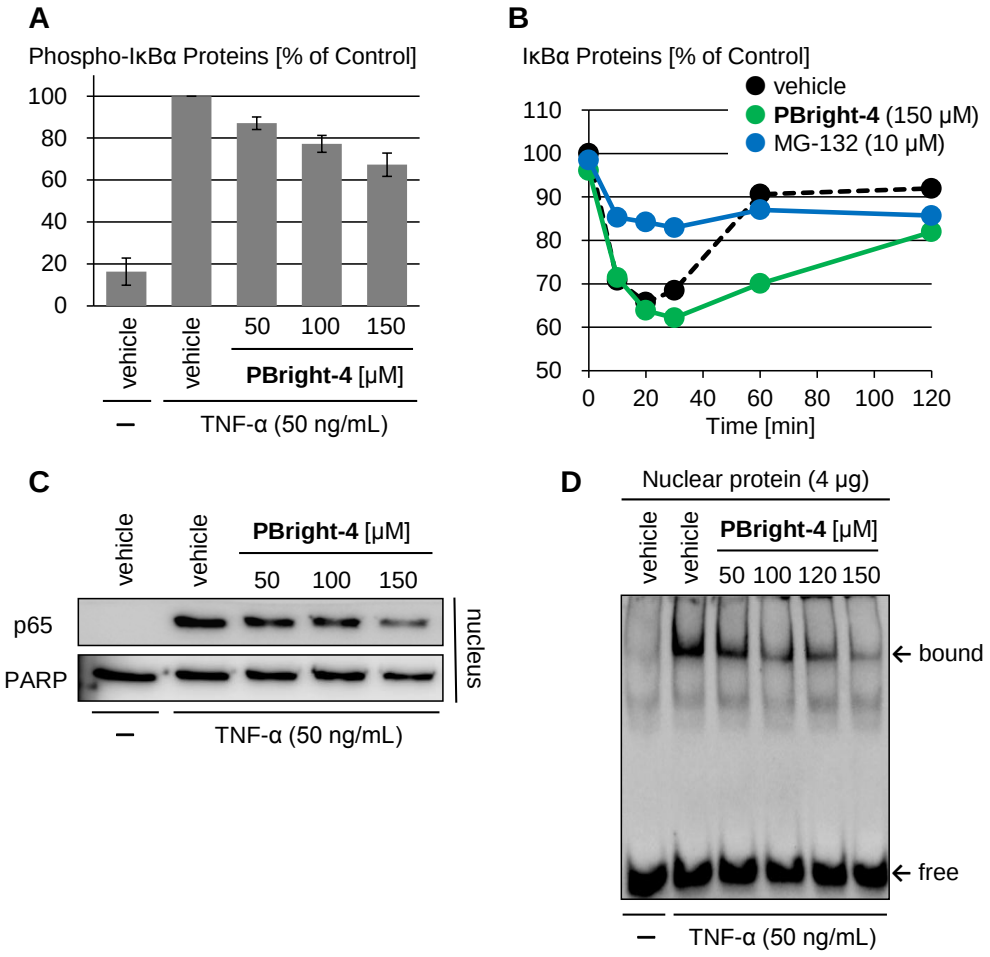


Figure S6. A) Inhibition of IκBα phosphorylation. HeLa cells were stimulated with TNF-α (50 ng/mL) for 7.5 min after pretreatment with 10 μM MG-132 (proteasome inhibitor, 30 min) and then treated with **PBright-4** (30 min). The control was treated in the same way, but with vehicle instead of **PBright-4**. Phosphorylated IκBα was quantified using the PathScan® ELISA method. B) Inhibition of IκBα degradation. HeLa cells were stimulated with TNF-α (250 ng/mL) for various times after pretreatment with **PBright-4** (150 μM), MG-132 (10 μM, proteasome inhibitor) or vehicle (DMSO) for 30 min. Total IκBα proteins were quantified using the DuoSet® ELISA method. C, D) Effect of **PBright-4** on nuclear translocation and DNA binding of RelA/p50 proteins. C) Western blotting analysis of nuclear proteins by immunoblotting with anti-RelA and anti-PARP antibodies (marker for nuclear fraction). D) EMSA using labeled DNA probe and nuclear fraction of HeLa cells. HeLa cells were stimulated with TNF-α (50 ng/mL) for 15 min (C) or 30 min (D) after pretreatment with either various concentrations of **PBright-4** or DMSO for 30 min.

Effect of Wit-1 (withanolides F) on the expression of *Renilla* Luciferase in HeLa/NF-κB-luc (firefly) cells

To investigate the possibility of non-specific inhibition of the transcriptional and translational machineries (such as the RNA polymerase activity or the protein synthesis) by **Wit-1** (withanolides F), we conducted the control experiments using the *Renilla* luciferase under the control of general CMV promoter. The *Renilla* luciferase was expressed in HeLa/NF-κB-luc cells, which produce firefly luciferase under the control of NF-κB promoter. The relative activity of the firefly luciferase against the *Renilla* luciferase was increased 8-fold when cells were treated with TNF-α. Treatment of **Wit-1** at lower concentration decreased the relative ratio of the activities of these luciferases (firefly/*Renilla*), indicating that **Wit-1** inhibited the transcriptional activity promoted by NF-κB activation with little influence for constitutive transcriptional and translational machineries.

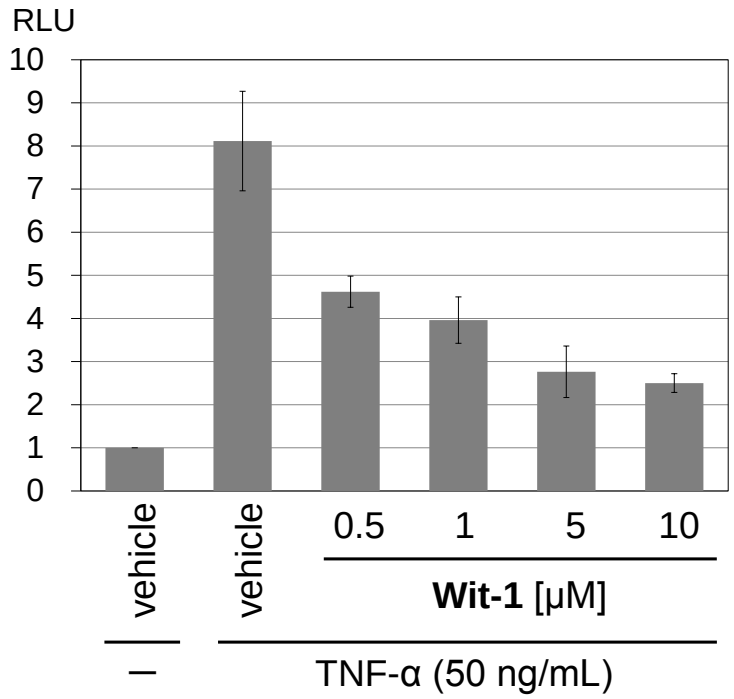
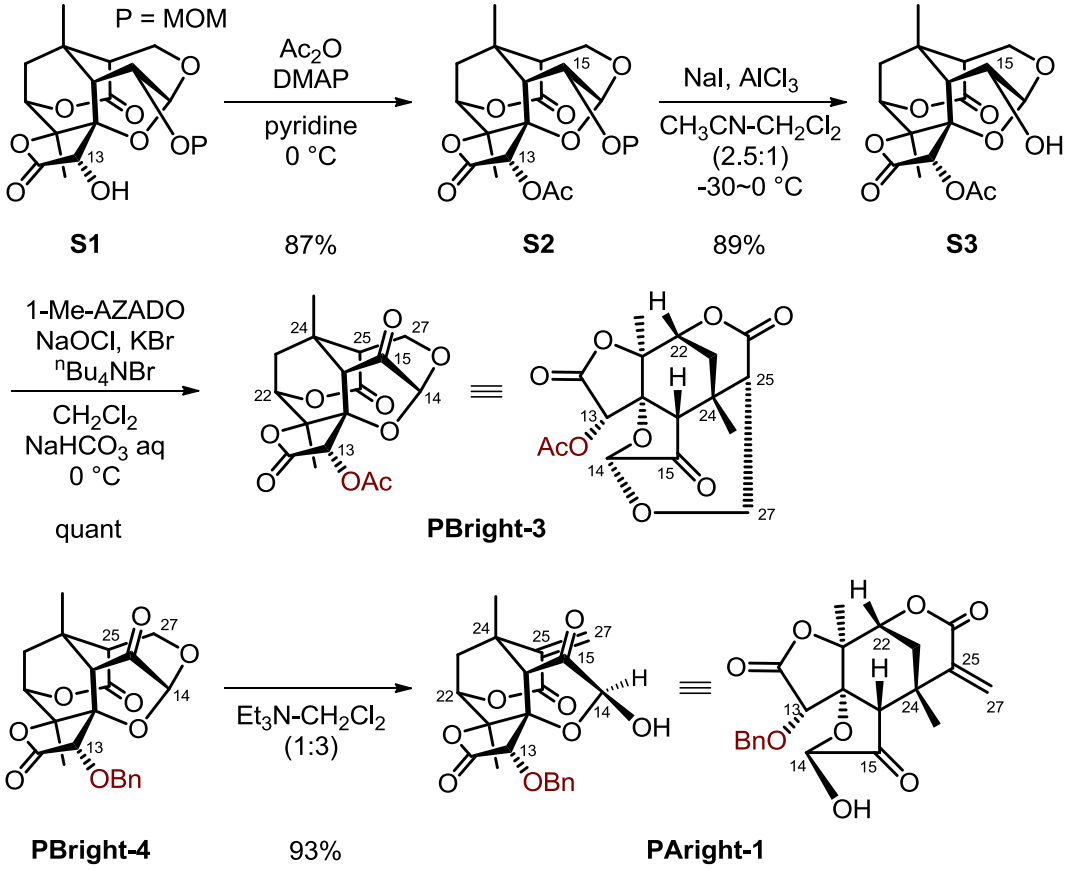


Figure S7. Effect of **Wit-1** (withanolide F) on the expression and the enzymatic activity of *Renilla* luciferase. Expression gene of *Renilla* luciferase (pGL4.75-RLuc) was transfected with HeLa/NF-κB-luc cells. Resulting cells were treated with **Wit-1** at the indicated concentrations. After incubation for 30 min, the cells were stimulated with 50 ng/mL TNF-α and incubated for 7 h at 37 °C under 5 % CO_2 in air. Firefly and *Renilla* luciferase activities were detected with a SpectraMax™ L microplate reader, and relative light unit (RLU) were shown (RLU was calculated by normalizing firefly luciferase activity to *Renilla* luciferase activity).

Synthesis of PBright-3 and PAright-1

Scheme S1. Synthesis of PBright-3 and PAright-1.



The synthetic procedure and characterization of **S1**, **PBright-1**, **PBright-2**, and **PBright-4** have already been reported (Ohkubo, M.; Hirai, G.; Sodeoka, M. *Angew. Chem. Int. Ed.* **2009**, *48*, 3862; Morita, M.; Hirai, G.; Ohkubo, M.; Koshino, H.; Hashizume, D.; Maruoka, K.; Sodeoka, M. *Org. Lett.* **2012**, *14*, 3434). **PBright-3** was synthesized from **S1** in the same way as described for the synthesis of **PBright-4**. The PA-type compound **PAright-1** was synthesized by treatment of **PBright-4** with Et_3N in dichloromethane. The synthetic procedure and characterization are described in the "Organic Synthesis" section.

Isolation and Identification

Experimental Procedure & Spectra Data

General; NMR spectra were recorded on a JEOL JNM-AL400 spectrometer or JEOL JNM-ECP500 spectrometer, operating at 400 MHz and 500 MHz for ^{1}H-NMR, 100.9 MHz and 125.8 MHz for ^{13}C-NMR. Chemical shifts were reported in the scale relative to $CDCl_3$, CD_3OD or d_6-DMSO as an internal reference. ESI-MS was taken on a Bruker micrOTOF-QII or a JEOL JMS-T100LC. Optical rotations were measured on a JASCO DIP-370 polarimeter. HPLC analysis was performed on TOSOH HPLC systems consisting of a CCPS pump, UV8020 detector set at 254 nm, and Senshu-Pak PEGASIL Silica 120-5 column (mobile phase, hexane/ethyl acetate or $CHCl_3/CH_3OH$). Column chromatography was performed with silica gel 60 (40-50 μm) purchased from Kanto Chemical Co.

Plant materials

Physalis alkekengi var. *franchetii* and *Physalis peruviana* were grown in RIKEN (Saitama, Japan).

Extraction and isolation

Physalis alkekengi* var. *franchetii. Entire plants of *P. alkekengi* var. *franchetii* (398 g, wet) were crushed and extracted with CH_3OH. The CH_3OH-soluble materials (12 g) were partitioned between 10 % aqueous CH_3OH and hexane. The aqueous CH_3OH phase was evaporated, and the residue was partitioned between H_2O and EtOAc. The EtOAc-soluble materials (2.4 g) were separated by column chromatography (Wakogel 50C18, H_2O/CH_3CN = 7/3—0/1, CH_3OH, and $CHCl_3$) to obtain 7 fractions ($F_1$1~$F_1$7). Fraction $F_1$3 (423 mg) was further separated by column chromatography (Silica gel 60, $CHCl_3/CH_3OH$ = 99/1) to obtain 5 fractions ($F_1$301~$F_1$305). Fraction $F_1$301 was purified by HPLC (SenshuPak PEGASIL Silica SP100, hexane/*i*-PrOH = 9/1) to give physalin C[1] (**PA-1**, 1.2 mg, *t*R = 11 min), and physalin B[2] (**PB-1**, 4.8 mg, *t*R = 23 min). Fraction $F_1$305 was purified by silica gel column chromatography (hexane/EtOAc = 7/3 then $CHCl_3/CH_3OH$ = 99/1) to give physalin III (**PC-1**, 4.1 mg), and physalin F[3] (**PB-4**, 8.7 mg).

---

(1) Kawai, M.; Matsuura, T. The structure of physalin C. A bitter principle of *Physalis alkekengi* var. *francheti*. *Tetrahedron* **1970**, *26*, 1743-1745.

(2) Kawai, M.; Ogura, T.; Makino, B.; Matsumoto, A.; Yamamura, H.; Butsugan, Y.; Hayashi, M. Physalins N and O from *Physalis alkekengi*. *Phytochemistry* **1992**, *31*, 4299-4302.

(3) Jacobo-Herrera, N. J.; Bremner, P.; Márquez, N.; Gupta, M. P.; Gibbons, S.; Muñoz, E.; Heinrich, M. Physalins from *Witheringia solanacea* as modulators of the NF-κB cascade. *J. Nat. Prod.* **2006**, *69,* 328-331.

Natural products in the calyces of *P. alkekengi* var. *franchetii* (6.0 g, dry) were also investigated. The CH_3OH-soluble materials (1.8 g) were partitioned between H_2O and hexane. The H_2O-soluble materials were partitioned with EtOAc. The EtOAc-soluble materials (320 mg) were separated by column chromatography (Wakogel 50C18, H_2O/CH_3CN = 2/3—0/1, CH_3OH, and $CHCl_3$) to obtain 3 fractions (F_21~F_23). Fraction F_21 was separated using silica gel column chromatography ($CHCl_3/CH_3OH$ = 95/5, silica gel 60) to obtain 5 fractions (F_2101~F_2105). Fraction F_2102 was further separated using silica gel column chromatography (Silica gel 60, hexane/EtOAc = 4/1—3/2) to obtain 8 fractions (F_3101~F_3108). Fraction F_3103 was purified by HPLC (SenshuPak PEGASIL Silica SP100, $CHCl_3/CH_3OH$ = 98/2) to give physalin N^2 (**PB-2**, 6.7 mg, *t*R = 7.2 min), and a mixture of physalin A^2 (**PA-2**) and physalin O^2 (**PC-2**) (*t*R = 8.5 min). The mixture was separated by recycling HPLC (SenshuPak PEGASIL Silica SP100, $CHCl_3/CH_3OH$ = 98/2) to give physalin A (**PA-2**, 1.1 mg) and physalin O (**PC-2**, 0.7 mg).

Physalis peruviana. The leaves of *P. peruviana* (106 g, dry) were crushed and then extracted with CH_3OH. The CH_3OH-soluble materials (27 g) were partitioned between 10 % aqueous CH_3OH and hexane. The aqueous CH_3OH-soluble materials were evaporated, and partitioned between H_2O and EtOAc. The EtOAc-soluble materials (3.2 g) were separated by column chromatography (Wakogel 50C18, H_2O/CH_3CN = 7/3—0/1, CH_3OH, and $CHCl_3$,) to obtain 2 fractions (F_31 and F_32). Fraction F_31 (909 mg) was further separated by column chromatography (Silica gel 60, CH_3Cl/CH_3OH, 99/1—95/5) to obtain 13 fractions (F_3101~F_3113). Fraction F_3104 (90 mg) was purified by silica gel column chromatography (hexane/EtOAc = 1/1) and HPLC (SenshuPak PEGASIL ODS SP100, CH_3CN/H_2O) to give withanolide E[4] (**Wit-4**, 6.3 mg), withanolide F[5] (**Wit-1**, 3.8 mg), and perulactone H[7] (**Per-2**, 1.4 mg). Fraction F_3108 (49 mg) was purified by silica gel column chromatography (hexane/EtOAc = 3/7) and HPLC (SenshuPak PEGASIL ODS SP100, CH_3CN/H_2O = 65/35) to give perulactone B[6] (**Per-1**, 15 mg, *t*R = 7.5 min). Fraction F_3111 (185 mg) was purified by silica gel column chromatography (hexane/EtOAc = 2/8) and HPLC (SenshuPak PEGASIL ODS SP100, CH_3CN/H_2O = 3/2) to give 4β-hydroxywithanolide E[7,8] (**Wit-S1**, 27 mg, *t*R = 12.2 min).

Natural products in stems of *P. peruviana* (121 g, dry) were also investigated. The EtOAc-soluble materials (1.2 g) were separated by column chromatography (Wakogel 50C18, H_2O/CH_3CN = 3/2—0/1, CH_3OH, and $CHCl_3$) to obtain 2 fractions (F_41 and F_42). Fraction F_41 (920 mg) was further separated by silica gel column chromatography (Silica gel 60, CH_3Cl/CH_3OH, 99/1—8/2) to obtain 8 fractions (F_4101~F_4108). Fraction F_4103 (253 mg) was purified by silica gel column chromatography (hexane/EtOAc = 3/2) and HPLC (SenshuPak PEGASIL ODS SP100, CH_3CN/H_2O = 7/3) to give withanolide G[4] (**Wit-2**, 11 mg, *t*R = 11 min), and Δ^{16}-withanolide[9] (**Wit-3**, 3.2 mg, *t*R =13 min).

(4) Gottlieb, H. E.; Kirson, I. ^{13}C NMR spectroscopy of withanolides and other highly oxygenated C_{28} steroids. *Org. Magn. Res*. **1981**, *16*, 20-25.

(5) Abdeljebbar, L. H.; Humam, M.; Christen, P.; Jeannerat, D.; Bruno, V.; Amzazi, S.; Benjouad, A.; Hostettmann, K.; Bekkouche, K. Withanolides from *Withania adpressa*. *Helv. Chim. Acta* **2007**, *90*, 346-352.

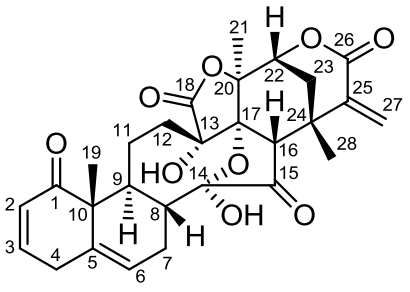
(6) Fang, S. T.; Liu, J. K.; Li, B. Ten new withanolides from *Physalis peruviana*. *Steroids* **2012**, *77*, 36-44.

(7) Sakurai, K.; Ishii, H.; Kobayashi, S.; Iwao, T. Isolation of 4β-hydroxywithanolide E, a new withanolide from *Physalis perviana* L. *Chem. Pharm. Bull*. **1976**, *24*, 1403-1405.

(8) Kirson, I.; Abraham, A.; Sethi, P. D.; Subramanian, S.; Glotter, E. 4β-hydroxywithanolide E, a new natural steroid with a 17α-oriented side-chain. *Phytochemistry* **1976**, *15*, 340-342.

(9) Velde, V. V.; Lavie, D. A Δ^{16}-withanolide in *Withania somnifera* as a possible precursor for α-side-chain. *Phytochemistry* **1982**, *21*, 731-733.

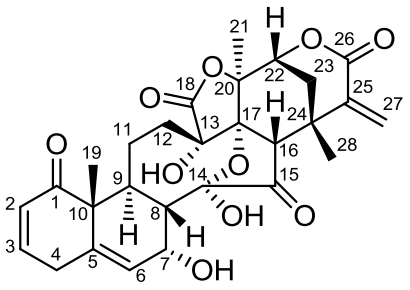
^{1}H and ^{13}C NMR data of PA-1 (Physalin C)



position	δ_H (*J* in Hz) (500 MHz, DMSO-d_6)	δ_C (125 MHz, DMSO-d_6)	δ_H (*J* in Hz) (Ref) (DMSO-d_6)
1		202.6	
2	5.80 dd (10.2, 2.3)	127.0	5.82 br d (9)
3	6.91 ddd (10.2, 4.6, 2.3)	146.6	6.91 dm (9)
4	2.88 dd (21.8, 4.6)	32.2	
	3.25 br d (21.8)		
5		135.8	
6	5.58 br d (5.7)	124.3	5.62 m
7	2.23 m	25.9	
	1.96 m		
8	1.81 td (11.3, 3.4)	42.6	
9	2.78 dd (11.3, 7.2)	34.5	
10		52.5	
11	1.97 m	23.8	
	1.10 m		
12	2.26 m	29.2	
	1.89 dd (15.6, 5.9)		
13		78.7	
14		101.0	
15		213.9	
16	2.93 s	53.1	
17		81.9	
18		172.5	
19	1.04 s	16.4	1.07 s
20		82.3	
21	1.77 s	20.9	1.80 s
22	4.53 m	76.0	4.55 m
23	1.93-2.02 m, 2H	30.7	
24		35.6	
25		138.5	
26		161.9	
27	5.56 s	131.6	5.57 br s
	6.36 s		6.39 br s
28	1.53 s	26.7	1.55 s
13-OH	5.95 s		5.90 s
14-OH	6.16 s		6.13 s

Ref) Kawai, M.; Matsuura, T. The structure of physalin C. A better principle of *Physalis alkekengi* var. *francheti*. *Tetrahedron* **1970**, *26*, 1743-1745.

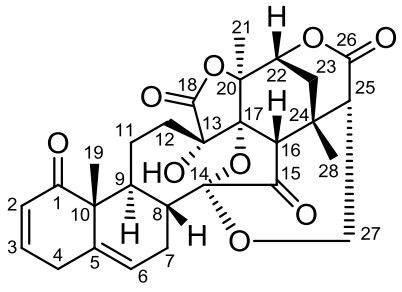
^{1}H and ^{13}C NMR data of PA-2 (Physalin A)



position	δ_H (J in Hz) (500 MHz, DMSO-d_6)	δ_C (125 MHz, DMSO-d_6)	δ_H (J in Hz) (Ref) (400 MHz, DMSO-d_6)	δ_C (Ref) (100 MHz, DMSO-d_6)
1		202.0		201.7
2	5.83 dd (10.3, 2.3)	126.8	5.83 dd (10, 2)	126.7
3	6.93 ddd (10.3, 4.6, 2.3)	146.6	6.93 ddd (10, 5, 2.5)	146.3
4	2.90 dd (21.8, 4.6)	32.0	2.91dd (22, 5)	31.9
	3.25 br d (21.8)		3.26 brd (22)	
5		139.4		139.2
6	5.69 dd (6.3, 1.7)	127.3	5.69 dd (6, 1.5)	127.0
7	4.46 br	61.4	4.47 br t (5)	61.3
8	1.81 dd (11.5, 1.7)	46.4	1.81 dd (12, 1.5)	46.3
9	2.99 dd (11.5, 8.6)	29.0	3.00 dd (12, 9)	28.9
10		53.9		53.8
11	2.04 m, 1.15 m	23.3	2.04 m, ~1.15m	23.1
12	2.23 ddd (15.5, 12.0, 8.0)	29.7	2.23 ddd (16, 12, 8)	29.6
	1.93 dd (15.5, 5.2)		1.93 dd (16, 5.5)	
13		82.1		81.9[a]
14		100.7		100.6
15		213.7		213.3
16	3.08 s	52.6	3.08 s	52.6
17		79.3		79.2[a]
18		171.8		171.5
19	1.02 s	14.0	1.02 s	13.9
20		82.0		81.9[a]
21	1.70 s	21.3	1.71 s	21.1
22	4.58 dd (4.0, 1.7)	75.6	4.59 dd (4, 2)	75.5
23	2.02 dd (14.6, 4.0)	30.6	2.03 dd (14, 4)	30.6
	2.07 dd (14.6, 1.7)		2.06 dd (14, 2)	
24		35.5		35.4
25		137.9		137.8
26		161.9		161.7
27	5.59 br s	132.2	5.59 br s	132.0
	6.42 s		6.43 s	
28	1.55 s	26.4	1.55 s	26.3
7-OH	5.00 d (4.0)		5.00 d (4.5)	
13-OH	5.60 s		5.60 s	
14-OH	6.40 s		6.38 s	

[a]Interchangeable data. Ref) Kawai, M.; Ogura, T.; Makino, B.; Matsumoto, A.; Yamamura, H.; Butsugan, Y.; Hayashi, M. Physalins N and O from *Physalis alkekengi*. *Phytochemistry* **1992**, *31*, 4299-4302.

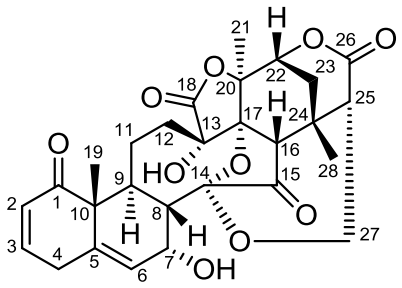
^{1}H and ^{13}C NMR data of PB-1 (Physalin B)



position	δ_H (J in Hz) (500 MHz, DMSO-d_6)	δ_C (125 MHz, DMSO-d_6)	δ_H (J in Hz) (Ref) (400 MHz, DMSO-d_6)	δ_C (Ref) (100 MHz, DMSO-d_6)
1		202.4		202.4
2	5.80 dd (10.0, 1.8)	126.9	5.80 dd (10, 2)	126.9
3	6.89 ddd (10.0, 4.9, 2.3)	146.2	6.89 ddd (10, 5, 2)	146.1
4	2.90 m	32.3	2.89 dd (20, 5)	32.3
	3.27 br d (21.8)		3.27 br d (20)	
5		135.6		135.5
6	5.59 br d (6.3)	123.4	5.59 br d (6)	123.4
7	1.99 m	24.4	~2.0 m	24.4
	2.20 m		2.21 m	
8	1.95 m	40.2	1.92 m	40.2
9	2.95 m	33.1	2.95 dd (11, 9)	33.1
10		51.9		51.9
11	2.16 m	24.1	2.18 m	24.1
	1.10 m		~1.1 m	
12	2.14 m	25.5	2.17 m	25.6
	1.45 m		1.45 m	
13		80.7		80.7
14		106.3		106.3
15		209.4		209.3
16	2.88 s	54.2	2.86 s	54.1
17		78.2		78.2
18		171.8		171.8
19	1.08 s	16.8	1.09 s	16.8
20		80.3		80.3
21	1.77 s	21.7	1.78 s	21.7
22	4.57 dd (3.4, 2.3)	76.3	4.56 dd (3, 2)	76.3
23	1.92 m	31.4	1.96 m	31.4
	2.10 dd (14.3, 3.4)		2.14 m	
24		30.5		30.5
25	2.89 m	49.4	2.88 br d (4)	49.4
26		167.3		167.2
27	4.25 dd (13.5, 4.0)	60.6	4.26 dd (14, 4)	60.6
	3.60 dd (13.5, 1.2)		3.60 dd (14, 1)	
28	1.16 s	24.5	1.16 s	24.4
13-OH	6.31 s		6.28 s	

Ref) Kawai, M.; Ogura, T.; Makino, B.; Matsumoto, A.; Yamamura, H.; Butsugan, Y.; Hayashi, M. Physalins N and O from *Physalis alkekengi*. *Phytochemistry* **1992**, *31*, 4299-4302.

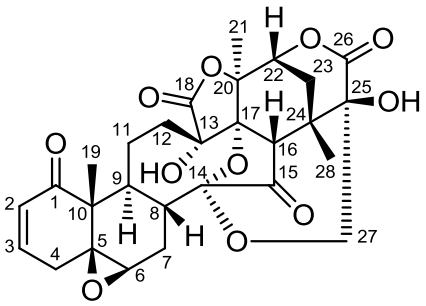
^{1}H and ^{13}C NMR data of PB-2 (Physalin N)



position	δ_H (*J* in Hz) (500 MHz, $CDCl_3$)	δ_C (125 MHz, $CDCl_3$)	δ_H (*J* in Hz) (Ref) (400 MHz, DMSO-d_6)	δ_C (Ref) (100 MHz, DMSO-d_6)
1		203.7		201.5
2	5.93 ddd (10.2, 2.9, 1.2)	127.4	5.83 dd (10, 2)	126.9
3	6.80 ddd (10.2, 4.6, 2.9)	145.6	6.93 ddd (10, 5, 2.5)	146.2
4	2.99 br dd (21.8, 4.6)	32.7	~2.9 m	32.3
	3.34 br d (21.8)		~3.3 m	
5		139.1		139.1
6	5.76 dd (5.7, 1.7)	124.9	5.69 br d (6)	125.5
7	4.59 m	62.1	~4.35 m	61.4
8	2.18 dd (12.6, 3.4)	44.9	~1.95 m	44.2
9	3.27 dd (12.6, 9.5)	28.1	~3.25 m	27.6
10		52.8		52.7
11	2.15 m	24.7	~2.2 m	24.0
	1.21 m		~1.1 m	
12	2.44 ddd (17.0, 12.9, 5.7)	25.6	~2.15 m	25.7
	1.54 ddd (17.0, 10.6, 2.3)		1.45 m	
13		79.3		81.0[a]
14		107.3		106.3
15		207.1		208.7
16	2.23 s	55.3	2.96 s	52.9
17		80.4		78.0[a]
18		172.0		171.7
19	1.19 s	17.2	1.06 s	15.6
20		81.0		80.3[a]
21	1.98 s	21.6	1.76 s	21.9
22	4.57 dd (3.4, 2.3)	76.8	4.60 m	76.2
23	2.11 dd (14.9, 3.4)	33.0	1.94 dd (12, 2.5)	31.3
	2.03 dd (14.9, 2.3)		2.10 br d (12)	
24		31.3		30.7
25	2.49 br d (4.0)	50.7	2.94 br d (4)	49.3
26		166.7		167.4
27	4.61 dd (13.5, 4.0)	61.1	4.33 dd (13, 4)	61.1
	3.84 dd (13.5, 1.2)		3.66 d (13)	
28	1.29 s	26.4	1.18 s	24.3
7-OH	3.49 s		~3.3 m	
13-OH	4.02 br s		6.32 s	

[a]Interchangeable data. Ref) Kawai, M.; Ogura, T.; Makino, B.; Matsumoto, A.; Yamamura, H.; Butsugan, Y.; Hayashi, M. Physalins N and O from *Physalis alkekengi*. *Phytochemistry* **1992**, *31*, 4299-4302.

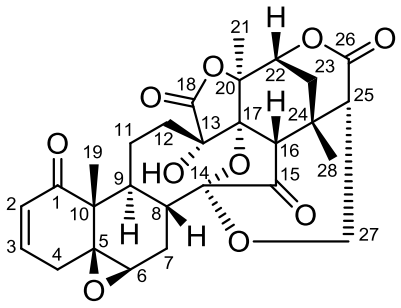
Data of PB-3 (25-OH-physalin F)



position	δ_H (J in Hz) (500 MHz, DMSO-d_6)	δ_C (125 MHz, DMSO-d_6)
1		201.5
2	6.01 dd (9.7, 2.9)	129.4
3	6.94 ddd (9.7, 6.3, 2.9)	144.2
4	2.80 br d (17.8)	31.7
	1.82 dd (17.8, 6.3)	
5		61.6
6	3.27 brs	61.4
7	2.30 m	25.2
	1.72 m	
8	1.86 m	36.5
9	2.47 m	34.9
10		49.1
11	2.48 m	20.4
	0.93 m	
12	1.89 m	24.9
	1.37 dd (16.0, 10.3)	
13		80.1
14		105.8
15		208.9
16	3.01 s	53.9
17		77.9
18		171.7
19	1.00 s	12.0
20		79.5
21	1.74 s	21.9
22	4.57 m	76.5
23	2.29 m	28.0
	1.71 m	
24		35.5
25		73.6
26		168.4
27	3.94 d (12.6)	64.6
	3.32 d (12.6)	
28	1.10 s	18.9
13-OH	6.48 s	
25-OH	6.44 s	

$[\alpha]_D^{24}$ -69.52 (*c* 0.22, MeOH); IR (neat) 3400, 1781, 1766, 1744, 1654, 1463, 1388, 1267, 1233, 1174, 1138, 1108, 1045, 1023, 965, 915, 879, 767 cm^{-1}; HRMS-ESI (m/z): $[M+Na^+]$ calcd for $C_{28}H_{30}O_{11}Na$, 565.1686; found, 565.1691.

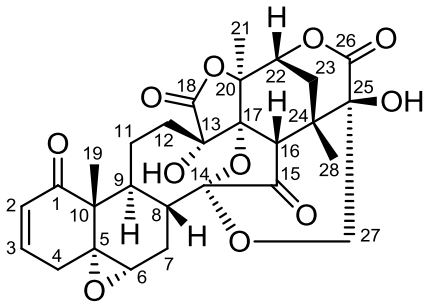
^{1}H and ^{13}C NMR data of PB-4 (Physalin F)



position	δ_H (*J* in Hz) (500 MHz, $CDCl_3$)	δ_C (125 MHz, $CDCl_3$)	δ_H (*J* in Hz) (Ref) (500 MHz, $CDCl_3$)	δ_C (Ref) (125 MHz, $CDCl_3$)
1		205.9		205.9
2	6.01 ddd (10.3, 2.9, 1.2)	127.8	6.02 dd (8.5, 1.0)	127.7
3	6.86 ddd (10.3, 5.2, 2.9)	146.3	6.87 ddd (8.5, 5.5, 3.0)	146.3
4	2.99 dt (20.6, 2.9)	33.3		33.3
	2.20 m			
5		61.7		61.7
6	3.26 br d (3.4)	64.9	3.25 d (3.5)	64.9
7	2.57 dt (14.3, 3.4)	24.8		24.8
	1.83 dd (14.3, 11.5)			
8	2.24 td(11.5, 3.4)	37.4		37.4
9	2.63 dd (11.5, 9.2)	34.2		34.2
10		50.0		50.0
11	2.14 m	23.5		23.6
	1.19 m			
12	2.36 ddd (17.2, 13.2, 5.2)	25.7		25.7
	1.53 br dd (17.2, 9.2)			
13		80.0		80.9
14		107.0		107.0
15		207.5		207.5
16	2.18 s	56.2	2.17 s	56.2
17		79.4		80.0
18		172.1		172.1
19	1.30 s	15.6	1.25 s	15.6
20		81.0		79.4
21	1.95 s	21.4	1.94 s	21.4
22	4.54 dd (3.4, 2.3)	76.9	4.53 dd (3.5, 2.5)	76.9
23	2.06 dd (14.9, 3.4)	33.0		33.0
	1.99 dd (14.9, 2.3)			
24		31.1		31.1
25	2.44 br d (4.0)	50.8	2.43 d (4.5)	50.8
26		166.6		166.6
27	4.51 dd (13.2, 4.0)	60.7	4.12 dd (14.5, 7.5)	60.7
	3.75 dd (13.2, 1.2)		3.76 d (13.5)	
28	1.26 s	26.5	1.30 s	26.5
13-OH	4.07 s		4.03 s	

Ref) Jacobo-Herrera, N. J.; Bremner, P.; Márquez, N.; Gupta, M. P.; Gibbons, S.; Muñoz, E.; Heinrich, M. Physalins from *Witheringia solanacea* as modulators of the NF-κB cascade. *J. Nat. Prod.* **2006**, *69,* 328-331.

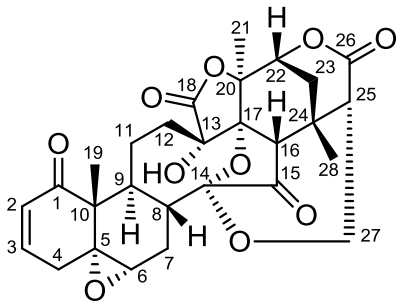
Data of PB-5 (25-OH-physalin J)



position	δ_H (J in Hz) (500 MHz, DMSO-d_6)	δ_C (125 MHz, DMSO-d_6)
1		202.0
2	5.84 dd (10.1, 2.3)	127.5
3	6.82 ddd (10.1, 4.8, 2.3)	144.1
4	3.15 dd (12.3, 4.8)	33.2
	1.78 m	
5		64.8
6	3.13 m	57.2
7	2.13 m	22.4
	1.72 m	
8	1.92 m	38.1
9	2.97 m	28.4
10		50.1
11	1.96 m	24.5
	0.94 m	
12	2.09 m	25.5
	1.40 dd (15.6, 9.7)	
13		80.6
14		106.3
15		209.3
16	2.97 s	53.9
17		78.1
18		171.6
19	1.18 s	13.5
20		79.6
21	1.79 s	22.0
22	4.57 dd (3.7, 1.8)	76.6
23	2.31 dd (14.7, 3.7)	28.1
	1.75 m	
24		35.5
25		73.5
26		168.4
27	3.92 d (12.4)	64.5
	3.31 d (12.4)	
28	1.10 s	18.9
13-OH	6.33 s	
25-OH	6.44 s	

$[\alpha]_D^{24}$ -73.52 (*c* 0.28, MeOH) IR (neat) 3415, 1780, 1763, 1747, 1668, 1464, 1386, 1271, 1234, 1173, 1138, 1094, 1061, 1038, 1017, 965, 946, 755, 669 cm^{-1}; HRMS-ESI (m/z): [M+Na^+] calcd for $C_{28}H_{30}O_{11}Na$, 565.1686; found, 565.1685.

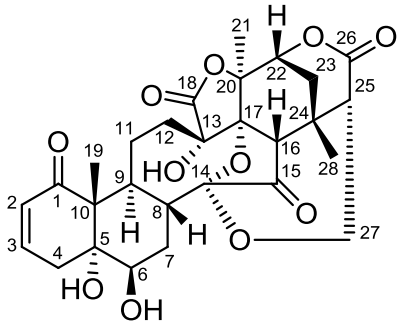
^{1}H and ^{13}C NMR data of PB-6 (Physalin J)



position	δ_H (*J* in Hz) (500 MHz, DMSO-d_6)	δ_C (125 MHz, DMSO-d_6)	δ_H (*J* in Hz) (Ref) (DMSO-d_6)
1		202.0	
2	5.84 dd (10.3, 2.3)	127.5	5.72 dd (10, 4)
3	6.82 ddd (10.3, 5.1, 2.3)	144.1	6.61 dm (10)
4	3.15 dt (19.8, 2.3)	33.2	
	1.78 m		
5		64.8	
6	3.13 d (5.1)	57.2	3.03 m
7	2.13 m	22.5	
	1.72 dd (15.6, 11.5)		
8	1.94 m	38.6	
9	2.96 dd (11.5, 8.7)	28.3	
10		50.2	
11	1.95 m	24.5	
	0.97 m		
12	2.13 m	25.9	
	1.41 dd (16.1, 9.7)		
13		78.1	
14		106.4	
15		209.5	
16	2.84 s	53.9	
17		80.9	
18		171.8	
19	1.19 s	13.6	
20		80.5	
21	1.79 s	21.9	
22	4.56 dd (3.7, 2.3)	76.3	4.59 m
23	2.08 m	31.4	
	1.91 m		
24		30.5	
25	2.87 br d (4.6)	49.3	
26		167.3	
27	4.23 dd (13.3, 4.6)	60.6	4.28 dd (14, 4)
	3.55 dd (13.3, 1.4)		3.60 d (14)
28	1.14 s	24.4	
13-OH	6.29 s		6.31 s

Ref) Row, L. R.; Sarma, N. S.; Reddy, K. S.; Matsuura, T.; Nakashima, R. The structure of physalins F and J from *Physalis angulata* and *P. lancifolia*. *Phytochemistry* **1978**, *17*, 1647-1650.

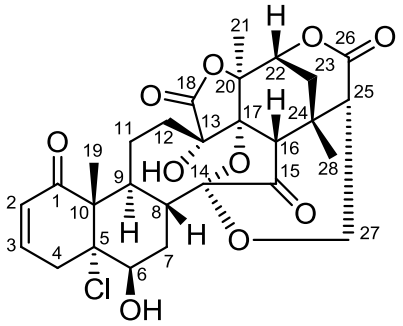
^{1}H and ^{13}C NMR data of PB-S1 (Physalin D)



position	δ_H (J in Hz) (500 MHz, DMSO-d_6)	δ_C (125 MHz, DMSO-d_6)	δ_H (J in Hz) (ref) (400 MHz, DMSO-d_6)	δ_C (Ref) (100 MHz, DMSO-d_6)
1		204.4		204.4
2	5.69 dd (102, 2.3)	127.3	5.68 dd (10, 2)	127.2
3	6.62 ddd (10.2, 5.2, 2.3)	142.9	6.61 ddd (10, 5, 2)	142.9
4	1.97 dd (19.5, 5.2)	35.3	1.96 dd (19, 5)	35.2
	3.10 br d (19.5)		3.10 br d (19)	
5		76.4		76.4
6	3.48 m	72.6	3.47 m	72.5
7	1.81 m, 1.79 m	26.7	1.80 m, 1.78 m	26.6
8	2.19 ddd (11.2, 11.2, 4.6)	38.3	2.19 td (11, 11, 5)	38.3
9	3.11m	30.0	3.10 m	29.9
10		53.5		53.5
11	1.75 m, 0.93 m	24.8	1.75 m, 0.93 m	24.7
12	2.09 m	25.9	2.08 m	25.8
	1.44 dd (16.3, 10.3)		1.44 dd (16, 10)	
13		78.7		78.7
14		106.9		106.9
15		210.0		209.8
16	2.79 s	54.0	2.77 s	54.0
17		80.7		80.7
18		171.9		171.8
19	1.10 s	13.3	1.10 s	13.3
20		80.5		80.5
21	1.80 s	21.7	1.80 s	21.6
22	4.57 m	76.5	4.55 m	76.3
23	2.09 dd (14.6, 4.0)	31.3	2.08 dd (14, 4)	31.3
	1.92 dd (14.6, 2.3)		1.92 br d (14)	
24		30.5		30.5
25	2.88 d (3.4)	49.4	2.86 d (4)	49.4
26		167.4		167.3
27	4.24 dd (12.6, 3.4)	60.5	4.23 dd (13, 3.5)	60.5
	3.57 d (12.6)		3.57 d (13)	
28	1.16 s	24.5	1.15 s	24.5
5-OH	4.24 s		4.21 s	
6-OH	4.91 d (4.0)		4.87 d (4)	
13-OH	5.79 s		5.70 s	

Ref) Kawai, M.; Yamamoto, T.; Makino, B.; Yamamura, H.; Araki, S.; Bustugan, Y.; Saito, K. The structure of physalin T from *Physalis alkekengi* var. *francheti*. *J. Asian Nat. Prod. Res.* **2001**, *3*, 199-205.

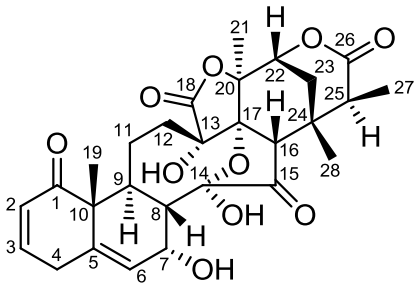
^{1}H and ^{13}C NMR data of PB-S2 (Physalin H)



position	δ_H (*J* in Hz) (500 MHz, DMSO-d_6)	δ_C (125 MHz, DMSO-d_6)	δ_H (*J* in Hz) (Ref) (400 MHz, DMSO-d_6)	δ_C (Ref) (100 MHz, DMSO-d_6)
1		200.3		200.3
2	5.82 dd (10.3, 2.3)	127.3	5.83 dd (10, 2.5)	127.2
3	6.78 ddd (10.3, 4.9, 2.3)	143.0	6.78 ddd (10, 5, 2)	142.8
4	2.46 dd (20.6, 4.9)	36.9	2.46 dd (21, 5)	36.9
	3.47 ddd (20.6, 2.3, 2.3)		3.48 ddd (21, 2.5, 2)	
5		82.4		82.3
6	3.88 m	72.7	3.88 dt (5, 3, 3)	72.6
7	2.02 m, 1.94 m	26.8	2.04 m, 1.95 m	26.7
8	2.26 ddd (11.9, 11.9, 3.4)	38.4	2.27 dt (11.5, 11.5, 4)	38.4
9	3.35 m	30.9	3.35 dd (11.5, 8)	30.9
10		54.4		54.3
11	2.13 m, 0.97 m	24.4	2.13 m, 0.97 m	24.3
12	1.89 m	25.9	1.89 m	25.8
	1.44 dd (16.0, 9.7)		1.44 br dd (16, 9)	
13		78.4		78.4
14		106.4		106.4
15		209.9		209.6
16	2.82 s	53.8	2.82 s	53.9
17		80.9		80.8
18		171.7		171.5
19	1.23 s	14.1	1.24 s	14.0
20		80.5		80.4
21	1.80 s	21.8	1.81 s	21.6
22	4.57 dd (3.4, 2.3)	76.4	4.57 dd (3, 2)	76.3
23	2.10 dd (14.3, 3.4)	31.3	2.10 dd (15, 3)	31.3
	1.93 m		1.93 dd (15)	
24		30.5		30.4
25	2.89 br d (4.0)	49.4	2.89 dd (4.5, 1)	49.4
26		167.4		167.1
27	4.26 dd (13.5, 4.0)	60.6	4.26 dd (13, 4.5)	60.5
	3.58 dd (13.5, 1.2)		3.59 dd (13, 1)	
28	1.16 s	24.4	1.17 s	24.4
6-OH	5.66 d (4.6)		5.66 d (5)	
13-OH	6.08 s		6.06 s	

Ref) Makino, B.; Kawai, M.; Ogura, T.; Nakanishi, M.; Yamamura, H.; Butsugan, Y. Structural revision of physalin H isolated from *Physalis angulata*. *J. Nat. Prod.* **1995**, *58*, 1668-1674.

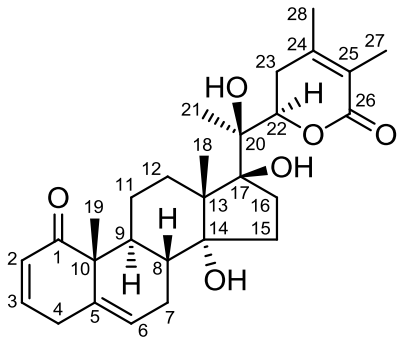
^{1}H and ^{13}C NMR data of PC-2 (Physalin O)



position	δ_H (J in Hz) (500 MHz, DMSO-d_6)	δ_C (125 MHz, DMSO-d_6)	δ_H (J in Hz) (Ref) (400 MHz, DMSO-d_6)	δ_C (Ref) (100 MHz, DMSO-d_6)
1		202.0		202.0
2	5.84 dd (10.0, 2.3)	126.8	5.84 dd (10, 2)	126.8
3	6.93 ddd (10.0, 4.6, 2.3)	146.6	6.94 ddd (10, 5, 2.5)	146.5
4	2.92 dd (22.3, 4.6)	32.0	2.93 dd (20, 5)	32.0
	3.27 br d (22.3)		3.27 br d (20)	
5		139.5		139.4
6	5.72 dd (6.3, 1.7)	127.2	5.72 dd (6, 1.5)	127.2
7	4.51 m	61.3	4.51 dd (6, 4.5)	61.3
8	1.87 dd (12.0, 1.7)	46.2	1.88 dd (12, 1.5)	46.2
9	3.01 dd (12.0, 8.6)	29.0	3.02 dd (12, 9)	29.0
10		53.9		53.9
11	2.03 m	23.3	2.03 m	23.3
	1.15 m		~1.15 m	
12	2.22 ddd (15.5, 12.3, 8.0)	29.6	2.23 ddd (16, 12, 8)	29.6
	1.93 dd (15.5, 5.2)		1.93 dd (16, 5.5)	
13		82.1[a]		82.1[b]
14		100.9		100.9
15		216.0		215.9
16	2.95 s	53.2	2.95 s	53.2
17		79.4[a]		79.3[b]
18		171.9		171.8
19	1.03 s	14.0	1.03 s	14.0
20		82.1[a]		82.1[b]
21	1.67 s	21.3	1.68 s	21.3
22	4.54 dd (4.6, 1.2)	76.2	4.53 br d (3)	76.2
23	2.08 dd (14.9, 4.6)	25.9	2.08 dd (15, 4)	25.9
	1.78 br d (14.9)		1.78 br d (15)	
24		34.5		34.5
25	2.59 q (7.5)	40.7	2.60 q (7.5)	40.7
26		171.9		171.9
27	1.14 d (7.5)	16.7	1.15 d (7.5)	16.7
28	1.29 s	25.0	1.30 s	25.0
7-OH	5.03 d (4.0)		5.00 d (4.5)	
13-OH	5.58 s		5.57 s	
14-OH	6.83 s		6.80 s	

[a,b]Interchangeable signals. Ref) Kawai, M.; Ogura, T.; Makino, B.; Matsumoto, A.; Yamamura, H.; Butsugan, Y.; Hayashi, M. Physalins N and O from *Physalis alkekengi*. *Phytochemistry* **1992**, *31*, 4299-4302.

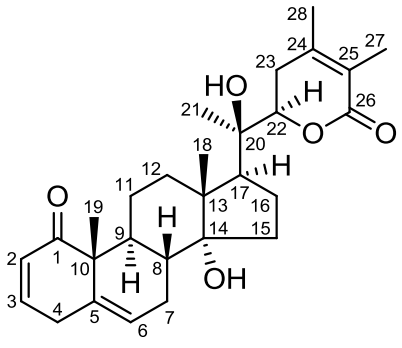
^{1}H and ^{13}C NMR data of Wit-1 (Withanolide F)



	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz) (Ref)	δ_C (Ref)
position	(400 MHz, $CDCl_3$)	(125 MHz, DMSO-d_6)	(DMSO-d_6)	(DMSO-d_6)
1		203.7		203.5
2	5.86 br dd (9.9, 1.8)	127.0	5.74-5.79 m	126.9
3	6.76 ddd (9.9, 4.6, 2.3)	146.7	6.88 ddd (9.8, 4.9, 2.2)	146.5
4	3.27 br dd (21.1, 2.3)	32.9	2.23-3.31 m	32.8
	2.83 dd (21.1, 4.6)		2.83 dd (21.6, 4.7)	
5		135.3		135.2
6	5.60 m	124.8	5.56-5.60 m	124.7
7		25.2	2.00-2.07 m, 1.70-1.75 m	25.1
8		36.7	1.68-1.74 m	36.6
9		35.3	2.02-2.15 m	35.2
10		50.2		50.1
11		22.9	2.00-2.07 m, 1.45-1.53 m	22.8
12		30.2	1.13-1.19 td (12.0, 12.0, 4.5)	30.1
			1.13-1.21 m	
13		53.8		53.7
14		81.5		81.4
15		32.0	1.32-1.40 m, 1.52-1.60 m	31.9
16		35.8	2.32-2.39 m, 1.49-1.55 m	35.7
17		87.4		87.3
18	1.13 s	20.4[a]	1.00 s	20.3
19	1.23 s	18.4	1.14 s	18.3
20		78.2		78.1
21	1.42 s	19.2	1.20-1.26 m	19.1
22	4.94 dd (9.4, 6.4)	81.1	4.65 dd (12.7, 3.6)	81.0
23		34.4	2.35-2.50 m, 2H	34.3
24		150.9		150.7
25		120.2		120.1
26		166.1		165.9
27	1.89 s	20.4[a]	1.85 s	20.2
28	1.94 s	12.2	1.72 s	12.1

[a]Interchangeable signals. Ref) Abdeljebbar, L. H.; Humam, M.; Christen, P.; Jeannerat, D.; Vitorge, B.; Amzazi, S.; Benjouad, A.; Hostettmann, K.; Beckkouche, K. Withanolides from *Withania adpressa*. *Helv. Chim. Acta* **2007**, *90*, 346-352.

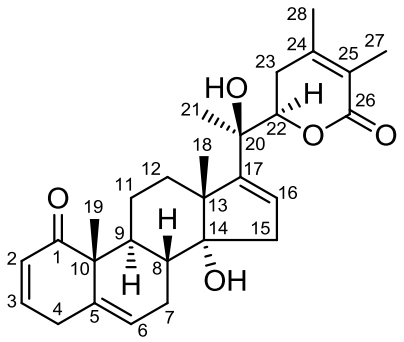
^{1}H and ^{13}C NMR data of Wit-2 (Withanolide G)



	δ_H (J in Hz)	δ_C	δ_C (Ref)
position	(400 MHz, $CDCl_3$)	(100 MHz, $CDCl_3$)	(22.6 MHz, $CDCl_3$)
1		204.3	204.3
2	5.88 ddd (10.0, 3.2, 0.9)	127.9	128.0
3	6.78 ddd (10.0, 4.6, 2.3)	145.3	145.3
4	3.29 br d (21.1)	33.4	33.5
	2.85 dd (21.1, 4.6)		
5		135.2	135.2
6	5.61 m	124.9	125.0
7		25.3	25.3
8		35.1[a]	35.2[b]
9		36.3[a]	36.3[b]
10		50.8	50.8
11		22.1	22.2
12		32.5	32.5
13		47.4	47.5
14		85.0	85.0
15		32.0	32.1
16		20.6	20.6
17		49.3	49.4
18	1.08 s	17.3	17.4
19	1.26 s	18.9	18.9
20		75.3	75.3
21	1.30 s	21.2	21.2
22	4.21 dd (13.3, 3.7)	81.3	81.4
23		31.8	31.7
24		149.1	149.2
25		121.9	121.9
26		166.2	166.2
27	1.88 s	12.5	12.4
28	1.94 s	20.5	20.5

[a,b]Interchangeable signals. Ref) Gottlieb, H. E.; Kirson, I. ^{13}C NMR spectroscopy of the withanolides and other highly oxygenated C_{28} steroids. *Org. Magn. Reson*. **1981**, *16*, 20-25.

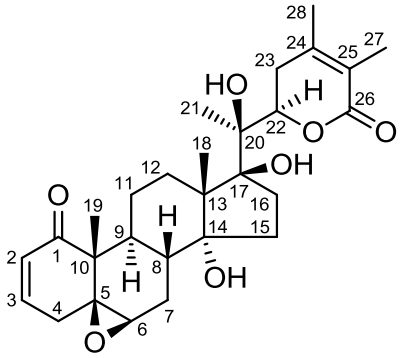
^{1}H and ^{13}C NMR data of Wit-3 (Δ^{16}-withanolide)



	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz) (ref)	δ_C (Ref)
position	(500 MHz, $CDCl_3$)	(125 MHz, $CDCl_3$)	(400 MHz, $CDCl_3$)	(22.6 MHz, $CDCl_3$)
1		204.2		198.0
2	5.86 ddd (10.2, 2.9, 1.2)	128.1	5.84 dd (9, 3.3)	128.2
3	6.77 ddd (10.2, 4.9, 2.3)	145.2	6.74 dq (9, 4.5)	145.2
4	3.27 br d (21.2)	33.5	3.26 br d (20)	33.5
	2.84 dd (21.2, 4.9)		2.82 dd (20, 4.5)	
5		135.1		135.2
6	5.62 m	125.3	5.59 $W_{1/2}$ (11)	125.4
7	2.32 m	25.5		25.5
	1.84 dddd (17.5, 5.4, 5.4, 2.3)			
8	1.93 dd (11.5, 5.4)	34.5		34.5
9	2.42 dd (11.5, 5.4)	36.0		36.0
10		50.6		50.7
11	2.32 m	22.3		22.3
	1.59 m			
12	2.32 m	28.7		28.7
	1.53 m			
13		52.4		52.4
14		84.3		84.4
15	2.34 m	40.0		40.0
	2.23 dd (15.5, 3.4)			
16	5.80 dd (3.4, 1.2)	124.2	5.79 $W_{1/2}$ (6.7)	124.3
17		156.4		156.5
18	1.18 s	22.5	1.18 s	22.5[a]
19	1.24 s	18.7	1.24 s	18.7
20		74.7		74.7
21	1.32 s	22.5	1.32 s	22.6[a]
22	4.47 dd (13.2, 3.4)	79.4	4.45 dd (11, 3.5)	79.5
23	2.78 br d (17.8)	29.9		29.9
	2.17 dd (17.8, 3.4)			
24		149.1		149.2
25		121.8		121.7
26		165.3		165.2
27	1.89 s	12.5	1.88 s	12.5
28	1.97 s	20.6	1.97 s	20.6

[a]Interchangeable signals. Ref) Velde, V. V.; Lavie, D. A Δ^{16}-withanolide in *Withania somnifera* as a possible precursor for α-side chains. *Phytochemistry* **1982**, *21*, 731-733.

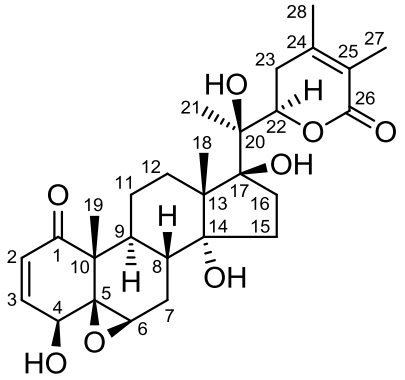
^{1}H and ^{13}C NMR data of Wit-4 (Withanolide E)



position	δ_H (J in Hz) (400 MHz, $CDCl_3$)	δ_C (100 MHz, $CDCl_3$)	δ_H (J in Hz) (Ref-a) ($CDCl_3$)	δ_C (Ref-b) (22.6 MHz, $CDCl_3$)
1		202.9		203.2
2	6.02 dd (10.1, 2.8)	129.8	6.00 dd (10, 2.5)	129.8
3	6.81 ddd (10.1, 6.4, 2.8)	143.6	6.82 ddd (10, 5, 2.5)	143.9
4		32.8		32.9
5		62.1		62.2
6	3.18 br s	64.1	3.18 br s	64.2
7		26.2		26.2
8		34.1		34.1
9		36.8		36.9
10		48.5		48.6
11		22.7		22.9
12		34.2		34.3
13		54.5		54.5
14		81.9		82.3
15		30.0		30.1
16		37.8		37.7
17		87.7		87.8
18	1.09 s	20.6	1.25 s	20.6
19	1.24 s	14.5	1.42 s	14.6
20		79.0		80.0
21	1.41 s	19.6	1.10 s	19.5
22	4.89 dd (11.5, 5.1)	79.6	4.88 br t (8)	80.3
23	2.51 m, 2H	32.4		32.5
24		150.7		151.1
25		121.4		121.4
26		165.9		166.6
27	1.88 s	12.3	1.95 s	12.3
28	1.94 s	20.5	1.95 s	20.6

Ref-a) Sakurai, K.; Ishii, H.; Kobayashi, S.; Iwao, T. Isolation of 4β-hydroxywithanolide E, a new withanolide from *Physalis peruviana* L. *Chem. Pharm. Bull*. **1976**, *24*, 1403-1405. Ref-b) Gottlieb, H. E.; Kirson, I. ^{13}C NMR spectroscopy of the withanolides and other highly oxygenated C_{28} steroids. *Org. Magn. Reson*. **1981**, *16*, 20-25.

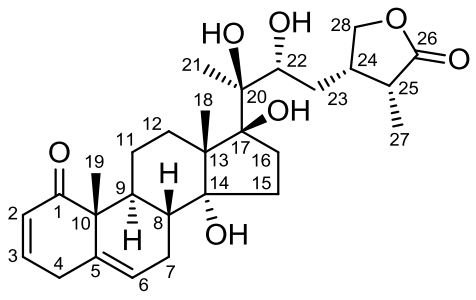
^{1}H and ^{13}C NMR data of Wit-S1 (4β-hydroxywithanolide E)



position	δ_H (J in Hz) (500 MHz, $CDCl_3$)	δ_C (125 MHz, $CDCl_3$)	δ_H (J in Hz) (Ref) ($CDCl_3$:CD_3OD=4:1)
1		201.8	
2	6.21 d (10.3)	133.0	6.18 d (10)
3	6.91 dd (10.3, 6.3)	141.4	7.02 dd (10, 6)
4	3.72 d (6.3)	70.2	3.67 d (6)
5		64.1	
6	3.27 br s	62.8	3.25 br s
7	2.01 m, 2H	25.8	
8	1.83 m	34.1	
9	1.69 m	36.6	
10		47.8	
11	1.70 m, 1.55 m	21.3	
12	2.26 ddd (12.0, 12.0, 4.0)	29.6	
	1.27 dd (12.0, 4.0)		
13		54.5	
14		81.8	
15	1.66 m, 1.55 m	32.3	
16	2.70 m, 1.44 m	37.8	
17		87.6	
18	1.06 s	20.2	1.37 s
19	1.40 s	16.6	1.43 s
20		79.0	
21	1.40 s	19.6	1.07 s
22	4.85 dd (11.5, 5.2)	79.7	4.85 dd (10, 7.5)
23	2.50 m, 2H	34.2	
24		150.7	
25		121.4	
26		166.0	
27	1.87 s	12.3	1.88 s
28	1.93 s	20.6	1.97 s
4-OH	2.70 s		

Ref) Sakurai, K.; Ishii, H.; Kobayashi, S.; Iwao, T. Isolation of 4β-hydroxywithanolide E, a new withanolide from *Physalis peruviana* L. *Chem. Pharm. Bull*. **1976**, *24*, 1403-1405.

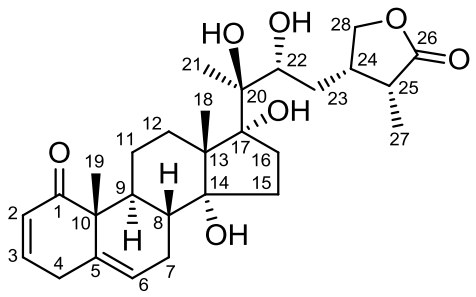
^{1}H and ^{13}C NMR data of Per-1 (Perulactone B)



position	δ_H (*J* in Hz) (400 MHz, $CDCl_3$)	δ_C (125 MHz, $CDCl_3$)	δ_H (*J* in Hz) (Ref) (400 MHz, $CDCl_3$)	δ_C (Ref) (100 MHz, $CDCl_3$)
1		204.7		204.7
2	5.88 dd (10.1, 1.8)	127.5	5.87 dd (10.0, 2.2)	127.5
3	6.80 ddd (10.1, 4.8, 2.3)	146.1	6.80 ddd (10.0, 4.7, 2.4)	146.1
4	3.28 br dd (21.4, 1.8)	33.4		33.4
	2.89 dd (21.4, 4.8)			
5		135.6		135.6
6	5.62 br d (5.5)	125.0	5.62 d (4.4)	124.9
7	2.08 m	25.5		25.5
	1.89 m			
8	2.24 ddd (11.5, 11.5, 4.1)	35.9		35.9
9	1.88 m	37.1		37.0
10		50.9		50.9
11	2.41 m, 1.72 m	23.0		23.0
12	1.73 m, 1.57 m	32.5		32.5
13		54.5		54.4
14		83.6		83.9
15	2.08 m, 1.25 m	31.5		31.6
16	2.67 m	37.6		37.6
	1.45 dd (14.9, 8.7)			
17		88.2		88.2
18	1.16 s	20.6	1.15 s	20.6
19	1.25 s	18.9	1.25 s	19.0
20		79.4		79.4
21	1.27 s	18.8	1.27 s	18.9
22	4.16 br d (10.1)	72.7	4.16 d (10.0)	72.6
23	2.41 m, 1.74 m	30.1		30.1
24	2.43 m	38.2		38.1
25	2.46 m	37.9		37.9
26		181.1		181.0
27	1.12 d (7.4)	10.5	1.12 d (7.0)	10.6
28	4.36 dd (8.5, 6.9)	73.4	4.36 dd (8.5, 7.0)	73.3
	4.02 dd (8.5, 8.5)		4.03 dd (8.5, 8.5)	

Ref) Fang, S. T.; Liu, J. K.; Li, B. Ten new withanolides from *Physalis peruviana*. *Steroids* **2012**, *77*, 36-44.

^{1}H and ^{13}C NMR data of Per-2 (Perulactone H)



position	δ_H (*J* in Hz) (500 MHz, $CDCl_3$)	δ_C (125 MHz, $CDCl_3$)	δ_H (*J* in Hz) (Ref) (600 MHz, $CDCl_3$)[b]	δ_C (Ref) (150 MHz, $CDCl_3$)[b]
1		203.7		205.0
2	5.90 dd (9.9, 1.7)	127.9	5.81 dd (10.2, 2.1)	127.8
3	6.79 ddd (9.9, 4.9, 2.3)	145.2	6.57 ddd (10.0, 4.8, 2.4)	146.1
4	3.30 br dd (21.2, 2.3)	33.4	3.23 br d (21.3)	33.6
	2.87 dd (21.2, 4.9)		2.80 dd (21.3, 4.9)	
5		135.8		135.3
6	5.61 m	124.0	5.55 d (5.0)	124.9
7	1.86-1.95 m, 2H	24.8	1.96 m, 1.78 m	24.9
8	1.92 m	36.1[a]	1.79 m	36.3
9	2.13 m	36.0[a]	2.14 td (12.0, 4.7)	35.9
10		50.8		50.9
11	2.36 m, 1.65 m	21.7	2.22 m, 1.54 m	22.0
12	2.34 m	26.2	2.27 td (13.1, 4.2)	26.3
	1.58 m		1.43 m	
13		50.1		50.1
14		87.3		86.2
15	1.77 m, 1.62 m	32.9	1.73 m, 1.51 m	32.8
16	2.63 ddd (15.2, 10.9, 1.7)	34.3	2.50 dd (14.4, 10.8)	33.9
	1.88 m		1.78 m	
17		92.2		92.2
18	0.99 s	19.1	0.91 s	19.2
19	1.27 s	19.0	1.20 s	19.1
20		77.2		76.8
21	1.27 s	23.4	1.20 s	23.3
22	3.74 d (10.3)	76.8	3.66 d (9.7)	76.6
23	1.79 m, 1.62 m	29.6	1.74 m, 1.57 m	29.4
24	2.78 m	37.1	2.70 m	37.0
25	2.68 m	38.0	2.63 m	38.0
26		180.7		181.6
27	1.20 d (7.5)	10.7	1.13 d (7.5)	10.7
28	4.48 dd (9.2, 7.2)	73.0	4.40 dd (9.0, 7.3)	73.1
	4.11 dd (9.2, 9.2)		4.04 dd (8.8, 8.8)	

[a]Interchangeable signals. [b]A few drops of CD_3OD were added to improve solubility. Ref) Fang, S. T.; Liu, J. K.; Li, B. Ten new withanolides from *Physalis peruviana*. *Steroids* **2012**, *77*, 36-44.

Organic Synthesis

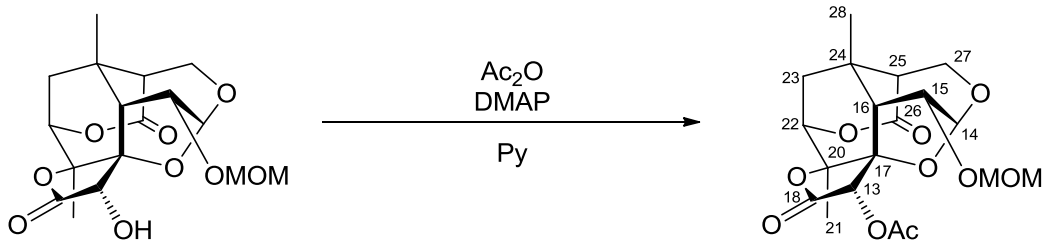
General; NMR spectra were recorded on a JEOL ECS400 spectrometer, or a Varian NMR System 500 spectrometer, operating at 400 or 500 MHz for ^{1}H-NMR and 100.4 or 125.8 MHz for ^{13}C-NMR. Chemical shifts were reported in the scale relative to $CHCl_3$ as an internal reference. ESI-MS was taken on a Bruker micrOTOF-QII, a JEOL JMS-T100LC, or Waters Synapt G2. Column chromatography was performed with silica gel 60 (40-50 mm) purchased from KANTO CHEMICAL CO., INC. High-performance liquid chromatography (HPLC) was performed with SHIMADZU LC-6AD system, SPD-10A detector set at 254 nm, and RID-10A refractive index detector. In general, reactions were carried out under a nitrogen atmosphere, unless noted otherwise. Dichloromethane (CH_2Cl_2) was purified by a Glass Contour Solvent Dispensing System.

The synthetic procedure and characterization of **S1**, **PBright-1**, **PBright-2**, and **PBright-4** have been reported.[10,11]

---

(10) Ohkubo, M.; Hirai, G.; Sodeoka, M. Synthesis of DFGH ring system of type B physalins: highly oxygenated, cage-shaped molecules. *Angew. Chem. Int. Ed*. **2009**, *48*, 3862-3866.
(11) Morita, M.; Hirai, G.; Ohkubo, M.; Koshino, H.; Hashizume, D.; Maruoka, K.; Sodeoka, M. Kinetically controlled one-pot formation of DEFGH-rings of type B physalins through domino-type transformations. *Org. Lett*. **2012**, *14*, 3434-3437.

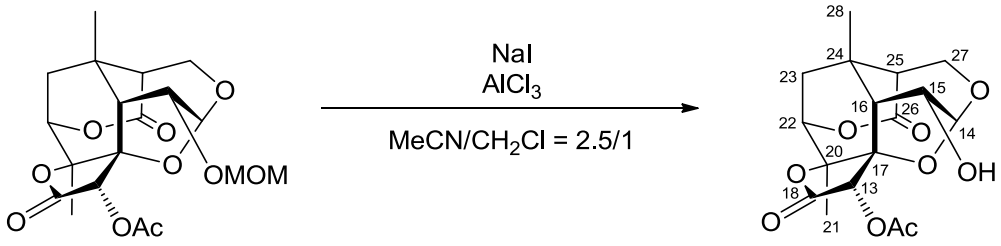
Compound **S2**



S1 **S2**

To a solution of **S1** (7.5 mg, 20 μmol) in pyridine (500 μl) were added acetic anhydride (500 μl, 5.3 μmol) and DMAP (1.1 mg, 9.0 μmol) at 0 ºC. After stirring for 20 min at 0 ºC, the reaction mixture was diluted with ethyl acetate, and water was added at 0 ºC. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over anhydrous magnesium sulfate and concentrated in vacuo. Further purification was carried out by silica gel column chromatography (eluent; hexane/ethyl acetate = 1/1 to 1/2) to give **S2** (7.3 mg, 87%) as a white amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.33 (s, 3H, *H*-28), 1.80 (s, 3H, *H*-21), 2.02 (dd, *J* = 2.9 Hz, 14.3 Hz, 1H, *H*-23), 2.06 (s, 1H, *H*-16), 2.07 (dd, *J* = 2.9 Hz, 14.3 Hz, 1H, *H*-23), 2.15 (s, 3H, 13-*OAc*), 2.42 (d, *J* = 4.0 Hz, 1H, *H*-25), 3.41 (s, 3H, 15-*OMOM*), 4.02 (d, *J* = 13.8 Hz, 1H, *H*-27), 4.51 (t, *J* = 2.9 Hz, 1H, *H*-22), 4.57 (dd, *J* = 4.0 Hz, 13.8 Hz, 1H, *H*-27), 4.65 (s, 1H, *H*-15), 4.68 (d, *J* = 6.9 Hz, 1H, 15-*OMOM*), 4.75 (d, *J* = 6.9 Hz, 1H, 15-*OMOM*), 5.35 (s, 1H, *H*-14), 5.88 (s, 1H, *H*-13); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.7 (13-*OAc*), 21.8 (*C*-21), 28.2 (*C*-28), 30.4 (*C*-24), 33.8 (*C*-23), 51.0 (*C*-25), 53.2 (*C*-16), 56.1 (15-*OMOM*), 60.5 (*C*-27), 71.6 (*C*-13), 75.2 (2C, *C*-15, *C*-22), 83.2 (*C*-17), 84.4 (*C*-20), 95.5 (15-*OMOM*), 106.3 (*C*-14), 167.2 (*C*-18), 168.0 (*13-OAc*), 169.1 (*C*-26); HRMS-ESI (m/z): [M+NH_4^+] calcd for $C_{19}H_{28}NO_{10}Na$, 430.1713; found, 430.1722.

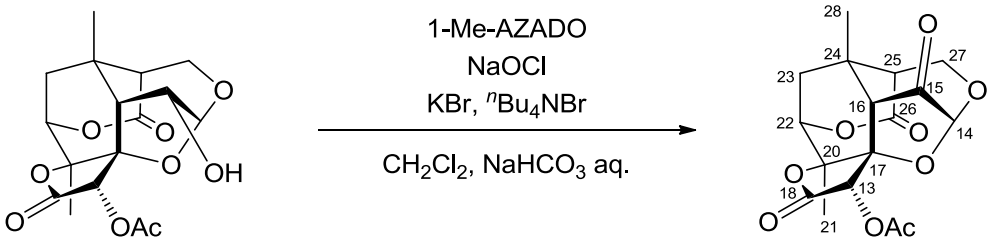
Compound **S3**



S2 **S3**

To a solution of **S2** (4.3 mg, 10 μmol) in acetonitrile (1.0 ml) and dichloromethane (0.4 ml) was added aluminium chloride (12.5 mg, 93.7 μmol) and sodium iodide (18.8 mg, 125 μmol) at -30 ºC. After stirring for 30 min at -30 ºC, the reaction mixture was warmed to 0 ºC. After stirring for 7.5 h at 0 ºC, the reaction mixture was diluted with ether, and brine was added at 0 ºC. The organic layer was separated and washed with brine. The combined aqueous layers were extracted with ethyl acetate. The combined organic layers were dried over anhydrous magnesium sulfate and concentrated in vacuo. Further purification was carried out by silica gel column chromatography (eluent; hexane/ethyl acetate = 1/1 to 1/2) to give **S3** (3.4 mg, 89%) as a white amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.36 (s, 3H, *H*-28), 1.76 (s, 3H, *H*-21), 2.00 (dd, *J* = 2.9 Hz, 14.9 Hz, 1H, *H*-23), 2.05 (dd, *J* = 2.9 Hz, 14.9 Hz, 1H, *H*-23), 2.13 (s, 1H, *H*-16), 2.25 (s, 3H, 13-*OAc*), 2.42 (d, *J* = 4.0 Hz, 1H, *H*-25), 3.98 (d, *J* = 13.2 Hz, 1H, *H*-27), 4.51 (t, *J* = 2.9 Hz, 1H, *H*-22), 4.56 (dd, *J* = 4.0 Hz, 13.2 Hz, 1H, *H*-27), 4.74 (d, *J* = 8.6 Hz, 1H, 15-*OH*), 4.94 (d, *J* = 8.6 Hz, 1H, *H*-15), 5.27 (s, 1H, *H*-14), 5.44 (s, 1H, *H*-13); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.7 (13-*OAc*), 21.8 (*C*-21), 28.6 (*C*-28), 30.5 (*C*-24), 33.9 (*C*-23), 51.2 (*C*-25), 55.4 (*C*-16), 60.7 (*C*-27), 72.1 (*C*-15), 73.3 (*C*-13), 74.9 (*C*-22), 82.9 (*C*-17), 85.0 (*C*-20), 108.9 (*C*-14), 167.1 (*C*-26), 167.8 (*C*-18), 171.8 (13-*OAc*); HRMS-ESI (m/z): [$M+NH_4^+$] calcd for $C_{17}H_{24}NO_9$, 386.1451; found, 386.1453.

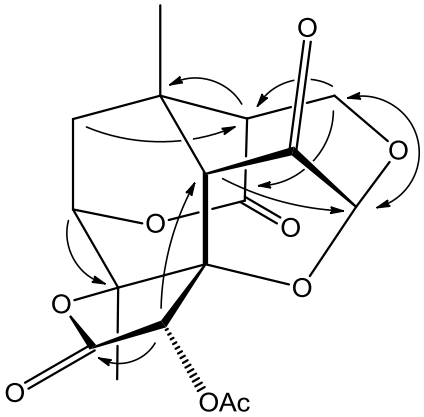
PBright-3



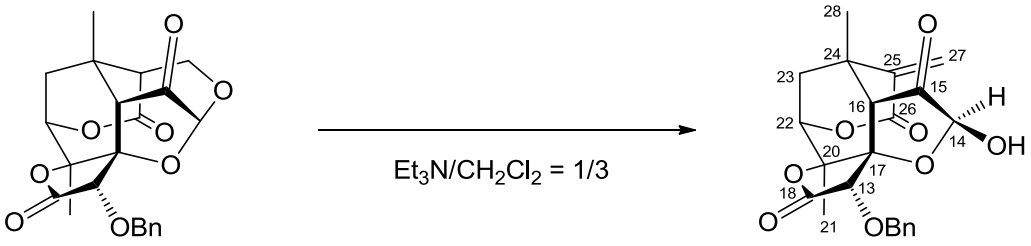
S3 **PBright-3**

To a mixture of a solution of **S3** (2.2 mg, 6.0 μmol) and 1-Me-AZADO (0.16 mg, 1.0 μmol) in dichloromethane (100 μl) and a saturated aqueous solution of sodium bicarbonate (20 μl) containing KBr (71 μg, 30 mM) and *n*-Bu_4NBr (96 μg, 15 mM), was added a pre-mixed solution of aqueous NaOCl (5% Cl) and a saturated aqueous solution of sodium bicarbonate (25.2 μl, 1:1.4 v/v) at 0 ºC. After stirring for 15 min at 0 ºC, was added a pre-mixed solution of aqueous NaOCl (5% Cl) and a saturated aqueous solution of sodium bicarbonate (25.2 ml, 1:1.4 v/v) at 0 ºC. After additional stirring for 10 min at 0 ºC, a saturated aqueous solution of sodium thiosulfate was added at 0 ºC. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over anhydrous magnesium sulfate and concentrated in vacuo. Further purification was carried out by silica gel column chromatography (eluent; hexane/ethyl acetate = 2/1) to give **PBright-3** (2.2 mg, quant.) as a white amorphous solid. A compound for biological assay was purified by HPLC (Senshu Pak PEGASIL Silica SP100, eluent; hexane/ethyl acetate = 3/2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.29 (s, 3H, *H*-28), 1.78 (s, 3H, *H*-21), 2.00 (dd, *J* = 2.9 Hz, 14.9 Hz, 1H, *H*-23), 2.15 (dd, *J* = 2.9 Hz, 14.9Hz, 1H, *H*-23), 2.18 (s, 3H, 13-*OAc*), 2.26 (s, 1H, *H*-16), 2.58 (d, *J* = 4.9 Hz, 1H, *H*-25), 3.88 (d, *J* = 13.7 Hz, 1H, *H*-27), 4.53 (dd, *J* = 4.9 Hz, 13.7 Hz, 1H, *H*-27), 4.62 (t, *J* = 2.9 Hz, 1H, *H*-22), 4.70 (s, 1H, *H*-13), 4.94 (s, 1H, *H*-14); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.3 (13-*OAc*), 20.5 (*C*-21), 26.7 (*C*-28), 31.3 (*C*-24), 32.8 (*C*-23), 51.1 (*C*-25), 56.4 (*C*-16), 61.7 (*C*-27), 72.6 (*C*-13), 74.9 (*C*-22), 77.5 (*C*-17), 83.3 (*C*-20), 98.6 (*C*-14), 166.3 (*C*-26), 167.7 (*C*-18), 169.4 (13-*OAc*), 202.8 (*C*-15); HRMS-ESI (m/z): [M+Na^+] calcd for $C_{17}H_{18}O_9Na$, 389.0849; found, 389.0842.

HMBC correlations for ***PBright-3***



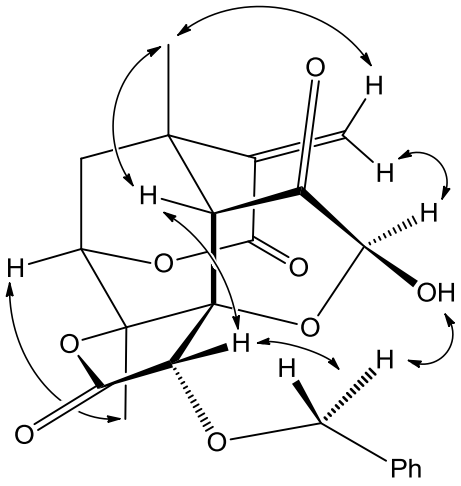
PAright-1



PBright-4 **PAright-1**

To a solution of **PBright-4** (1.4 mg, 3.4 μmol) in dichloromethane (300 μl) was added triethylamine (100 μl) at room temperature. After stirring for 3 h at the same temperature the reaction mixture was concentrated in vacuo to give **PAright-1** (1.3 mg, 93%) as a white amorphous solid. A compound for biological assay was purified by HPLC (Senshu Pak PEGASIL Silica SP100, eluent; hexane/ethyl acetate = 3/2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 3H, *H*-28), 1.87 (s, 3H, *H*-21), 1.98 (dd, *J* = 1.8 Hz, 14.7 Hz, 1H, *H*-23), 2.14 (dd, *J* = 4.1 Hz, 14.7 Hz, 1H, *H*-23), 2.21 (s, 1H, *H*-16), 3.33 (d, *J* = 3.7 Hz, 1H, 14-*OH*), 3.66 (s, 1H, *H*-13), 4.59 (dd, *J* = 1.8 Hz, 4.1 Hz, 1H, *H*-22), 4.61 (d, *J* = 11.0 Hz, 1H, 13-*OBn*), 4.91 (d, *J* = 3.7 Hz, 1H, *H*-14), 4.96 (d, *J* = 11.0 Hz, 1H, 13-*OBn*), 5.58 (s, 1H, *H*-27), 6.64 (s, 1H, *H*-27), 7.30-7.38 (m, 5H, 13-*OBn*); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 20.6 (*C*-21), 25.9 (*C*-28), 31.9 (*C*-23), 35.1 (*C*-24), 57.3 (*C*-16), 73.5 (13-*OBn*), 75.3 (*C*-22), 80.7 (*C*-13), 82.3 (*C*-17), 83.2 (*C*-20), 93.2 (*C*-14), 128.2 (13-*OBn*), 128.4 (13-*OBn*), 128.6 (13-*OBn*), 131.2 (*C*-27), 136.0 (13-*OBn*), 137.5 (*C*-25), 162.3 (*C*-26), 169.3 (*C*-18), 206.2 (*C*-15); HRMS-ESI (m/z): [M+Na^+] calcd for $C_{22}H_{22}O_8Na$, 437.1212; found, 437.1213.

NOE correlations for ***PAright-1***



Cell Biology

Cell Culture

Human cervical epithelial HeLa cells were maintained in D-MEM (Sigma) supplemented with 10 % fetal bovine serum (FBS), 1000 U/mL penicillin and 100 μg/mL streptomycin in a humidified chamber at 5 % CO_2. NF-κB reporter stable cell line derived from HeLa (HeLa/NF-κB-luc) cells (Panomics) were maintained in D-MEM (Sigma) supplemented with 10 % fetal bovine serum (FBS), 1000 U/mL penicillin (Sigma), 100 μg/mL streptomycin (Sigma), and 300 μg/mL hygromycin B (SIGMA) in a humidified chamber at 5 % CO_2.

Luciferase Reporter Assay

HeLa/NF-κB-luc cells were seeded at 2.5 x 10^4 cells/well in 96-well microtiter plates and incubated overnight at 37 °C under 5 % CO_2 in air. The medium was changed to serum-free D-MEM, and then cells were treated with compounds at the indicated concentrations. After incubation for 30 min, the cells were stimulated with 50 ng/mL TNF-α (REILA) and incubated for 7 h at 37 °C under 5 % CO_2 in air. The medium was removed, and the cells were washed with cold phosphate-buffered saline (PBS). Then Lysis buffer (Promega) was added to the wells. The resulting lysate was transferred to 96-well white microtiter plates and mixed with luciferase detection reagent (Promega). Luciferase activity was detected with a SpectraMax™ L microplate reader (Molecular Devices) using a 470 nm filter. The IC_{50} value for each cell line was determined from the sigmoid dose-response curve using the Origin8J program.

Cytotoxicity on HeLa/NF-κB-luc Cells

HeLa/NF-κB-luc cells were seeded at 2.5 x 10^4 cells/well in 96-well microtiter plates and incubated overnight at 37 °C under 5 % CO_2 in air. The medium was changed to serum-free D-MEM, and cells were treated with compounds at the indicated concentrations. After 6.5 h treatment, Alamar Blue™ (Invitrogen) was added to the wells, and the plate was incubated for 2 h at 37 °C under 5 % CO_2 in air. Fluorescence was measured with a SpectraMax™ M5 microplate reader (Molecular Devices) using a 530 nm-excitation filter and a 590 nm-emission filter.

Dual luciferase reporter gene assay (As an example: Rosenstiel, P. *et al. J. Cell Sci.* **2009**, *122*, 3522)
The mixture of FuGENE® HD Transfection Reagent (Promega) and pGL4.75-RLuc (Promega) was added to 40 mm dish containing 2 mL of HeLa/NF-κB-luc cells (2.5 x 10^5 cells) in growth medium. Methods for treatment of compounds and reporter assay were same with the above method (see ***Luciferase Reporter Assay***). Cells were divided into two plates, and firefly and *Renilla* luciferase activities were detected using two different substrates, luciferase detection reagent (Promega) and *Renilla*-Glo® Luciferase Assay System (Promega), with a SpectraMax™ L microplate reader (Molecular Devices) using a 470 nm filter.

Enzyme-Linked ImmunoSorbent Assay (ELISA)

***PathScan® ELISA*:** HeLa cells were seeded at 1.5 x 10^5 cells/well in 48-well plates and incubated overnight at 37 °C under 5 % CO_2 in air. The medium was changed to serum-free D-MEM, and cells were treated with proteasome inhibitor MG-132 (Wako) for 30 min (only phosphor-IκBα) followed by the test compounds for 30 min at 37 °C under 5 % CO_2 in air. After incubation, cells were stimulated with 50 ng/mL TNF-α for 7.5 min (for phosphor-IκBα) or 12.5 min (for total IκBα). The medium was removed, and the cells were washed with ice-cold PBS. The amounts of phosphor-IκBα and total IκBα were quantified according to the manufacturer's protocols (Cell Signaling). Absorbance was detected with a SpectraMax™ M2^e microplate reader (Molecular Devices) using a 450 nm filter (reference, 650 nm filter).

***DuoSet® ELISA*:** HeLa cells were seeded at 1.5 x 10^5 cells/well in 48-well plates and incubated overnight at 37 °C under 5 % CO_2 in air. The medium was changed to serum-free D-MEM, and cells were treated with test compound for 30 min at 37 °C under 5 % CO_2 in air. After incubation, HeLa cells were stimulated with 250 ng/mL TNF-α for various times (0, 10, 20, 30, 60, and 120 min). The medium was removed, and then the cells were washed with ice-cold PBS. The amount of total IκBα was quantified according to the manufacturer's protocols (R&D Systems). Absorbance was detected with a SpectraMax™ M2^e microplate reader (Molecular Devices) using a 450 nm filter (reference, 650 nm filter).

Western Blotting

Cell lysates were resolved on 10 % SDS-PAGE gels (Bio-Rad), and bands were transferred to PVDF membranes (Bio-Rad) and probed with specific antibodies. Detection was done with the ECL Western blotting detection system (Millipore) and a LAS-4000 (Fuji Film, Tokyo, Japan). Primary antibodies included anti-NF-κB RelA (ab7970; Abcam), anti-NF-κB p105/p50 (ab7971; Abcam), and anti-α-tubulin (T9026; Sigma). The secondary antibodies were horseradish peroxidase-conjugated goat anti-rabbit IgG (sc-2004; Santa Cruz Biotechnology) and goat anti-mouse IgG (170-6516; Bio-Rad).

Cell Fractionation

HeLa cells (for the experiment on nuclear translocation of NF-κB: 5 x 10^5 cells/1 mL/well, 12-well plate; for EMSA: 3 x 10^5 cells/2 mL/well, 6-well plate) were harvested in hypotonic lysis buffer (10 mM HEPES-KOH, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, pH 7.9), and supplemented with 1 mM DTT, 0.5 mM PMSF-serine protease inhibitor. Nonidet P-40 was added to a final concentration of 0.5 %, and extracts were incubated for 5 min on ice. Samples were centrifuged at 2,500 rpm for 5 min at 4 °C. The supernatant (cytoplasmic fraction) was collected, and the cell pellet (containing nuclei) was resuspended in ice-cold nuclear extraction buffer (20 mM HEPES-KOH, 400 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM PMSF-serine protease inhibitor, pH 7.9), incubated for 15 min on ice, and then centrifuged at 15,000 for 15 min at 4 °C. The supernatant containing nuclear proteins was used for western blotting and EMSA.

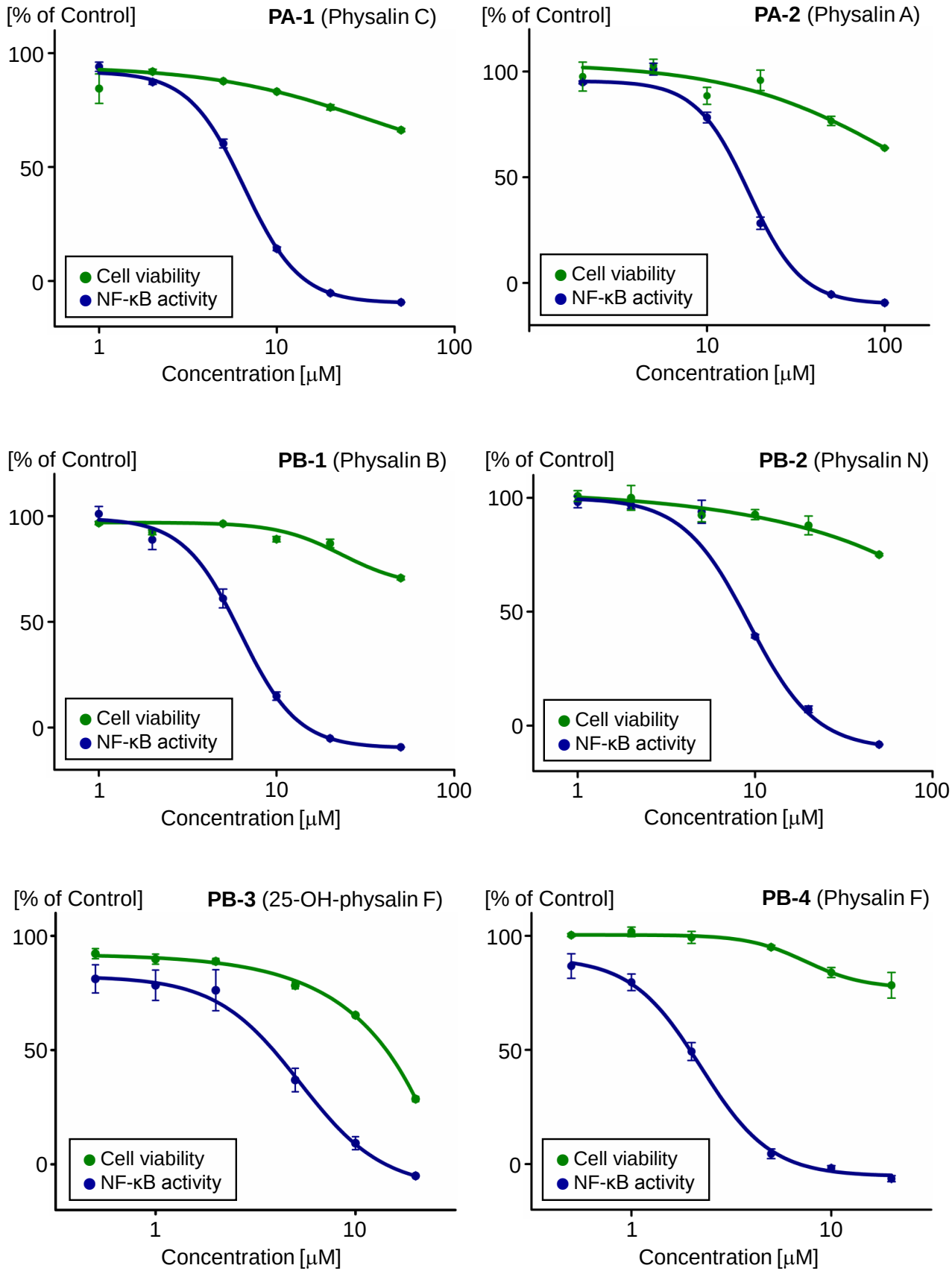
Quantitative Determination of Protein Concentration

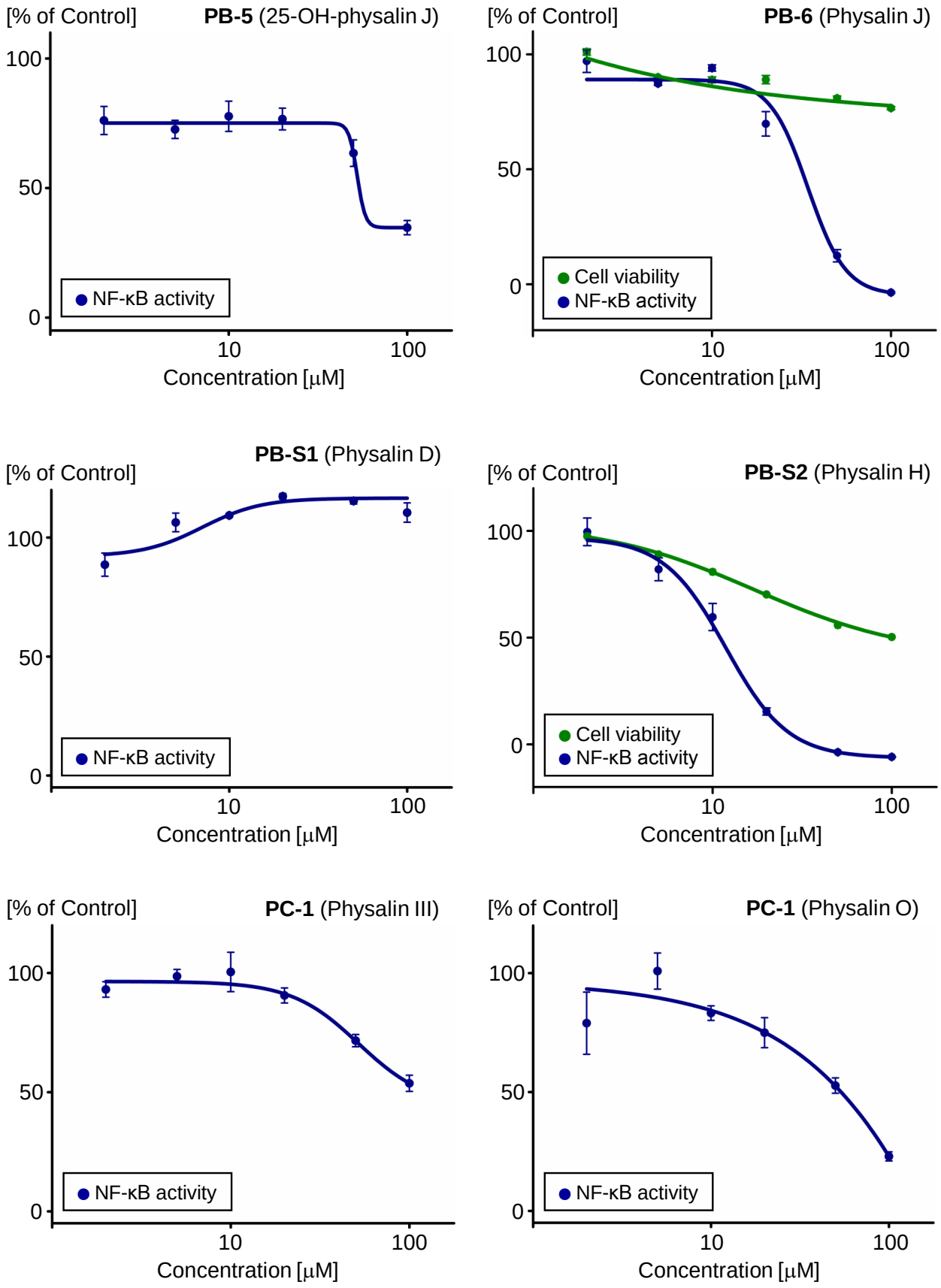
Quantitative determination of nuclear protein was performed using Quick Start™ Bradford (BIO-RAD). Absorbance was measured with a SpectraMax M5 (Molecular Devices) at 595 nm.

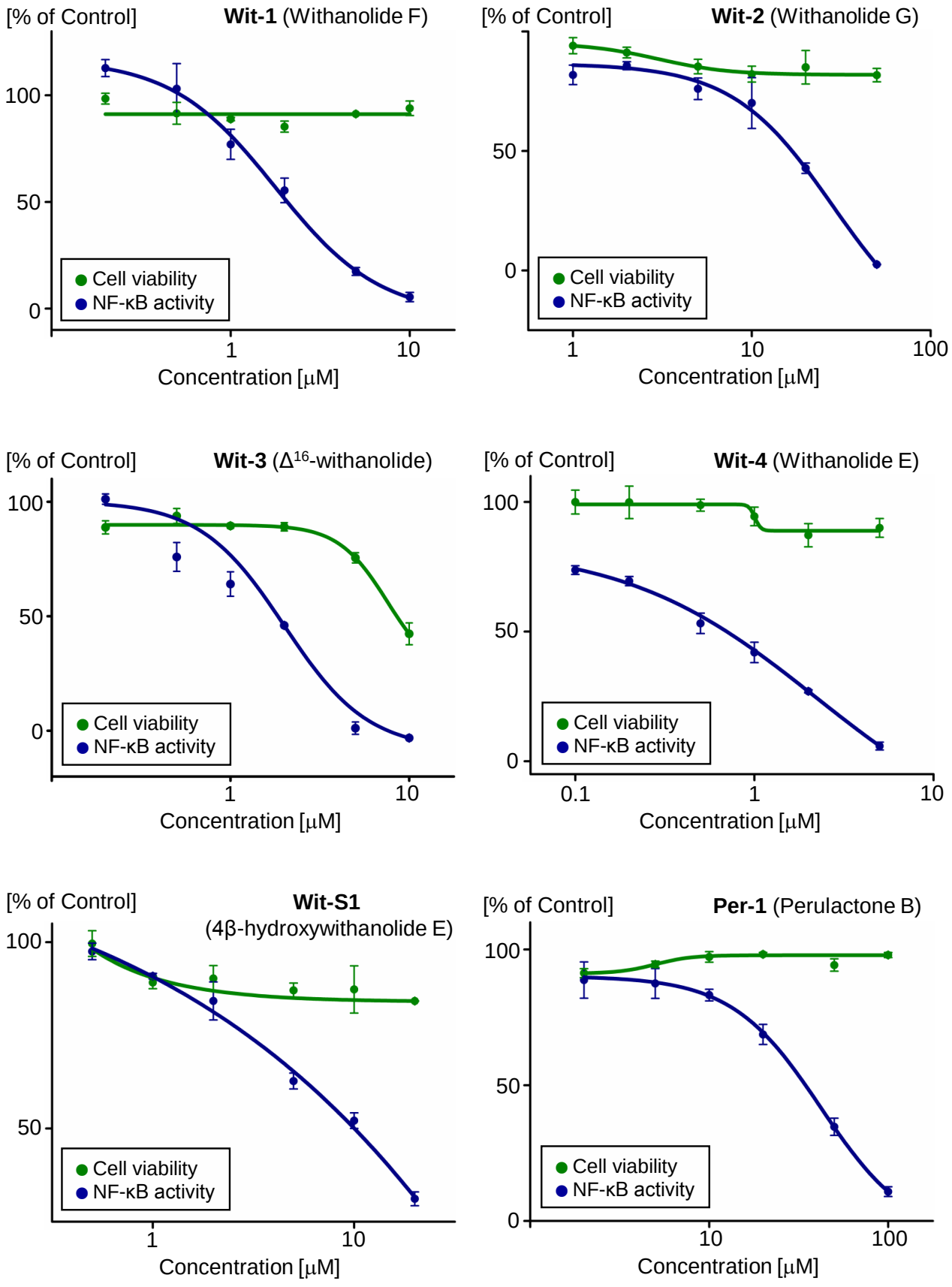
Electrophoretic Mobility Shift Assay (EMSA)

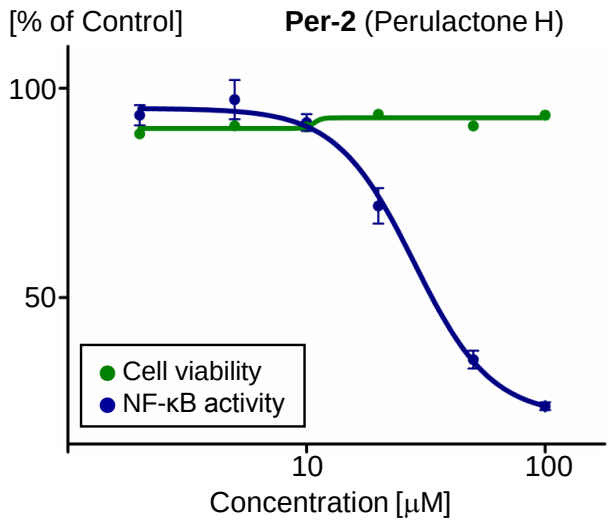
Gel-shift assay was performed using a DIG (digoxigenin) Gel Shift Kit, 2nd generation (Roche) according to the manufacturer's directions. The 3' end of NF-κB double-stranded consensus oligonucleotide (5'-AGTTGAGGGGACTTTCCCAGGC-3', Promega) was labeled with DIG-11-ddUTP using terminal transferase. Nuclear protein (4 μg) was incubated for 15 min at room temperature with 4 μL binding buffer, 1 μL poly [D(I-C)], 1 μL poly L-lysine, 2 μL DIG-labeled NF-κB oligonucleotide (400 pg/mL), and nuclease-free water (final volume, 20 μL). The samples were separated by electrophoresis (5 % nondenaturing polyacrylamide gel, 0.25 x TBE buffer, 150 V, 40 min at 4 °C). After electroblotting (0.25 x TBE buffer, 380 mA, 40 min at 4 °C) onto a nylon membrane, oligonucleotide was cross-linked to nylon membrane for 20 min at 120 °C. Then the membrane was treated with anti-digoxigenin antibody linked to alkaline phosphatase. Chemiluminescence generated from the substrate CSPD (disodium 3-(4-methoxyspiro{1,2-dioxetane-3,29-(5-chloro)tricyclo[3.3.1.137]decan}-4-yl)phenyl phosphate) by dephosphorylation was recorded on a LAS-4000 imaging device (Fuji Film).

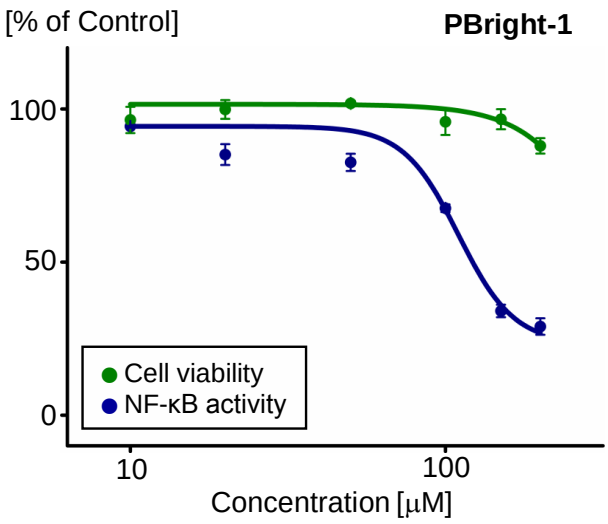
Dose-dependent Inhibition Curves of NF-κB Activation and Cytotoxicity on HeLa/NFkB-luc cells

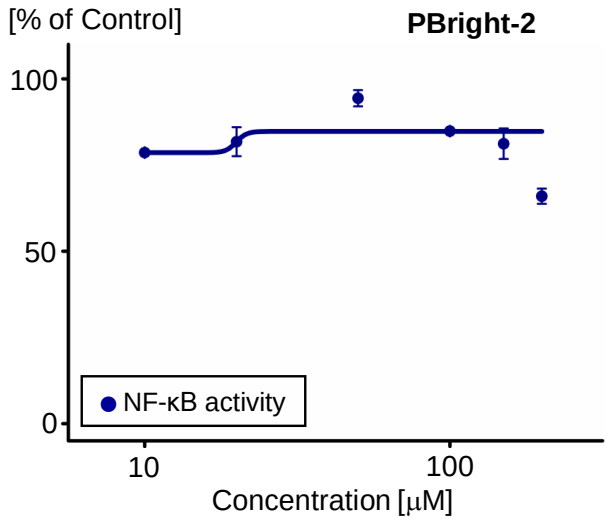


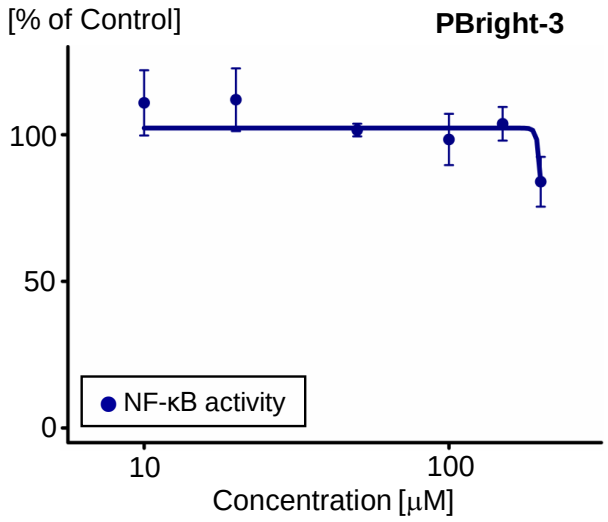

[% of Control]
PB-5 (25-OH-physalin J)
100
50
0
NF-κB activity
10
100
Concentration [μM]
[% of Control]
PB-6 (Physalin J)
100
50
0
Cell viability
NF-κB activity
10
100
Concentration [μM]
PB-S1 (Physalin D)
[% of Control]
100
50
0
NF-κB activity
10
100
Concentration [μM]
[% of Control]
PB-S2 (Physalin H)
100
50
0
Cell viability
NF-κB activity
10
100
Concentration [μM]
[% of Control]
PC-1 (Physalin III)
100
50
0
NF-κB activity
10
100
Concentration [μM]
[% of Control]
PC-1 (Physalin O)
100
50
0
NF-κB activity
10
100
Concentration [μM]

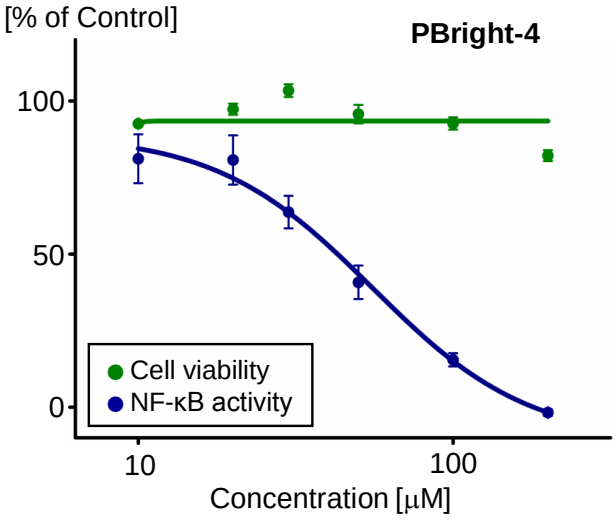

[% of Control]
Wit-1 (Withanolide F)
100
50
0
Cell viability
NF-κB activity
1
10
Concentration [μM]
[% of Control]
Wit-2 (Withanolide G)
50
0
Cell viability
NF-κB activity
1
10
100
Concentration [μM]
[% of Control]
Wit-3 (Δ16-withanolide)
100
50
0
Cell viability
NF-κB activity
1
10
Concentration [μM]
[% of Control]
Wit-4 (Withanolide E)
100
50
0
Cell viability
NF-κB activity
0.1
1
10
Concentration [μM]
[% of Control]
Wit-S1
(4β-hydroxywithanolide E)
100
50
Cell viability
NF-κB activity
1
10
Concentration [μM]
[% of Control]
Per-1 (Perulactone B)
100
50
0
Cell viability
NF-κB activity
10
100
Concentration [μM]

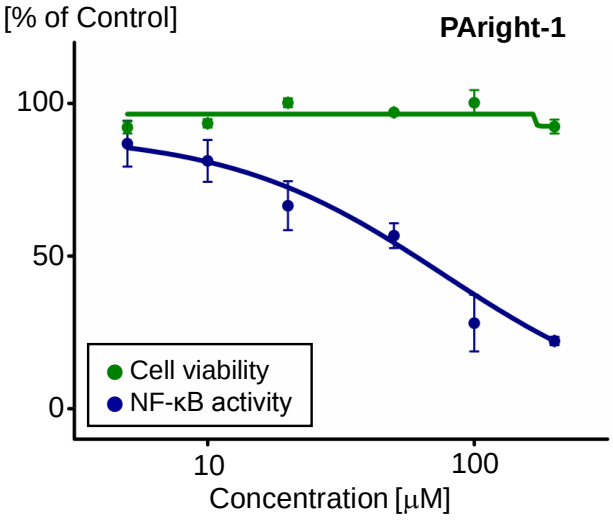

[% of Control]
Per-2 (Perulactone H)
100
50
Cell viability
NF-κB activity
10
100
Concentration [μM]


[% of Control]
PBright-1
100
50
0
Cell viability
NF-κB activity
10
100
Concentration [μM]

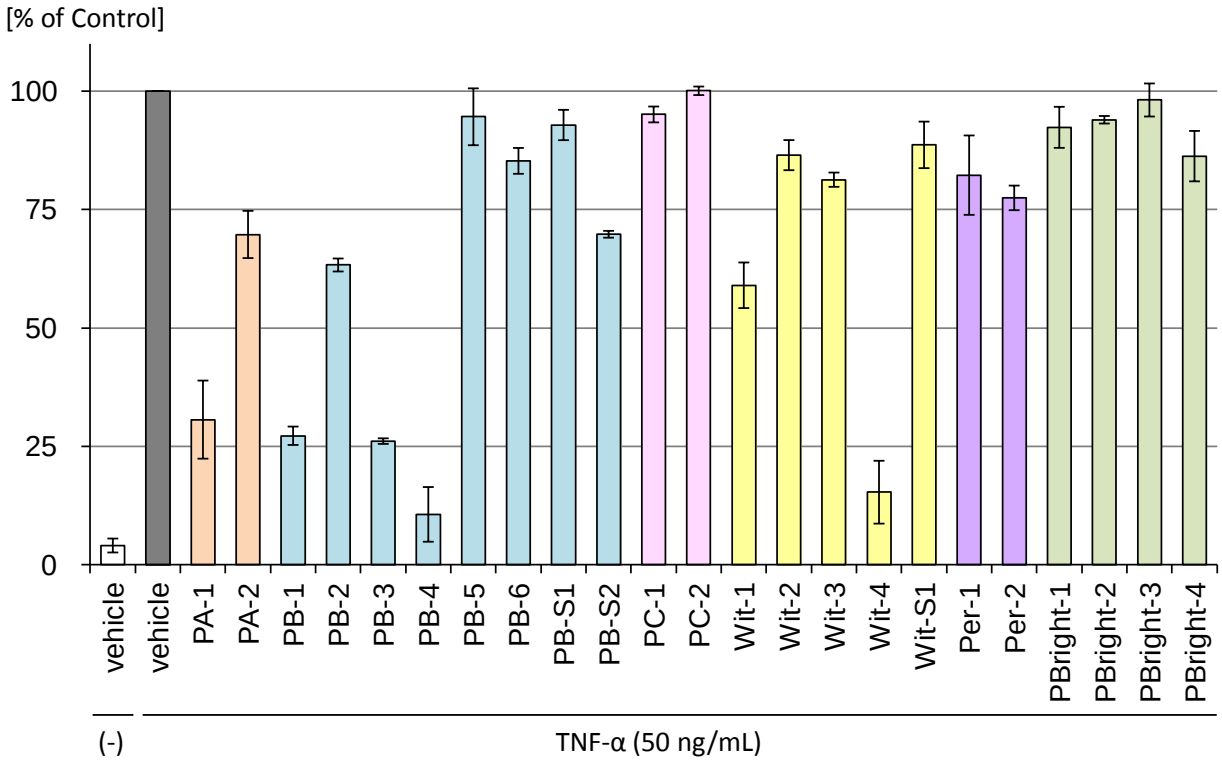

[% of Control]
PBright-2
100
50
0
NF-κB activity
10
100
Concentration [μM]


[% of Control]
PBright-3
100
50
0
NF-κB activity
10
100
Concentration [μM]


[% of Control]
PBright-4
100
50
0
Cell viability
NF-κB activity
10
100
Concentration [μM]

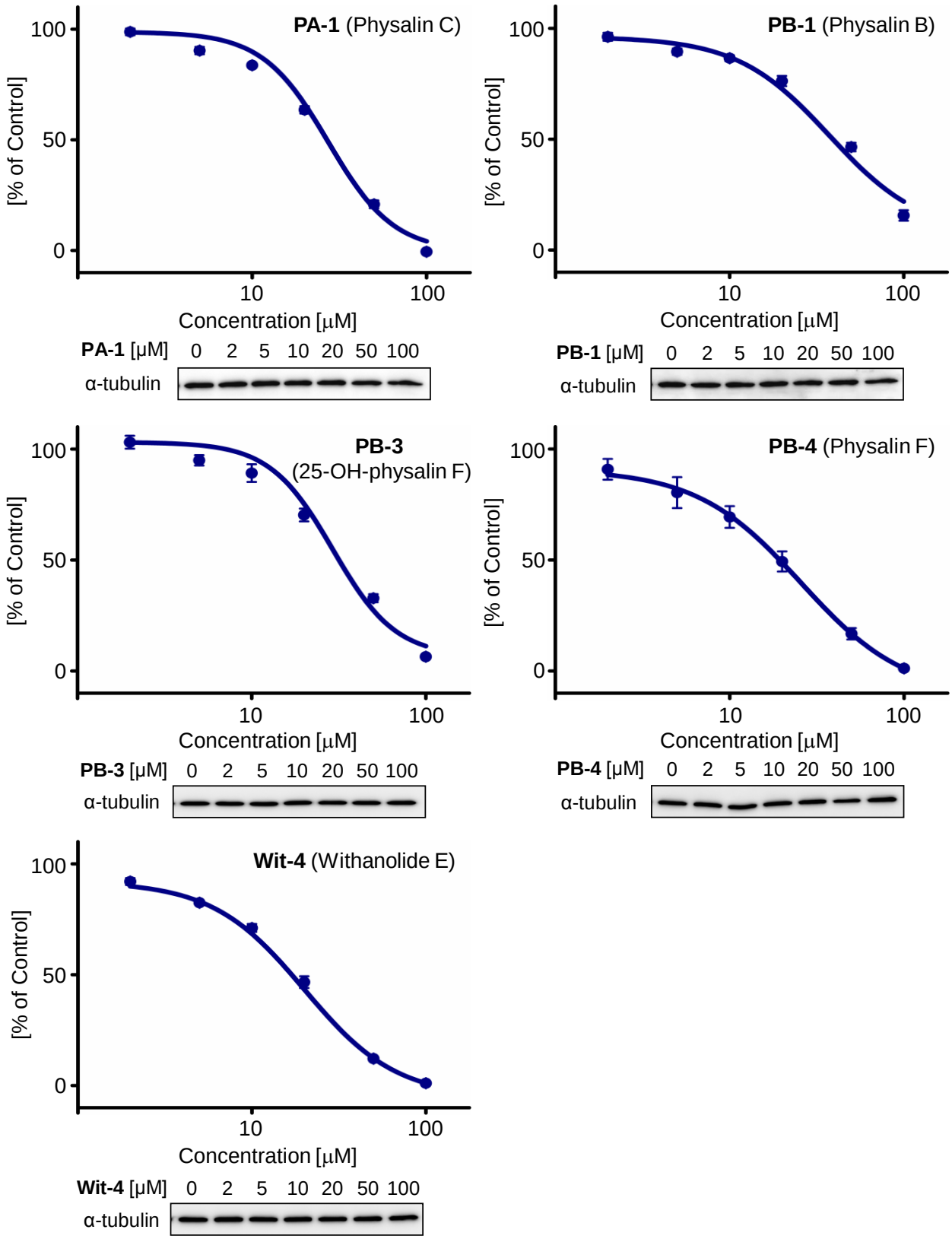

[% of Control]
PAright-1
100
50
0
Cell viability
NF-κB activity
10
100
Concentration [μM]

Inhibition of IκBα Phosphorylation by Oxygenated Steroids at 50 μM

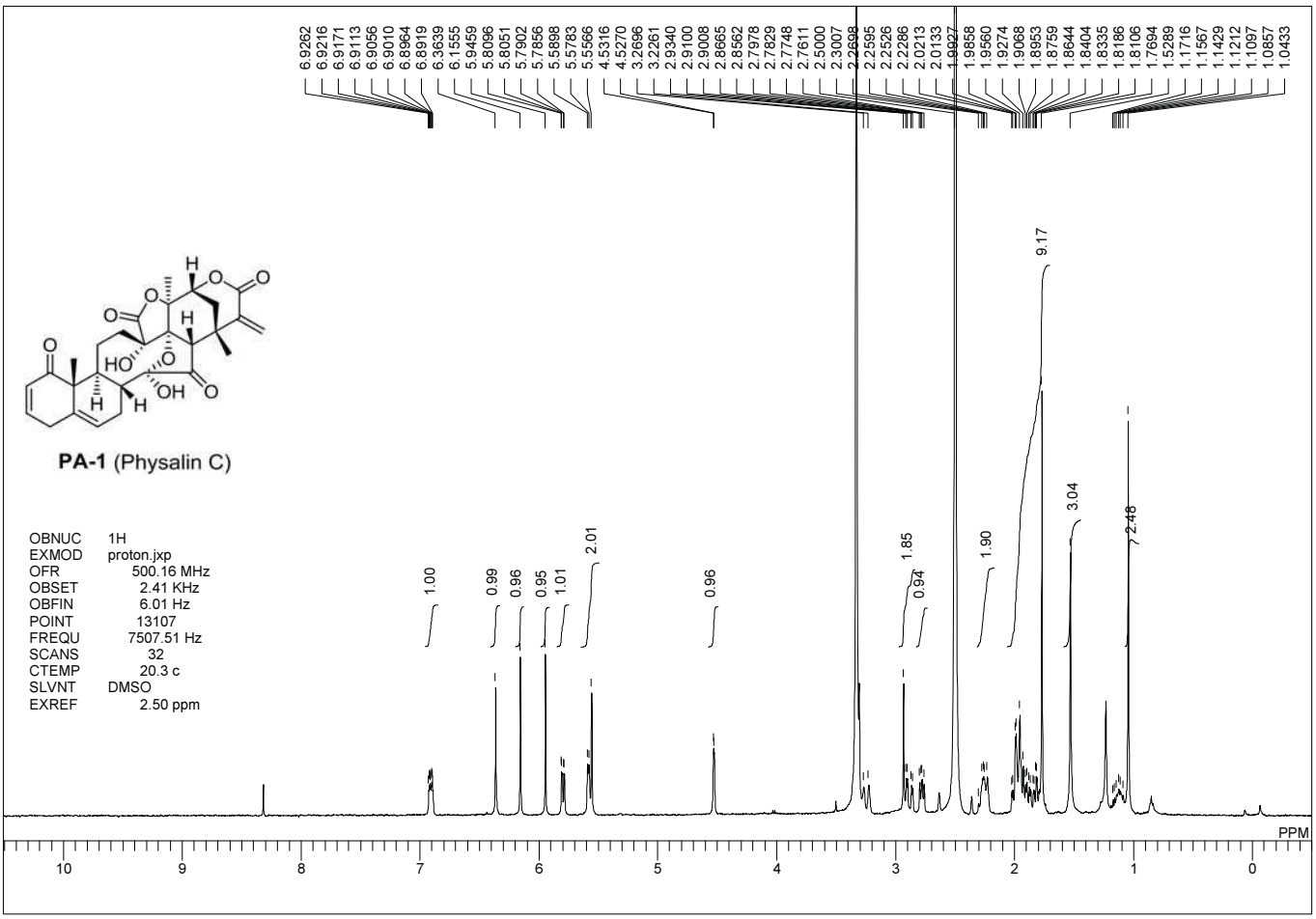


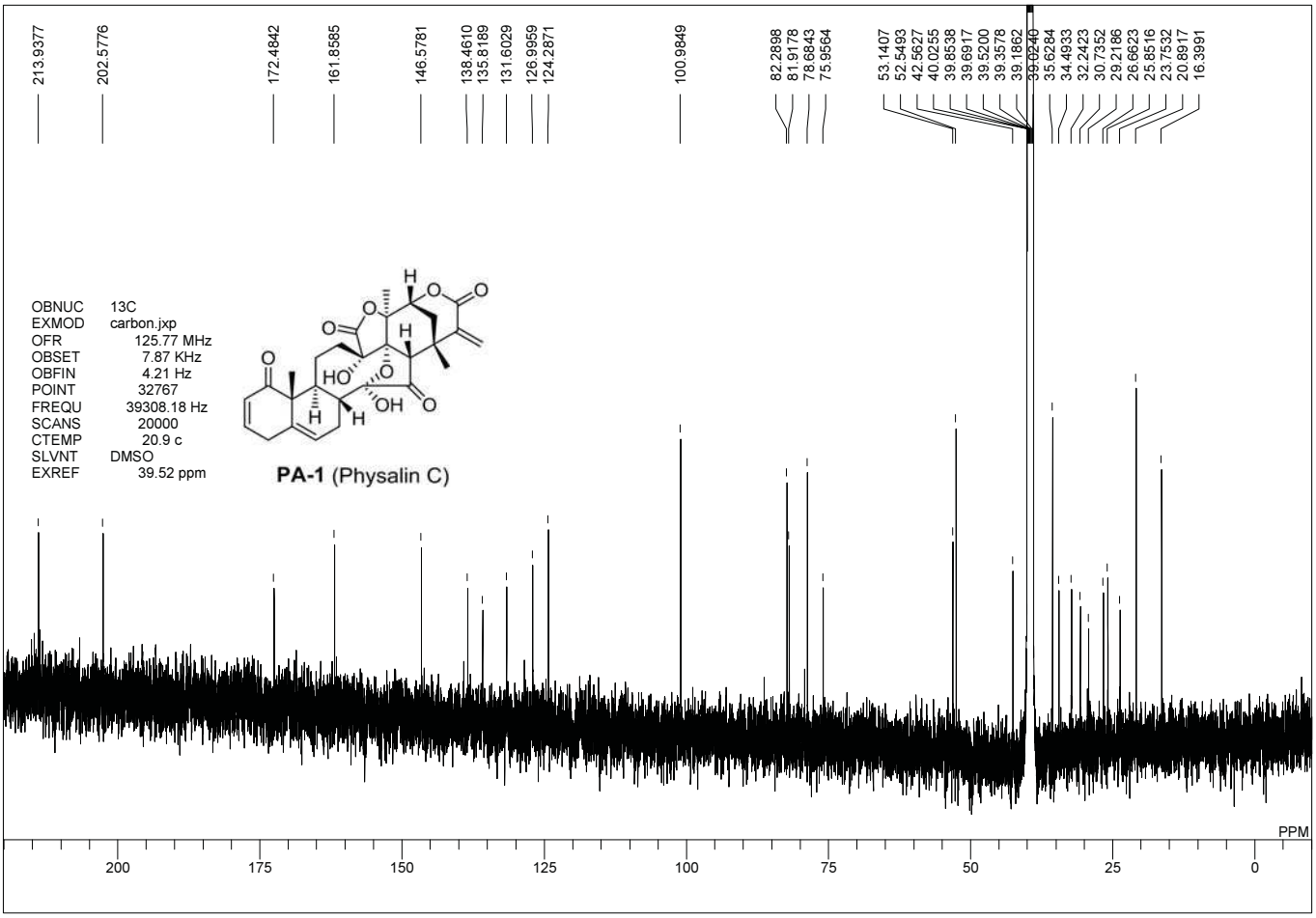
HeLa cells were stimulated with TNF-α (50 ng/mL) for 7.5 min after pretreatment with 10 μM MG-132 (proteasome inhibitor) for 30 min and then treated with DMSO or test compound (50 μM) for 30 min. Phosphorylated IκBα proteins were quantified using the PathScan® ELISA method.

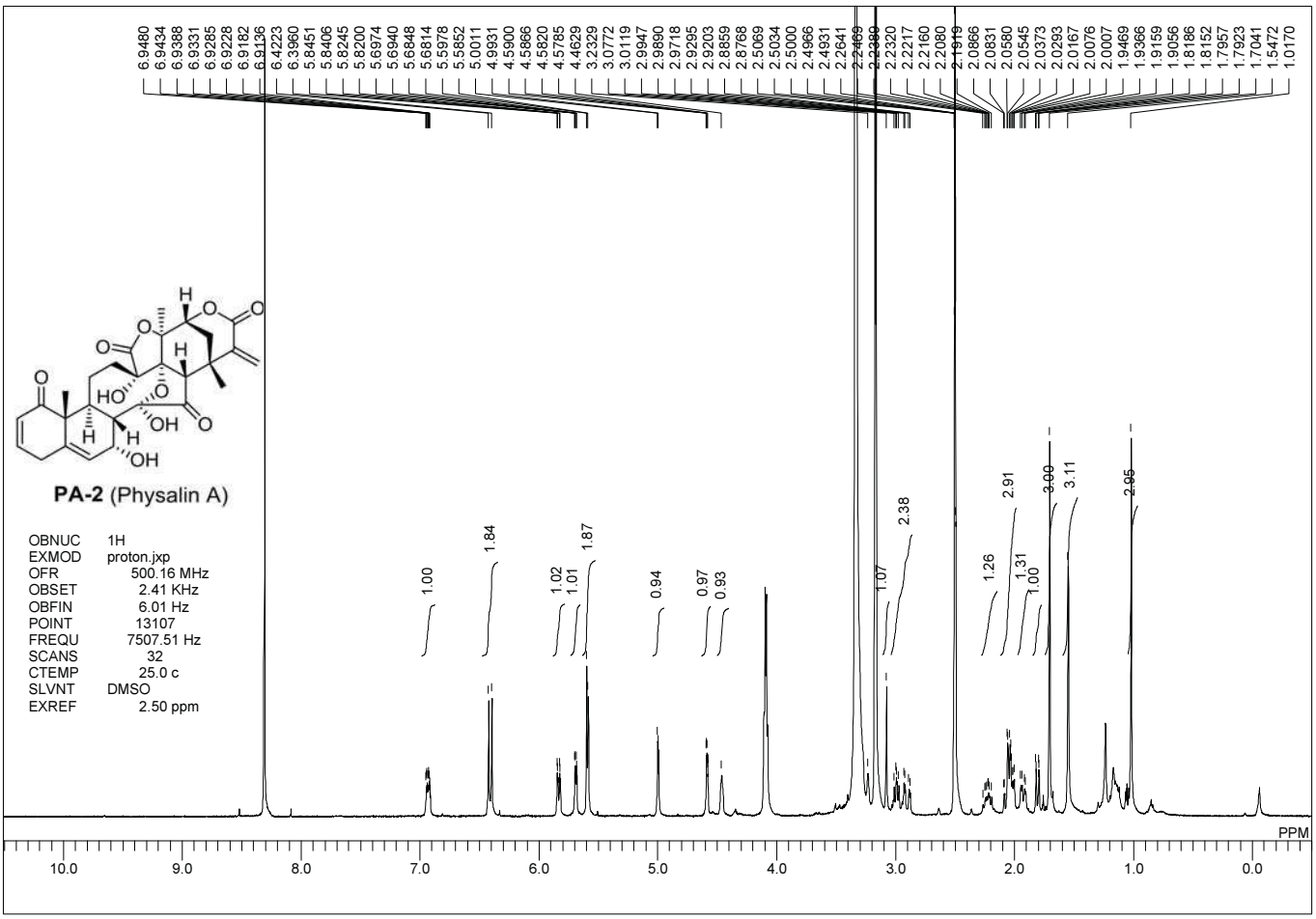
Dose-dependent Inhibition Curves for IκBα Phosphorylation

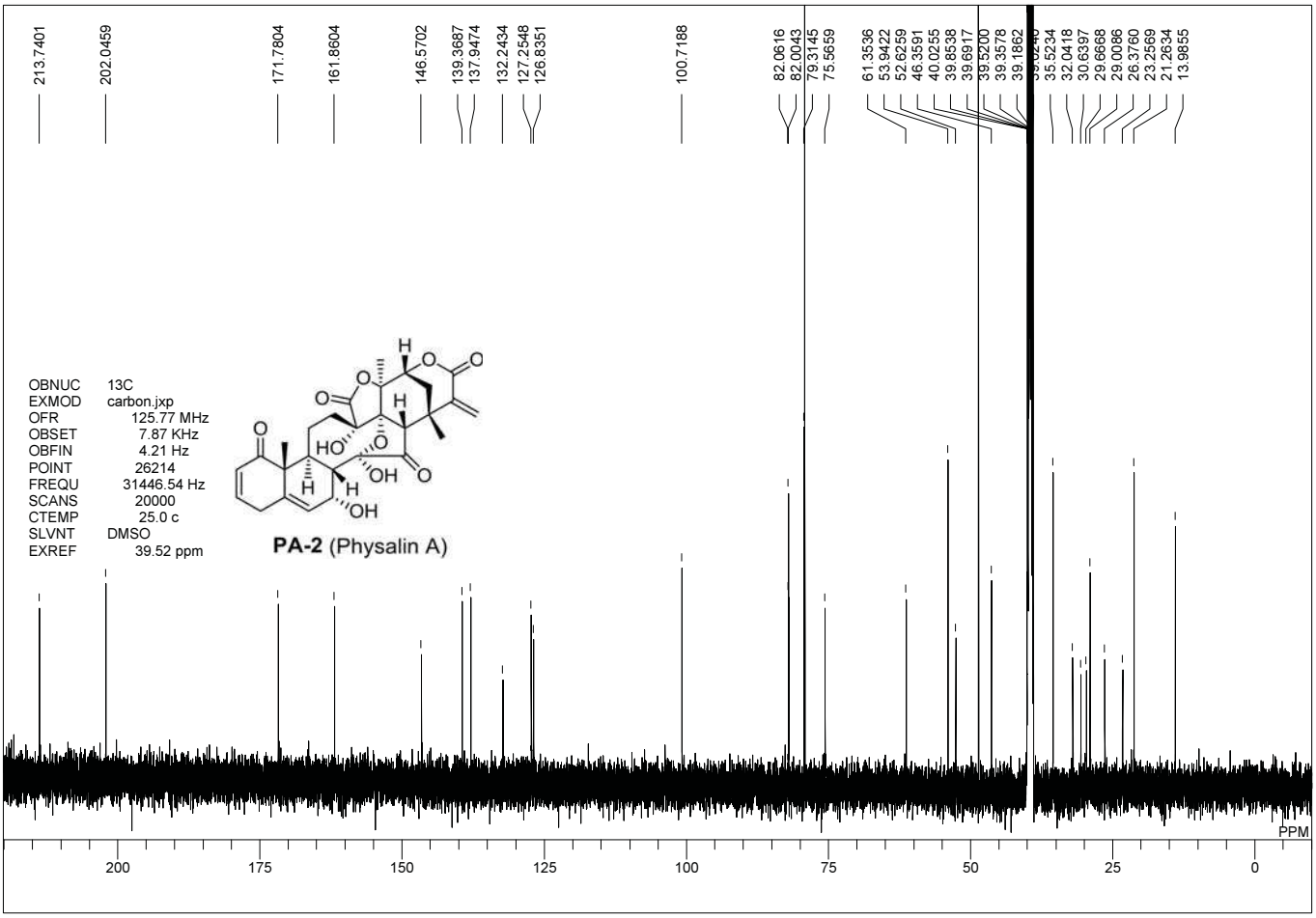


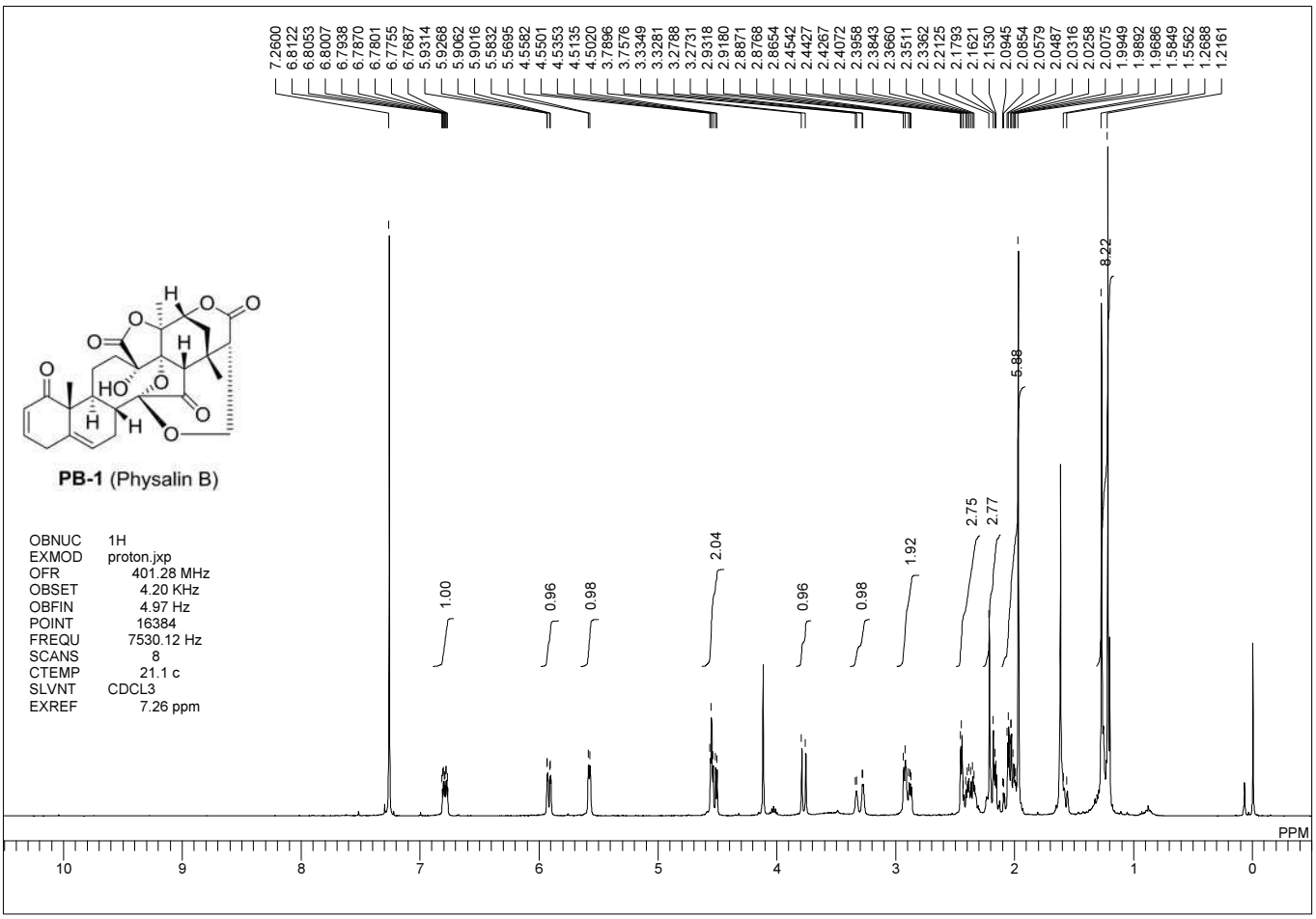
HeLa cells were stimulated with TNF-α (50 ng/mL) for 7.5 min after pretreatment with various concentration of test compounds or DMSO for 30 min. The phospho-IκBα proteins were quantified by ELISA method. Western blotting analysis of whole-cell proteins with anti-α-tubulin antibody.

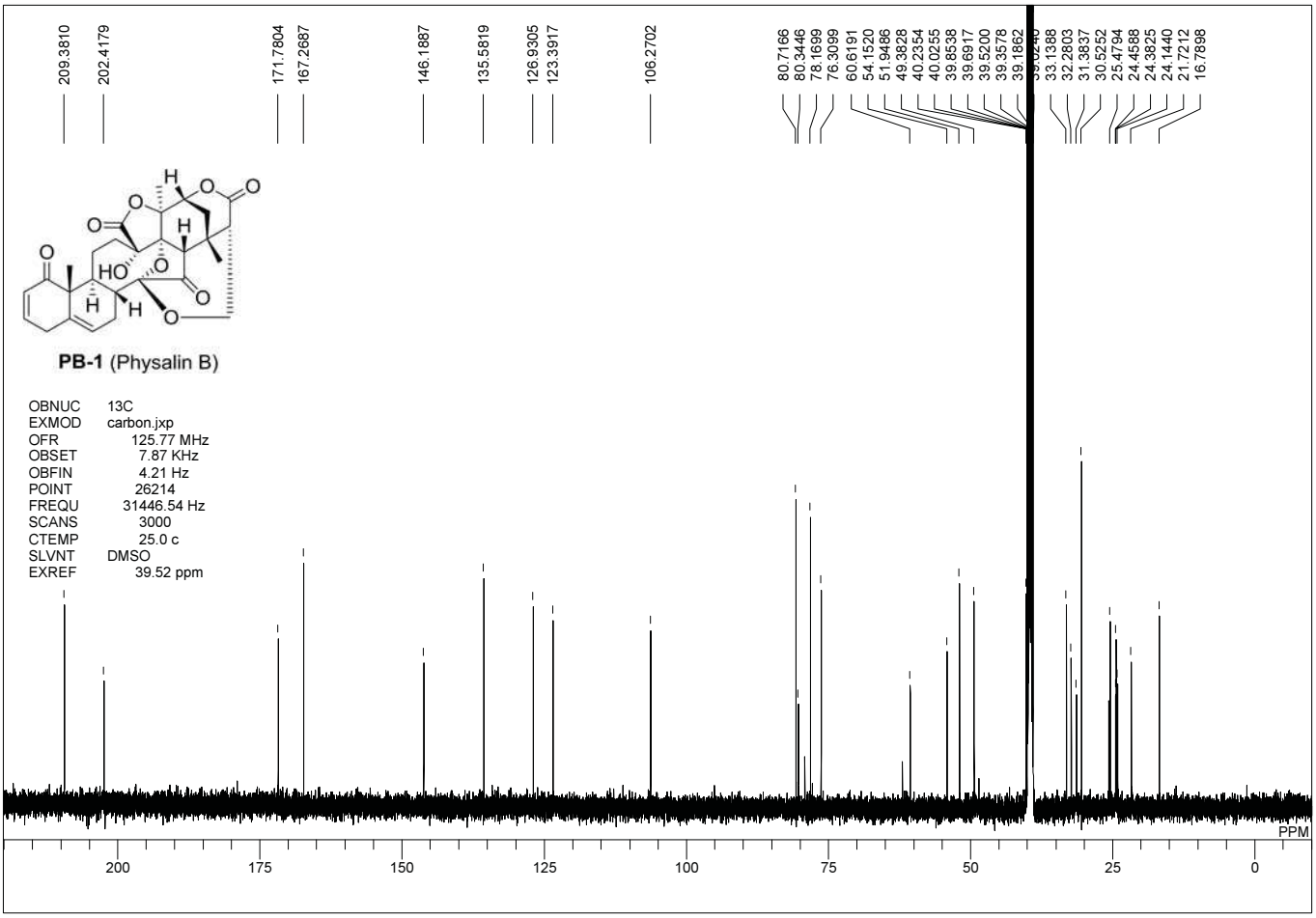

PA-1 (Physalin C)
OBNUC 1H
EXMOD proton.jxp
OFR 500.16 MHz
OBSET 2.41 KHz
OBFIN 6.01 Hz
POINT 13107
FREQU 7507.51 Hz
SCANS 32
CTEMP 20.3 c
SLVNT DMSO
EXREF 2.50 ppm
PPM

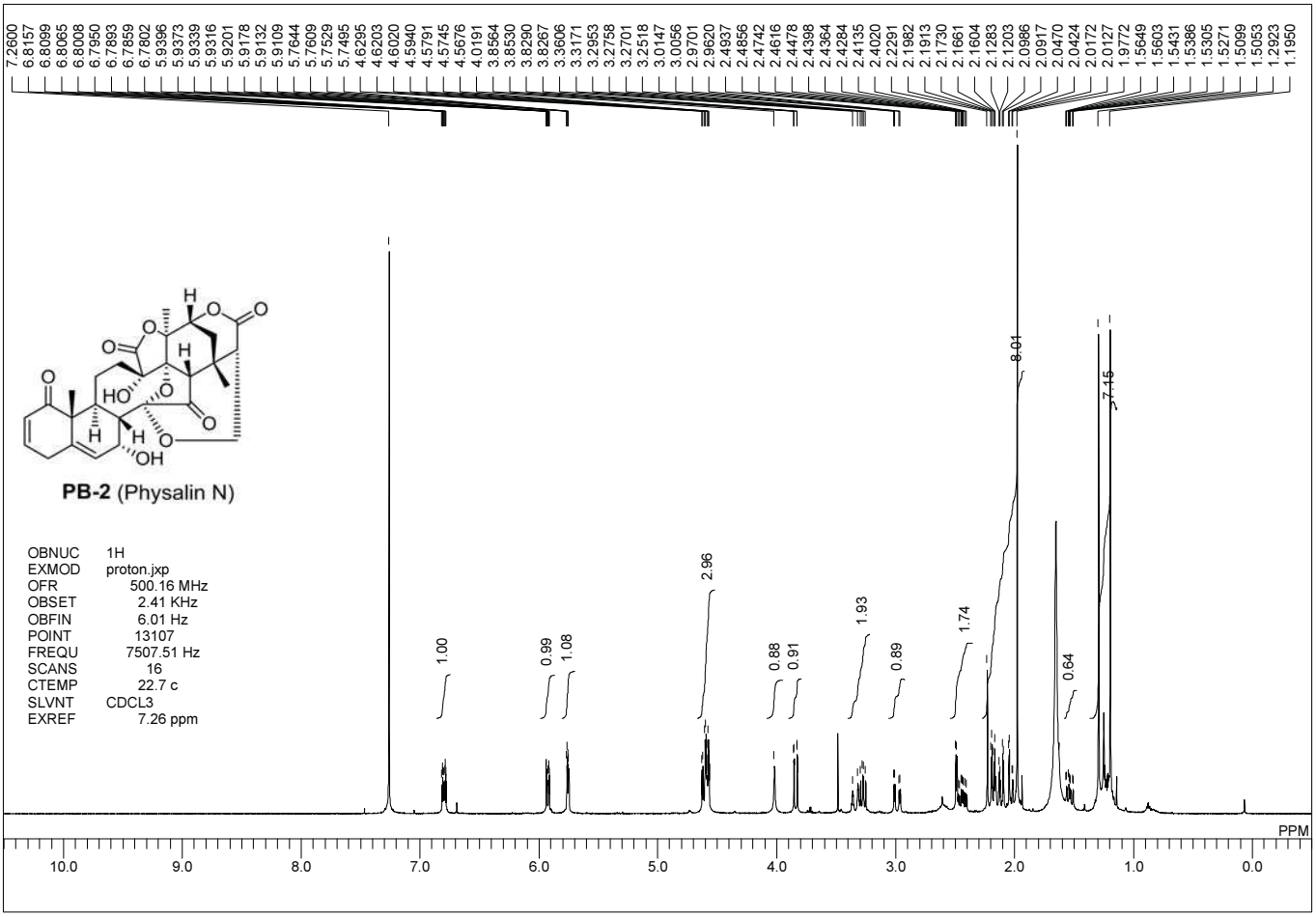

PA-1 (Physalin C)
OBNUC 13C
EXMOD carbon.jxp
OFR 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 32767
FREQU 39308.18 Hz
SCANS 20000
CTEMP 20.9 c
SLVNT DMSO
EXREF 39.52 ppm
PPM

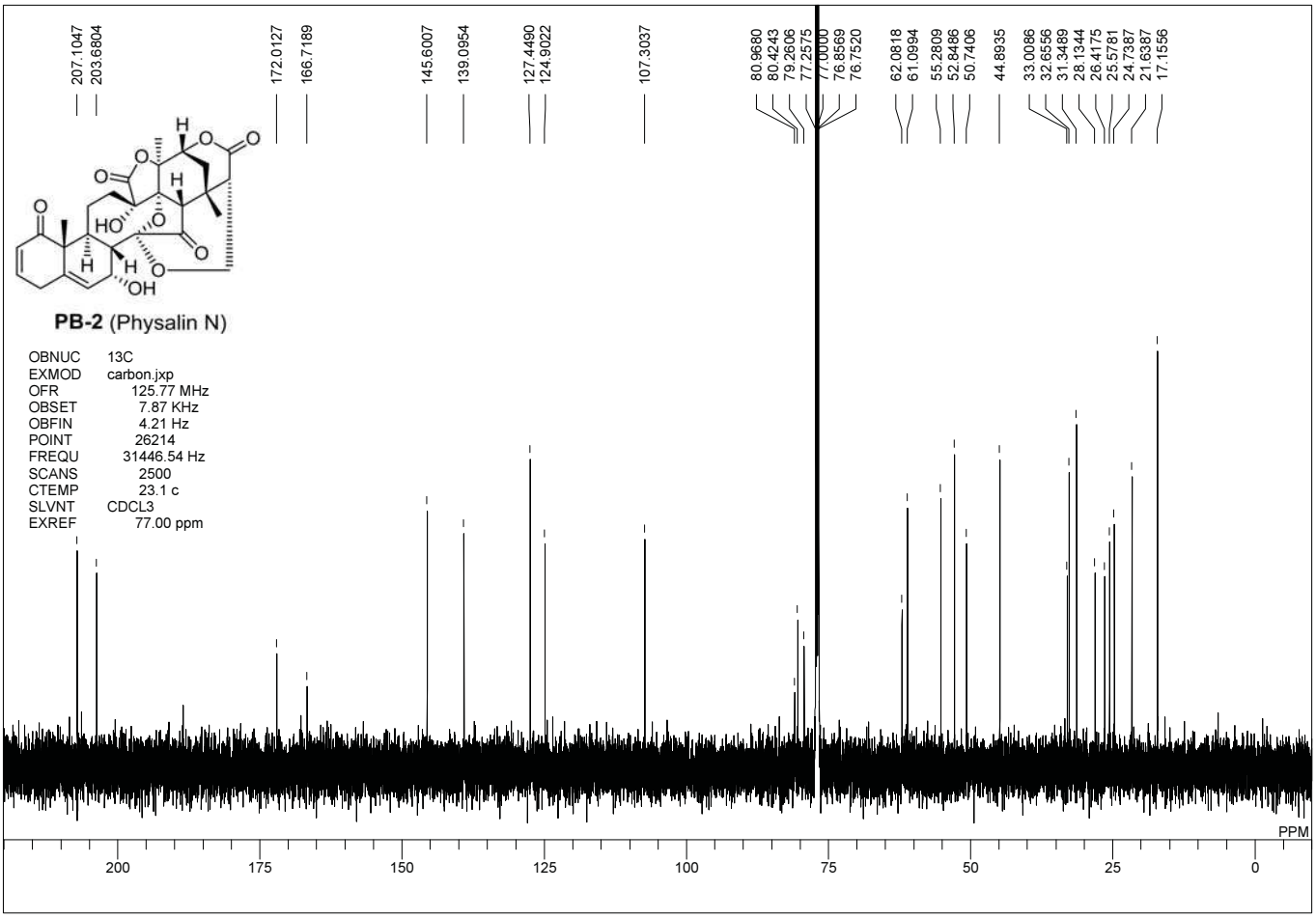

PA-2 (Physalin A)
OBNUC 1H
EXMOD proton.jxp
OFR 500.16 MHz
OBSET 2.41 KHz
OBFIN 6.01 Hz
POINT 13107
FREQU 7507.51 Hz
SCANS 32
CTEMP 25.0 c
SLVNT DMSO
EXREF 2.50 ppm
PPM

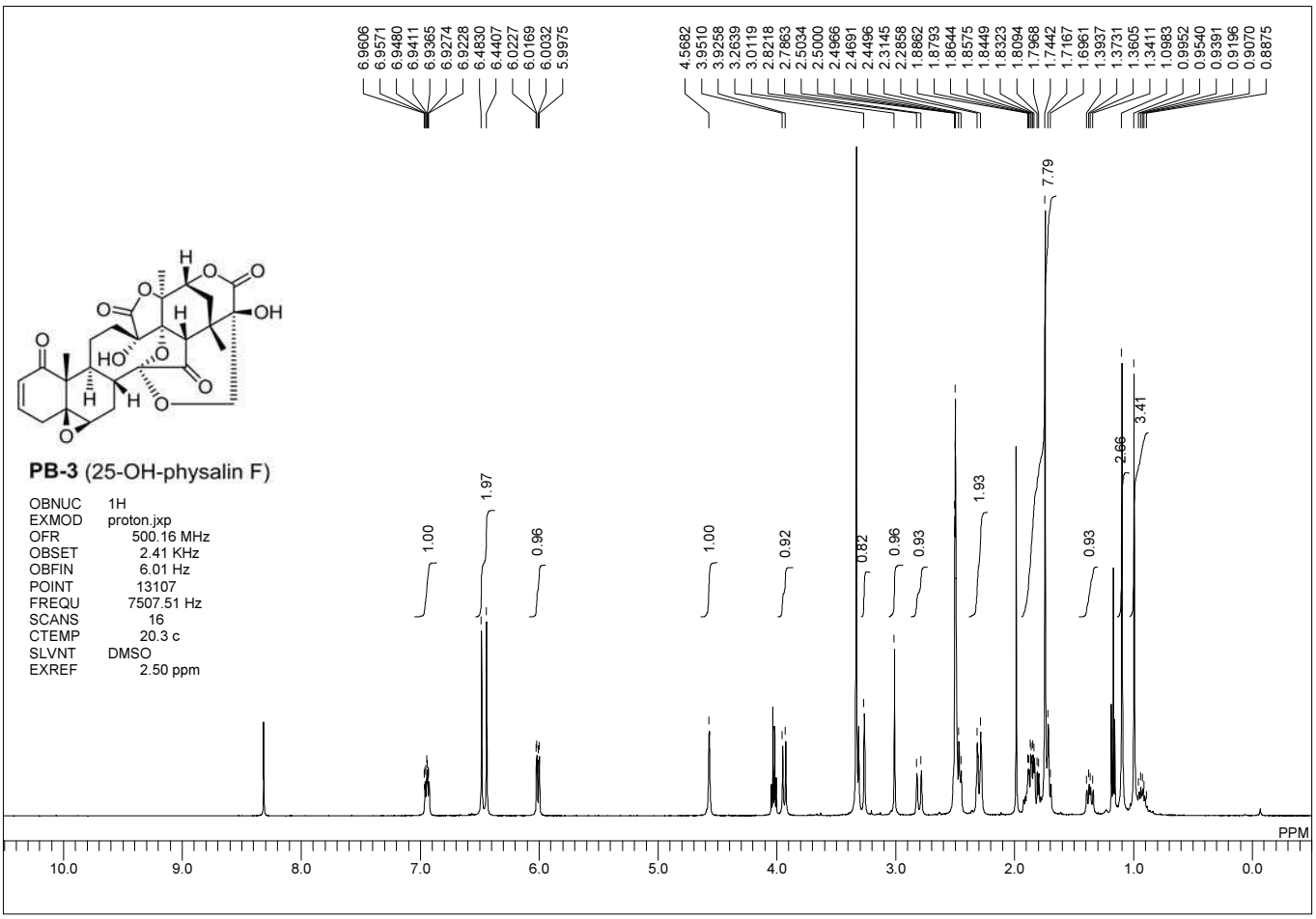

PA-2 (Physalin A)
OBNUC 13C
EXMOD carbon.jxp
OFR 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 26214
FREQU 31446.54 Hz
SCANS 20000
CTEMP 25.0 c
SLVNT DMSO
EXREF 39.52 ppm
PPM

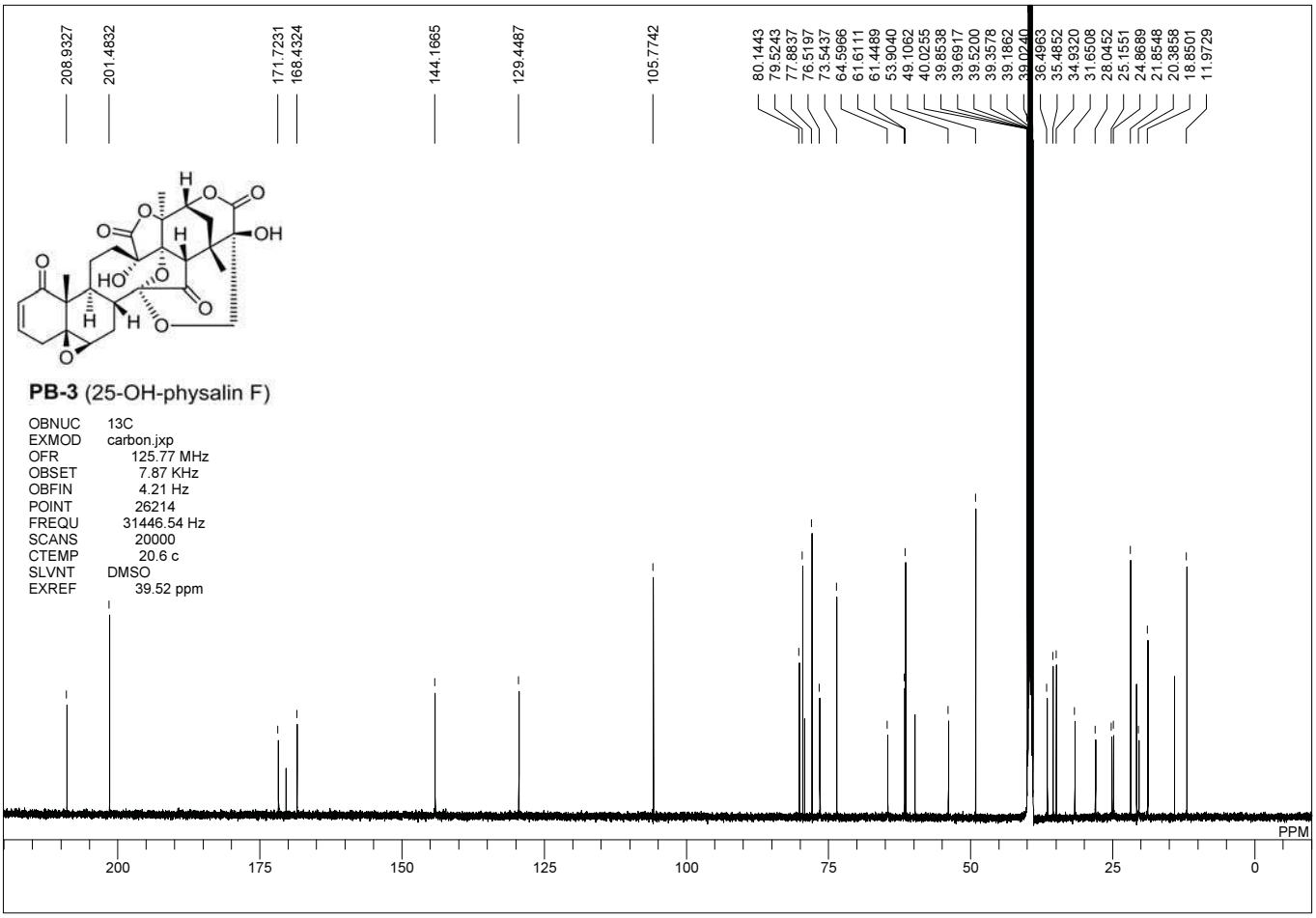

PB-1 (Physalin B)
OBNUC 1H
EXMOD proton.jxp
OFR 401.28 MHz
OBSET 4.20 KHz
OBFIN 4.97 Hz
POINT 16384
FREQU 7530.12 Hz
SCANS 8
CTEMP 21.1 c
SLVNT CDCL3
EXREF 7.26 ppm
PPM

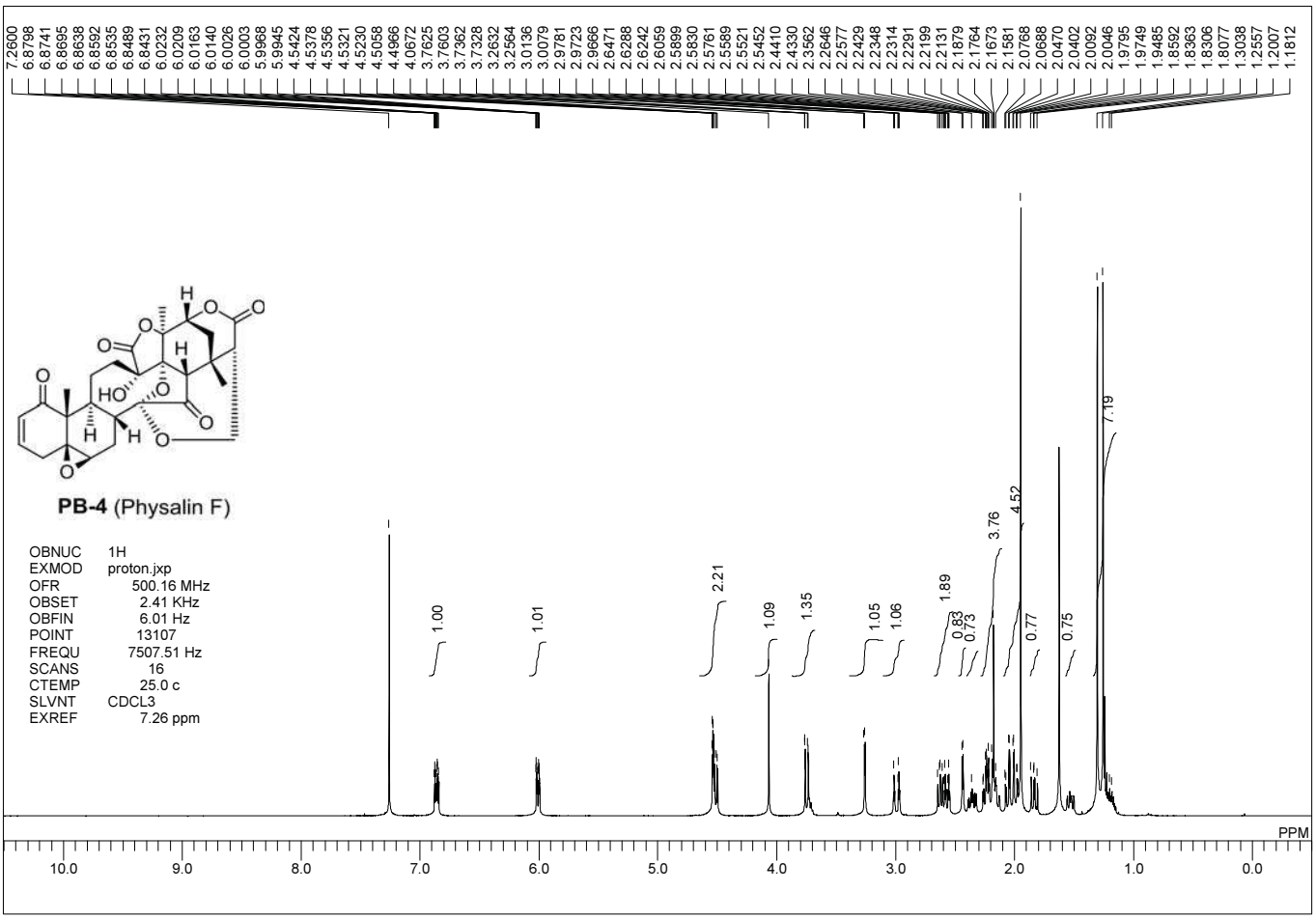

PB-1 (Physalin B)
OBNUC 13C
EXMOD carbon.jxp
OFR 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 26214
FREQU 31446.54 Hz
SCANS 3000
CTEMP 25.0 c
SLVNT DMSO
EXREF 39.52 ppm
PPM

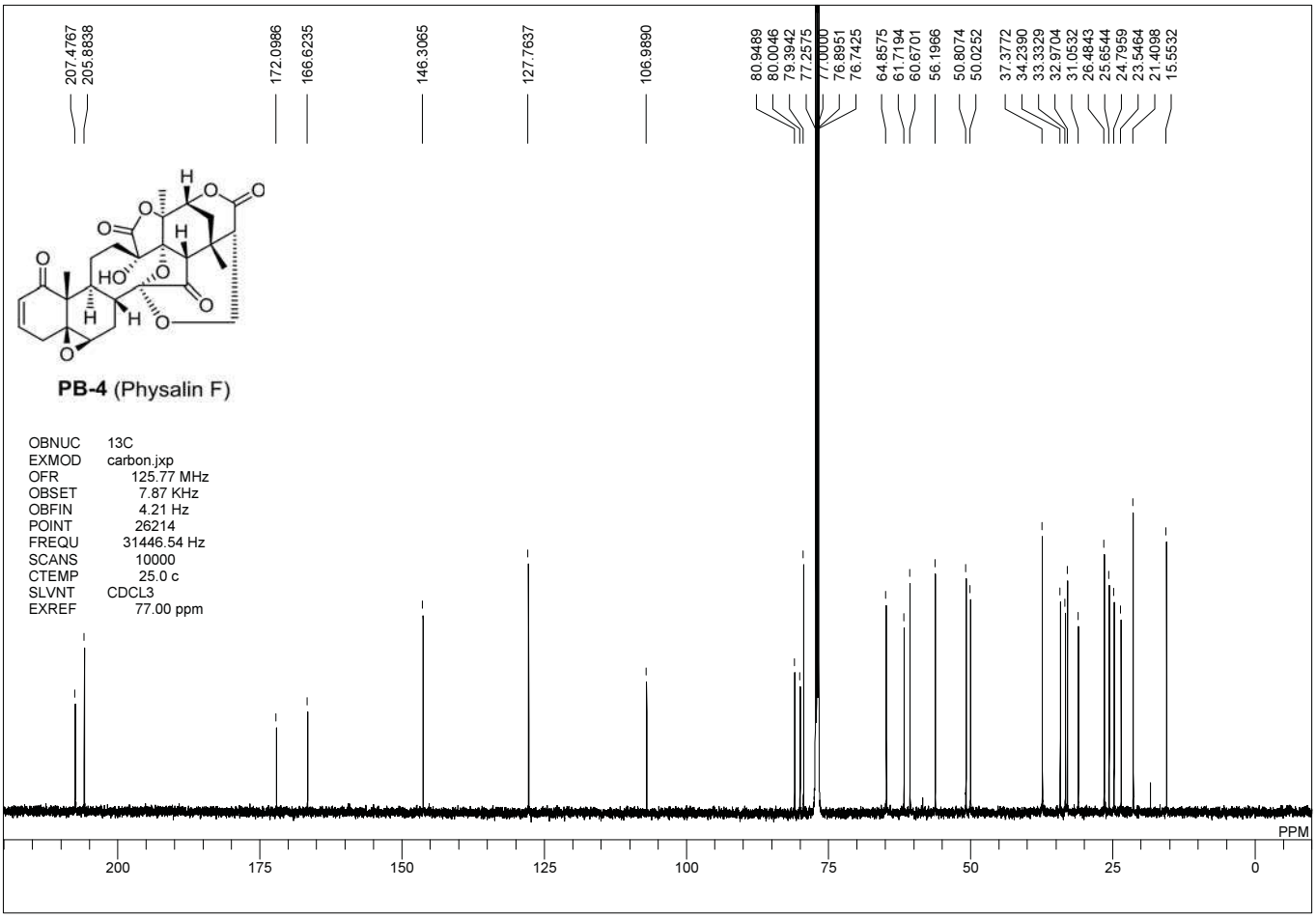

PB-2 (Physalin N)
OBNUC 1H
EXMOD proton.jxp
OFR 500.16 MHz
OBSET 2.41 KHz
OBFIN 6.01 Hz
POINT 13107
FREQU 7507.51 Hz
SCANS 16
CTEMP 22.7 c
SLVNT CDCL3
EXREF 7.26 ppm
1.00
0.99
1.08
2.96
0.88
0.91
1.93
0.89
1.74
8.01
0.64
7.15
PPM

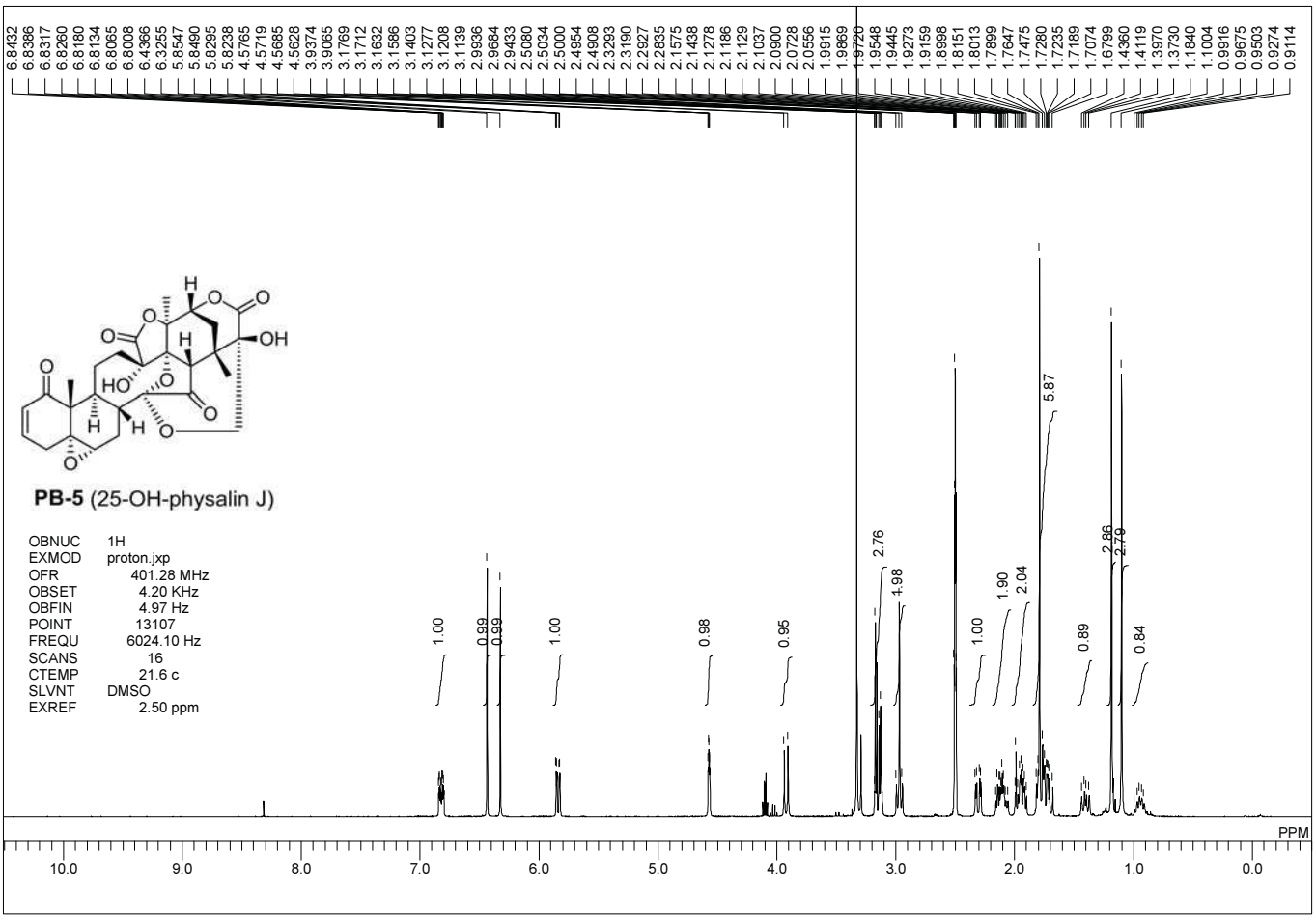

PB-2 (Physalin N)
OBNUC 13C
EXMOD carbon.jxp
OFR 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 26214
FREQU 31446.54 Hz
SCANS 2500
CTEMP 23.1 c
SLVNT CDCL3
EXREF 77.00 ppm
PPM

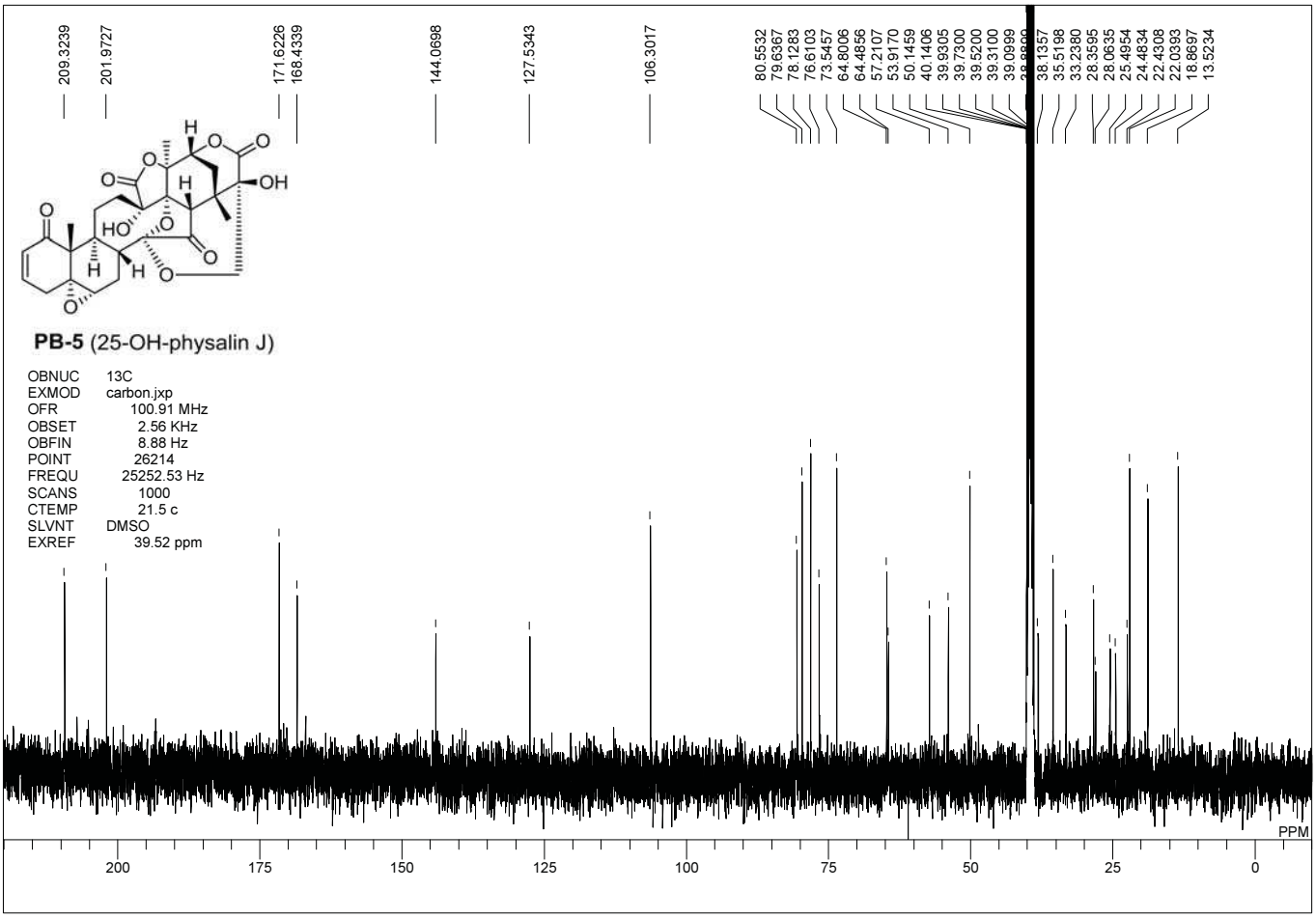

PB-3 (25-OH-physalin F)
OBNUC 1H
EXMOD proton.jxp
OFR 500.16 MHz
OBSET 2.41 KHz
OBFIN 6.01 Hz
POINT 13107
FREQU 7507.51 Hz
SCANS 16
CTEMP 20.3 c
SLVNT DMSO
EXREF 2.50 ppm
PPM

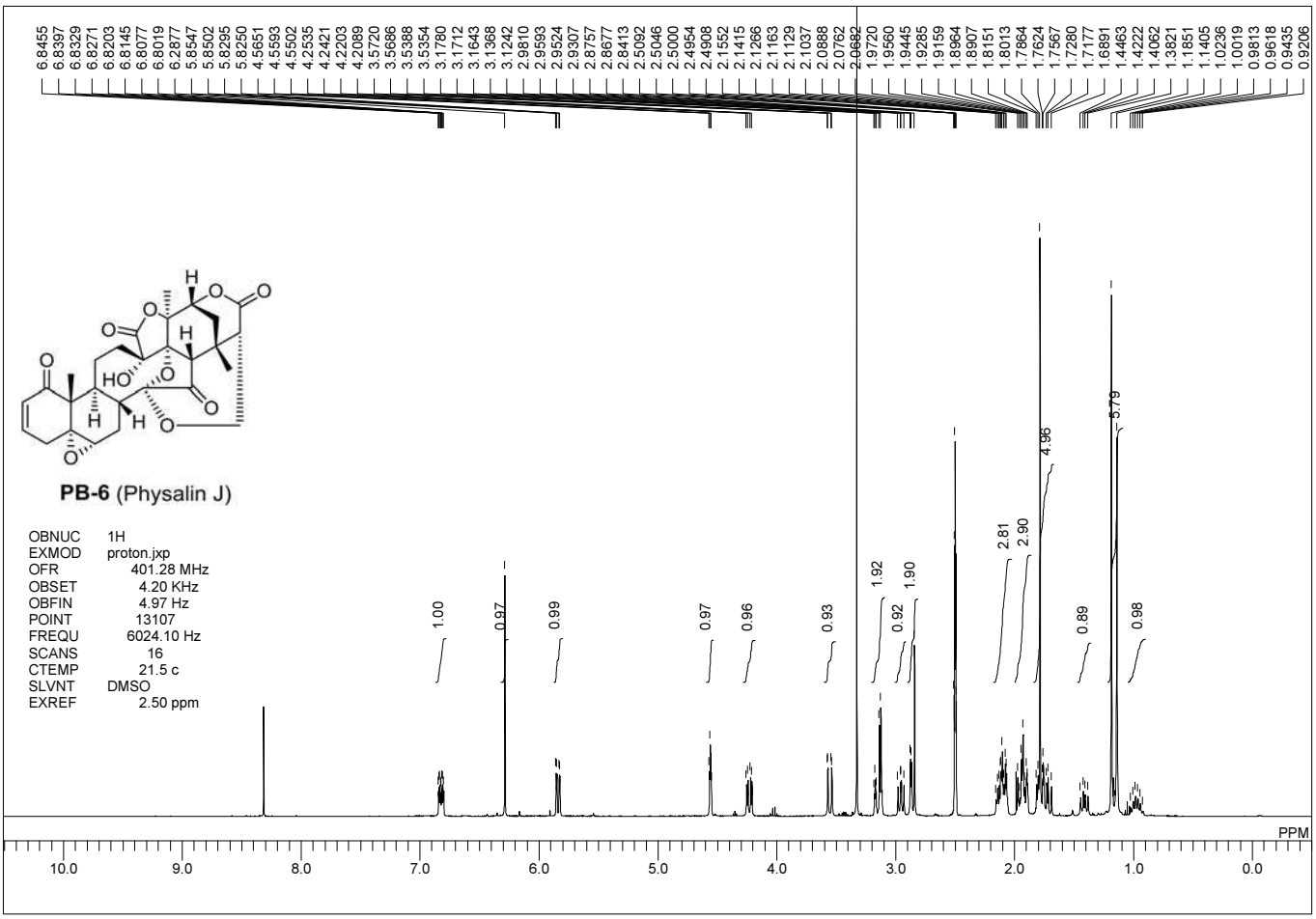

PB-3 (25-OH-physalin F)
OBNUC 13C
EXMOD carbon.jxp
OFR 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 26214
FREQU 31446.54 Hz
SCANS 20000
CTEMP 20.6 c
SLVNT DMSO
EXREF 39.52 ppm
PPM

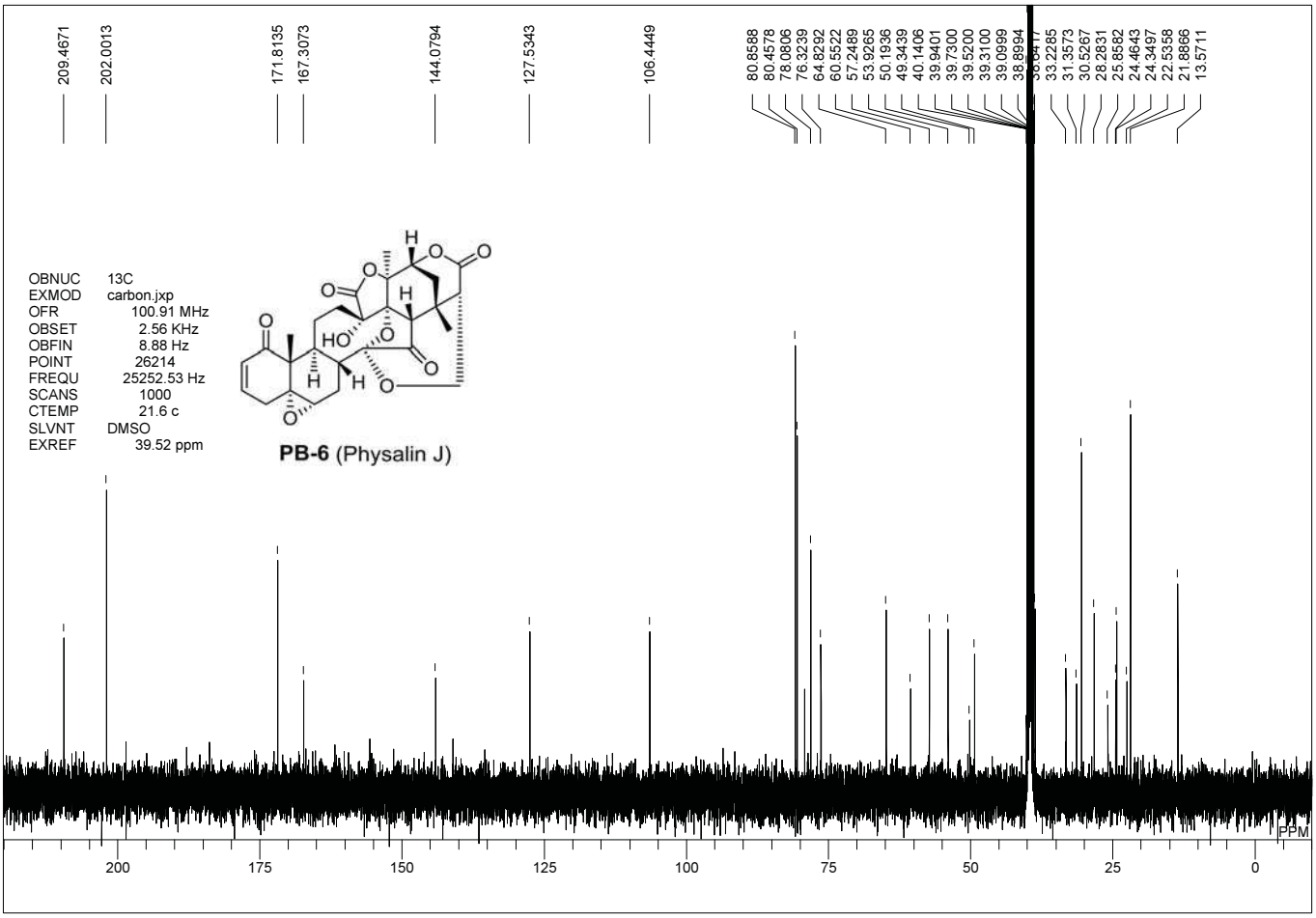

PB-4 (Physalin F)
OBNUC 1H
EXMOD proton.jxp
OFR 500.16 MHz
OBSET 2.41 KHz
OBFIN 6.01 Hz
POINT 13107
FREQU 7507.51 Hz
SCANS 16
CTEMP 25.0 c
SLVNT CDCL3
EXREF 7.26 ppm
PPM

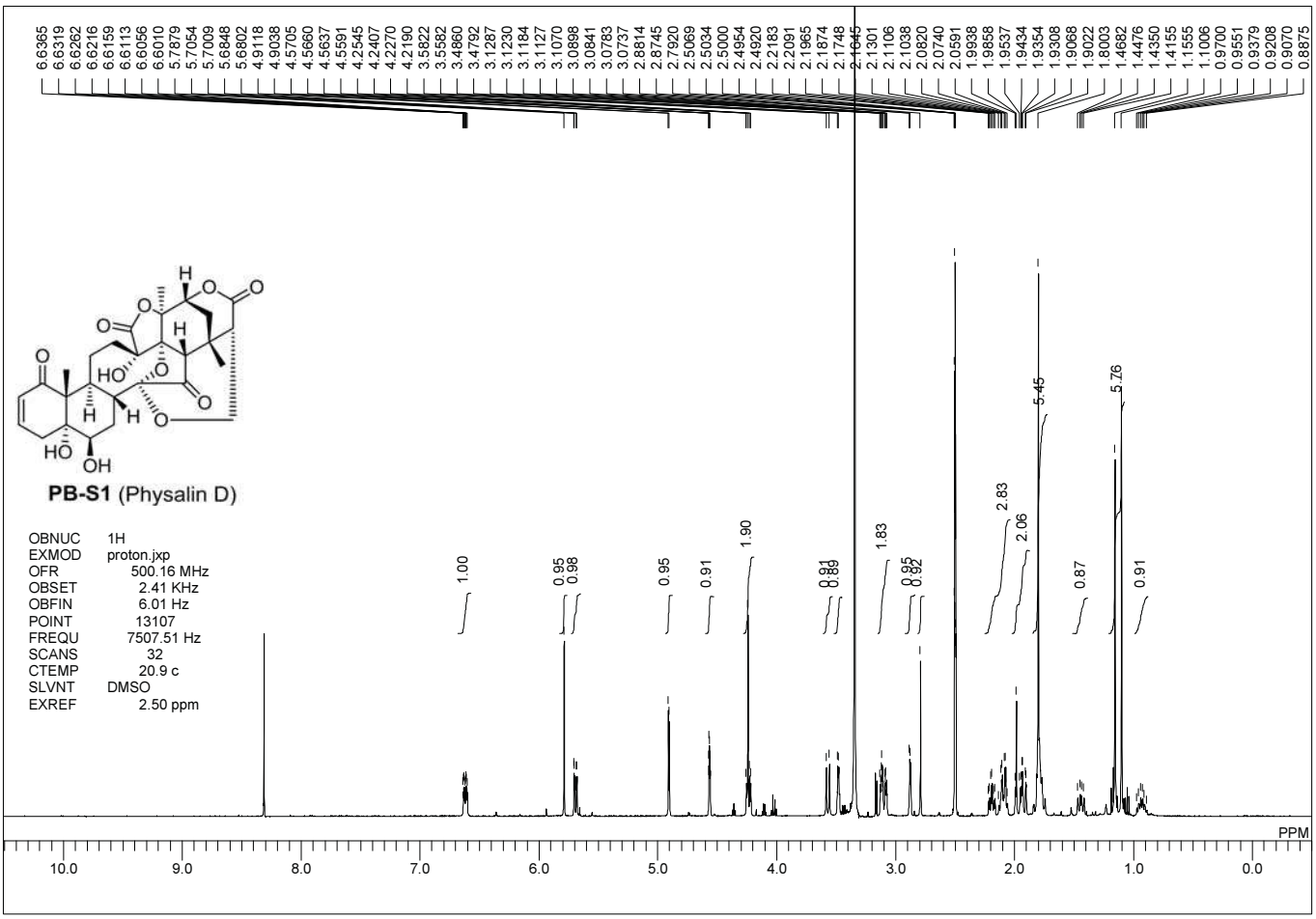

PB-4 (Physalin F)
OBNUC 13C
EXMOD carbon.jxp
OFR 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 26214
FREQU 31446.54 Hz
SCANS 10000
CTEMP 25.0 c
SLVNT CDCL3
EXREF 77.00 ppm
PPM

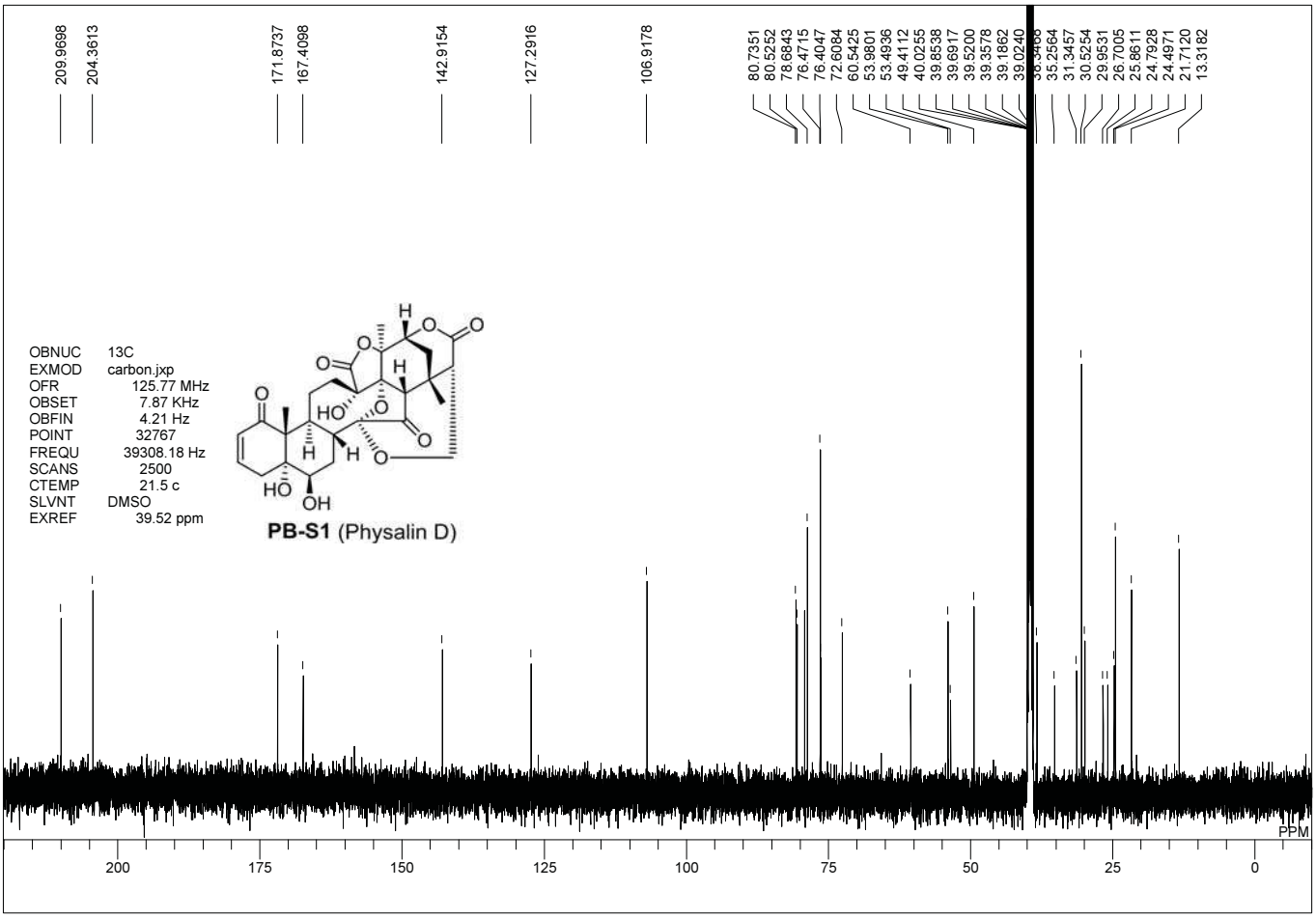

PB-5 (25-OH-physalin J)
OBNUC 1H
EXMOD proton.jxp
OFR 401.28 MHz
OBSET 4.20 KHz
OBFIN 4.97 Hz
POINT 13107
FREQU 6024.10 Hz
SCANS 16
CTEMP 21.6 c
SLVNT DMSO
EXREF 2.50 ppm
PPM

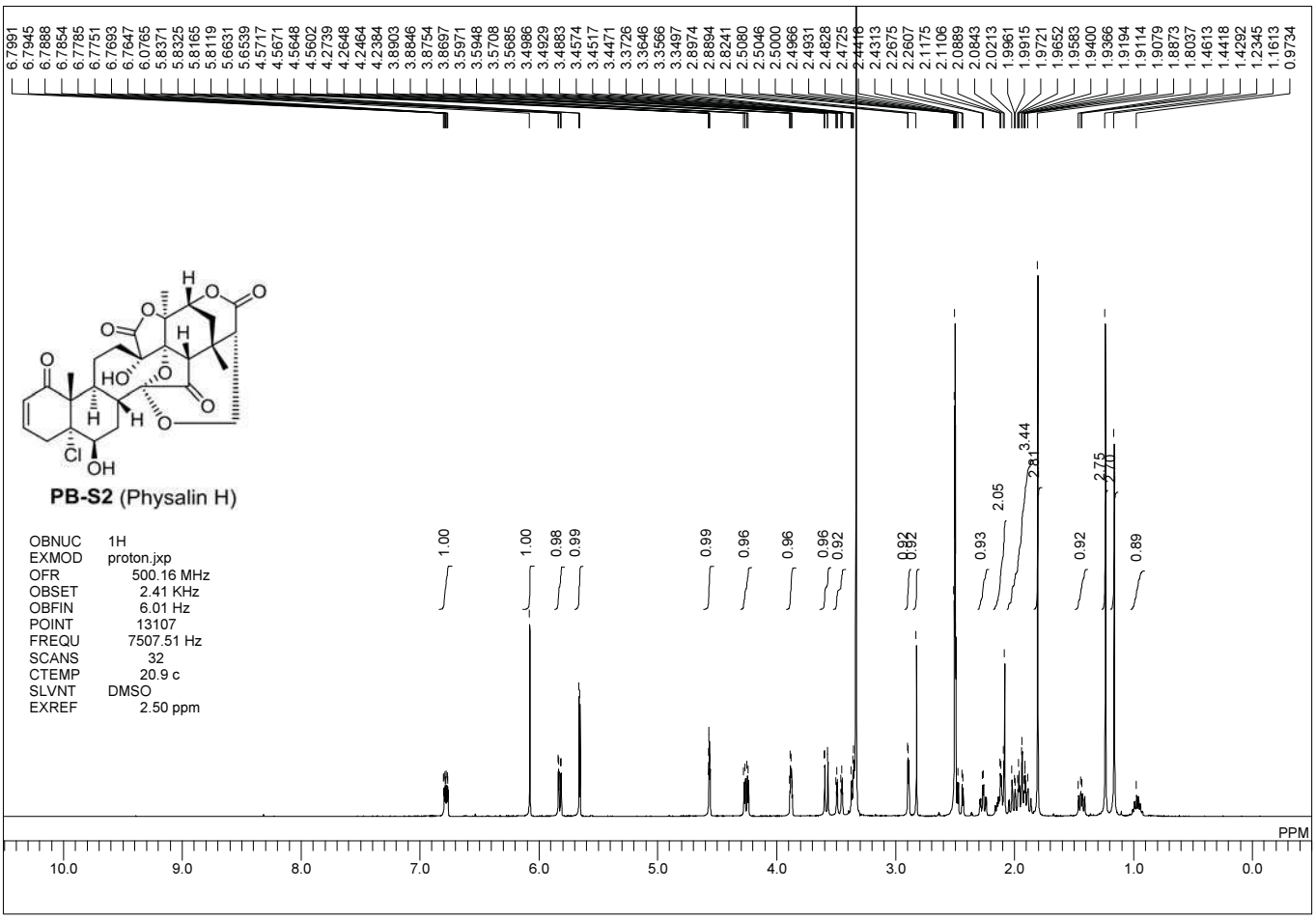

PB-5 (25-OH-physalin J)
OBNUC 13C
EXMOD carbon.jxp
OFR 100.91 MHz
OBSET 2.56 KHz
OBFIN 8.88 Hz
POINT 26214
FREQU 25252.53 Hz
SCANS 1000
CTEMP 21.5 c
SLVNT DMSO
EXREF 39.52 ppm
PPM

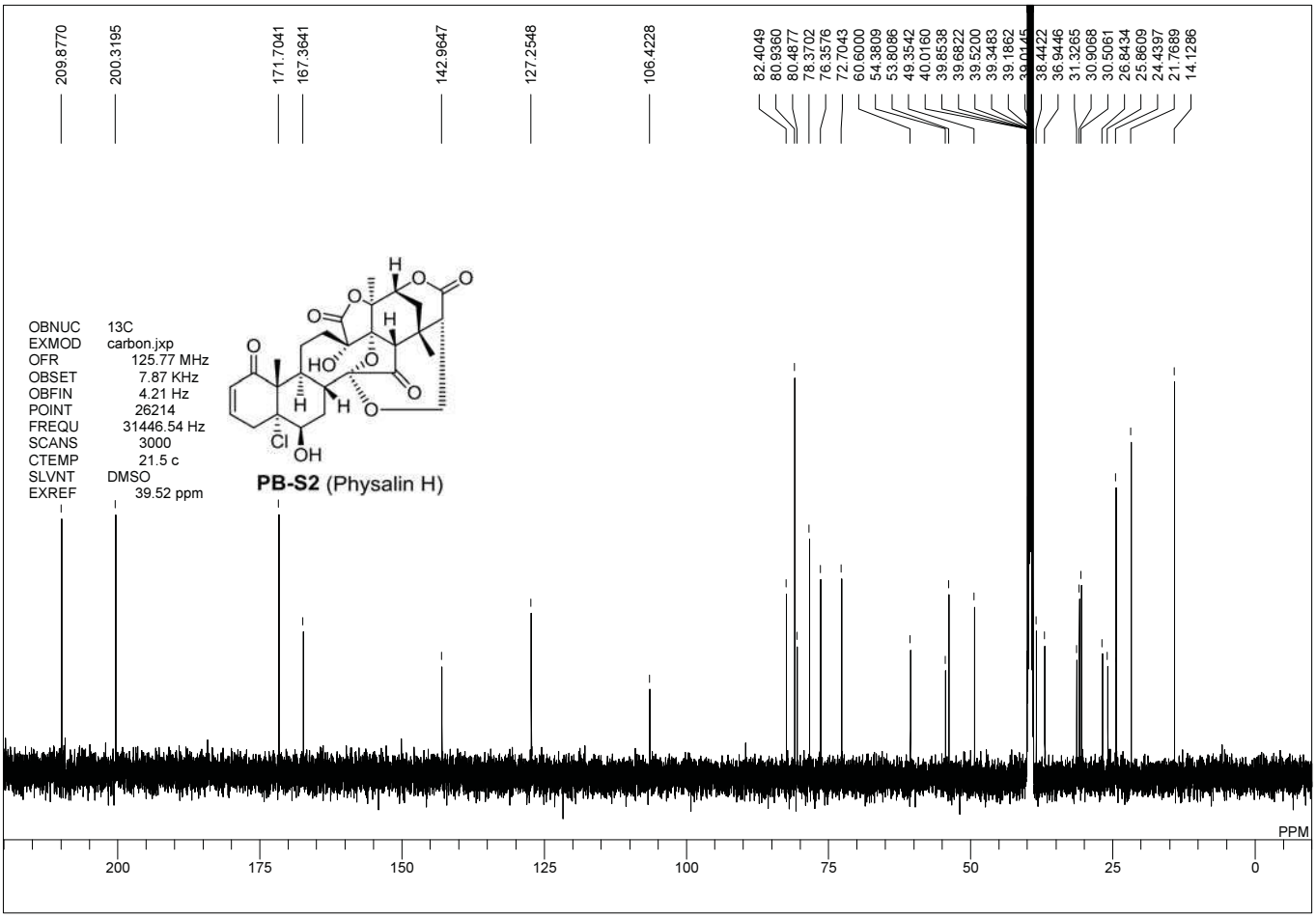

PB-6 (Physalin J)
OBNUC 1H
EXMOD proton.jxp
OFR 401.28 MHz
OBSET 4.20 KHz
OBFIN 4.97 Hz
POINT 13107
FREQU 6024.10 Hz
SCANS 16
CTEMP 21.5 c
SLVNT DMSO
EXREF 2.50 ppm
PPM

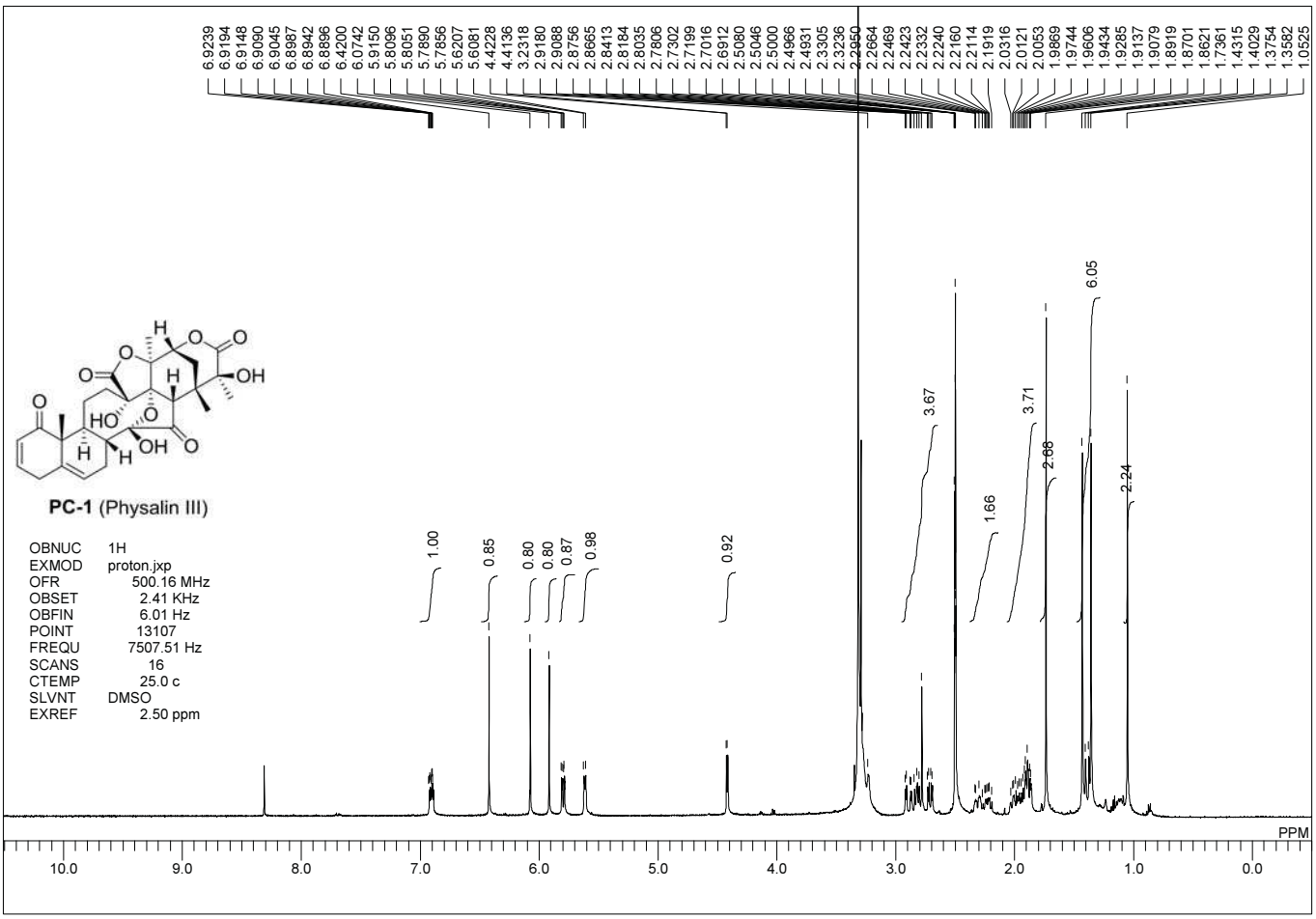

PB-6 (Physalin J)
OBNUC 13C
EXMOD carbon.jxp
OFR 100.91 MHz
OBSET 2.56 KHz
OBFIN 8.88 Hz
POINT 26214
FREQU 25252.53 Hz
SCANS 1000
CTEMP 21.6 c
SLVNT DMSO
EXREF 39.52 ppm
PPM

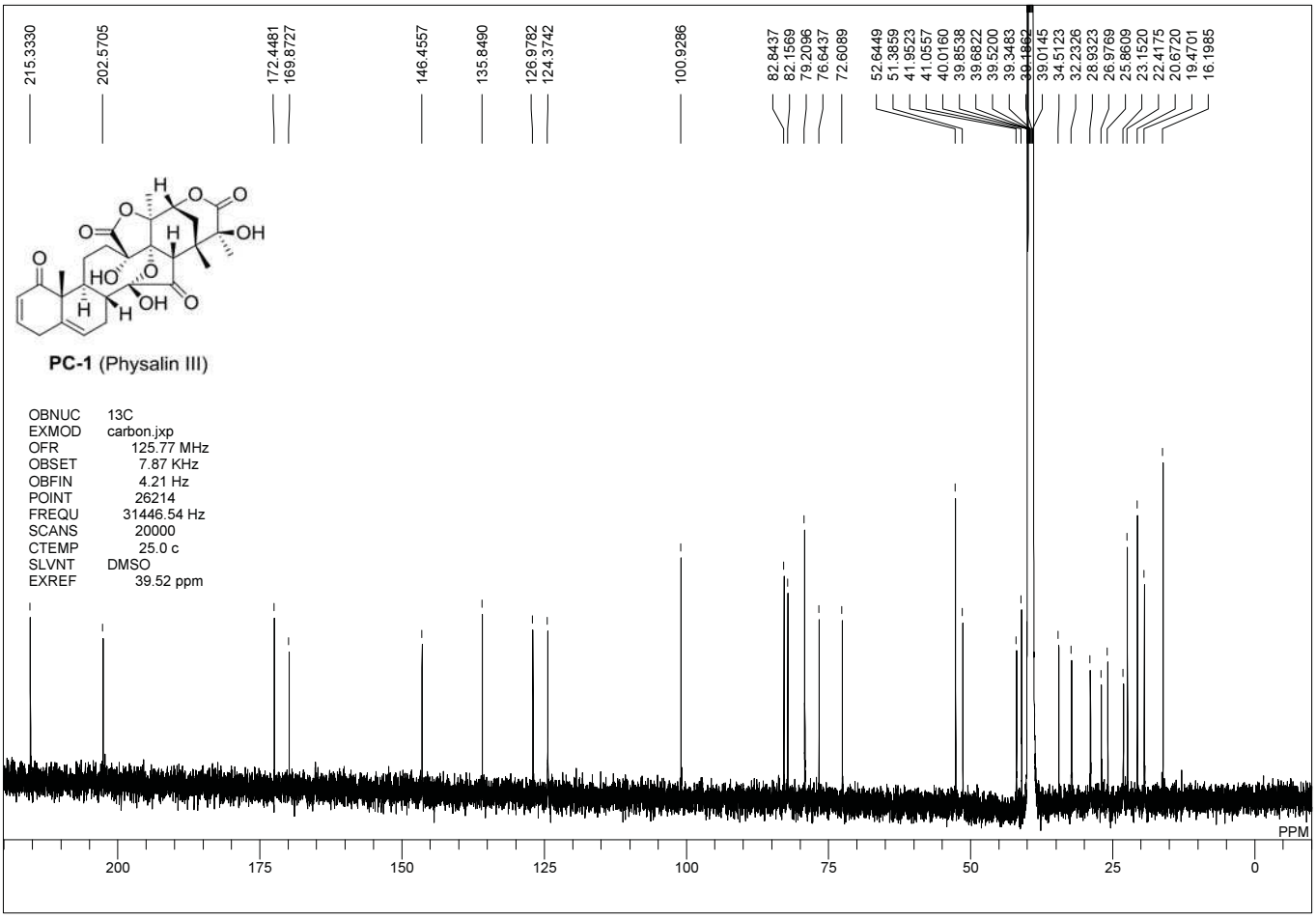

PB-S1 (Physalin D)
OBNUC 1H
EXMOD proton.jxp
OFR 500.16 MHz
OBSET 2.41 KHz
OBFIN 6.01 Hz
POINT 13107
FREQU 7507.51 Hz
SCANS 32
CTEMP 20.9 c
SLVNT DMSO
EXREF 2.50 ppm
1.00
0.95
0.98
0.95
0.91
1.90
1.83
2.83
2.06
5.45
5.76
0.87
0.91
PPM

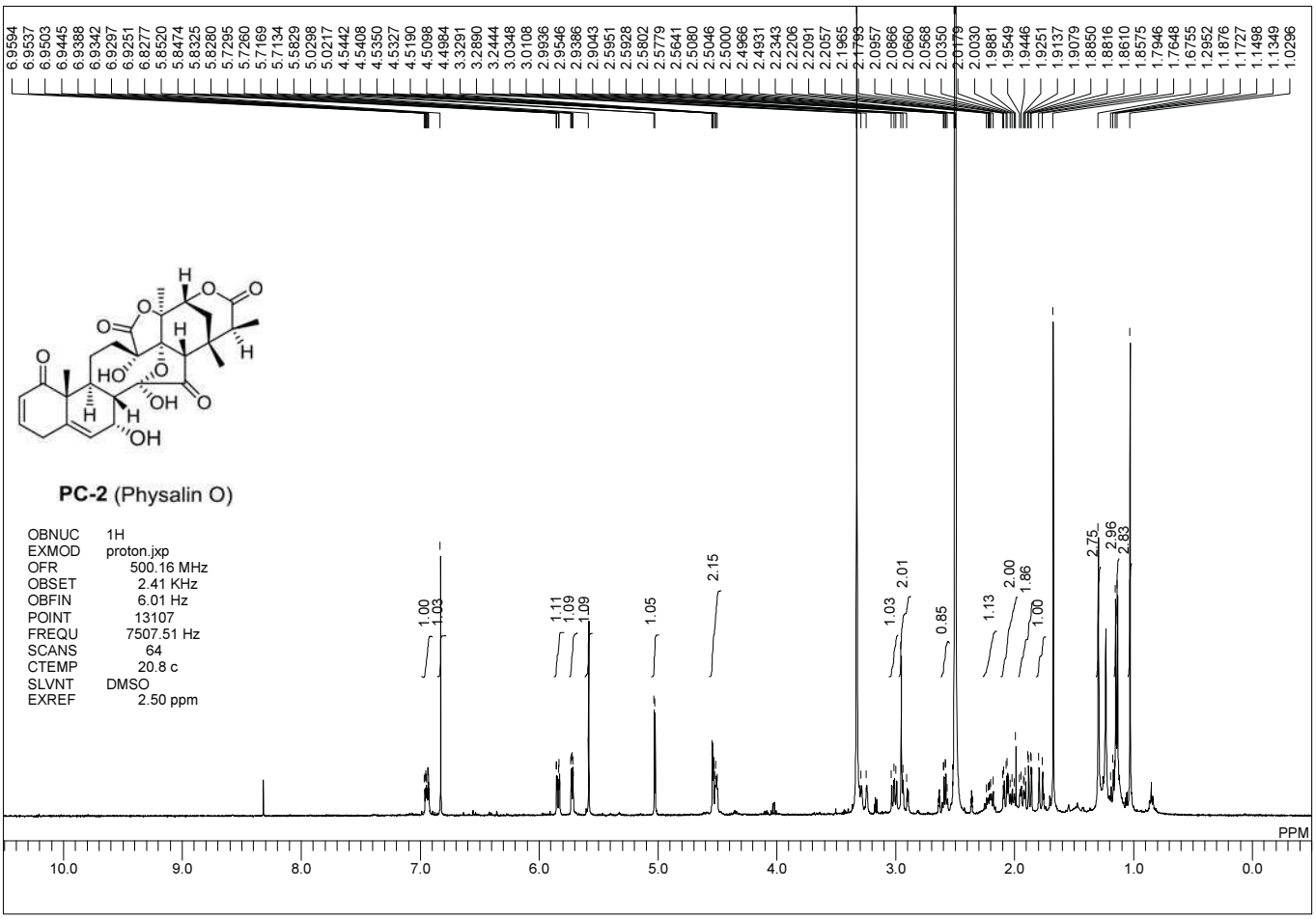

PB-S1 (Physalin D)
OBNUC 13C
EXMOD carbon.jxp
OFR 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 32767
FREQU 39308.18 Hz
SCANS 2500
CTEMP 21.5 c
SLVNT DMSO
EXREF 39.52 ppm
209.9698
204.3613
171.8737
167.4098
142.9154
127.2916
106.9178
PPM

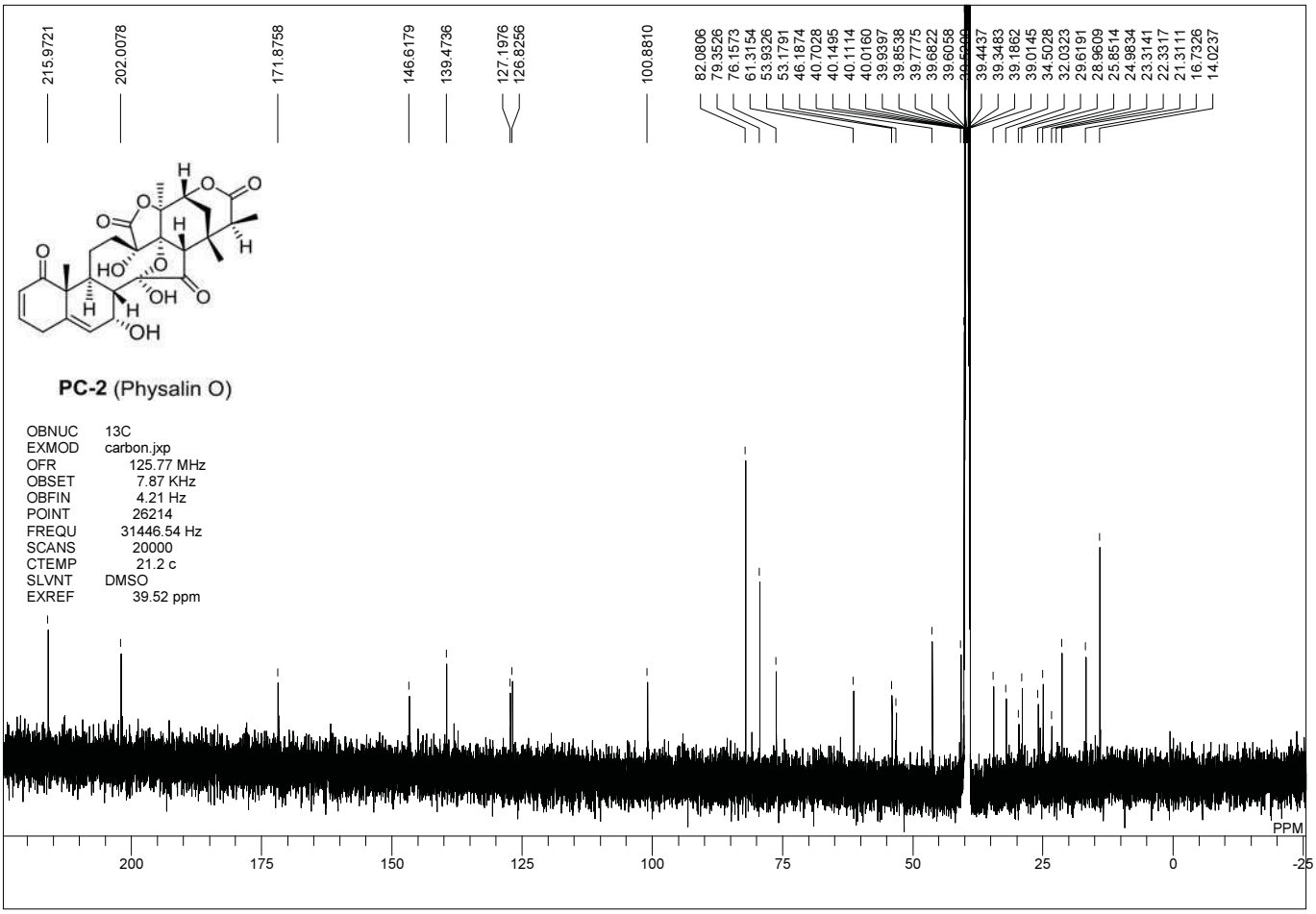

PB-S2 (Physalin H)
OBNUC 1H
EXMOD proton.jxp
OFR 500.16 MHz
OBSET 2.41 KHz
OBFIN 6.01 Hz
POINT 13107
FREQU 7507.51 Hz
SCANS 32
CTEMP 20.9 c
SLVNT DMSO
EXREF 2.50 ppm
PPM

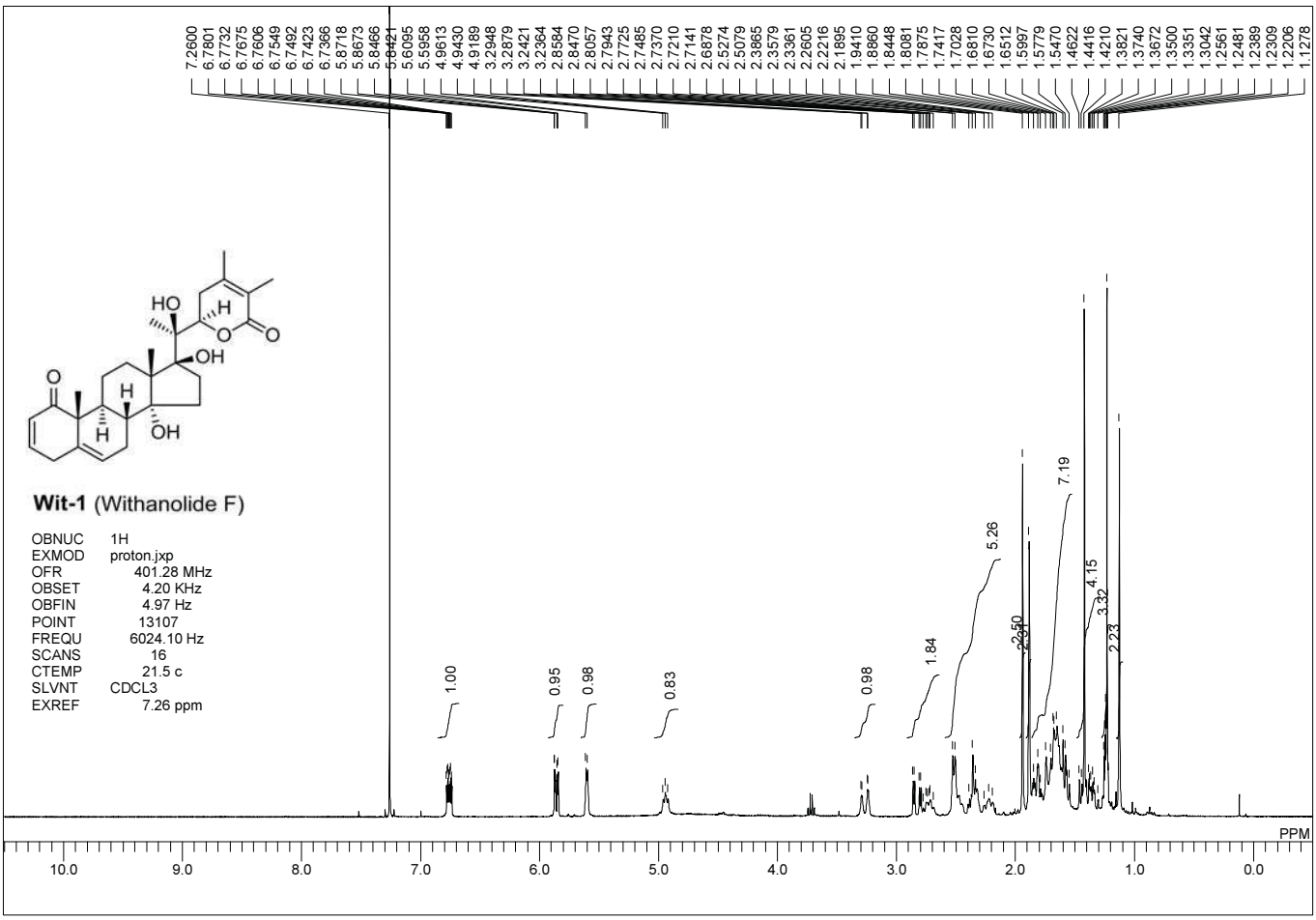

PB-S2 (Physalin H)
OBNUC 13C
EXMOD carbon.jxp
OFR 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 26214
FREQU 31446.54 Hz
SCANS 3000
CTEMP 21.5 c
SLVNT DMSO
EXREF 39.52 ppm
PPM

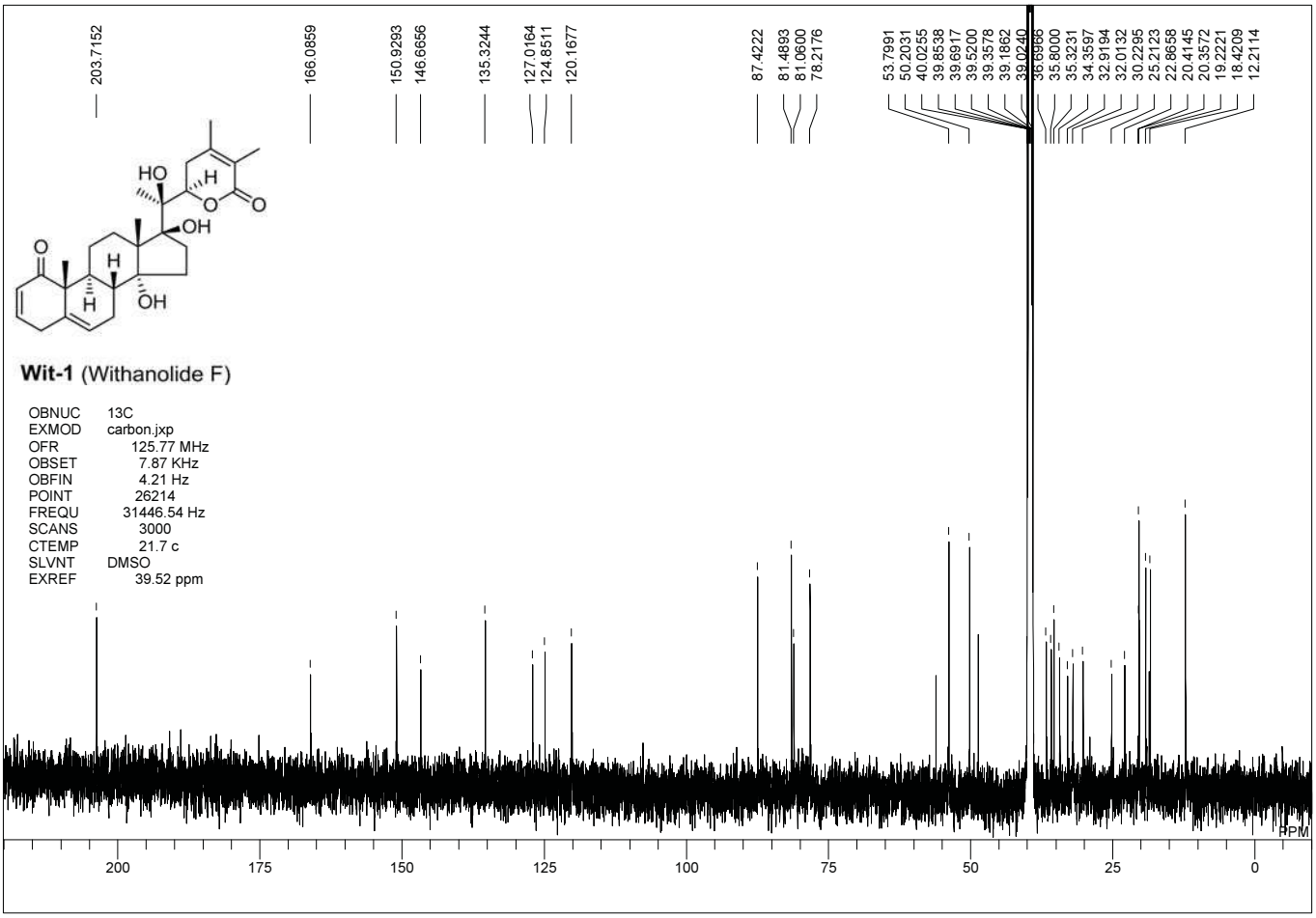

PC-1 (Physalin III)
OBNUC 1H
EXMOD proton.jxp
OFR 500.16 MHz
OBSET 2.41 KHz
OBFIN 6.01 Hz
POINT 13107
FREQU 7507.51 Hz
SCANS 16
CTEMP 25.0 c
SLVNT DMSO
EXREF 2.50 ppm
PPM

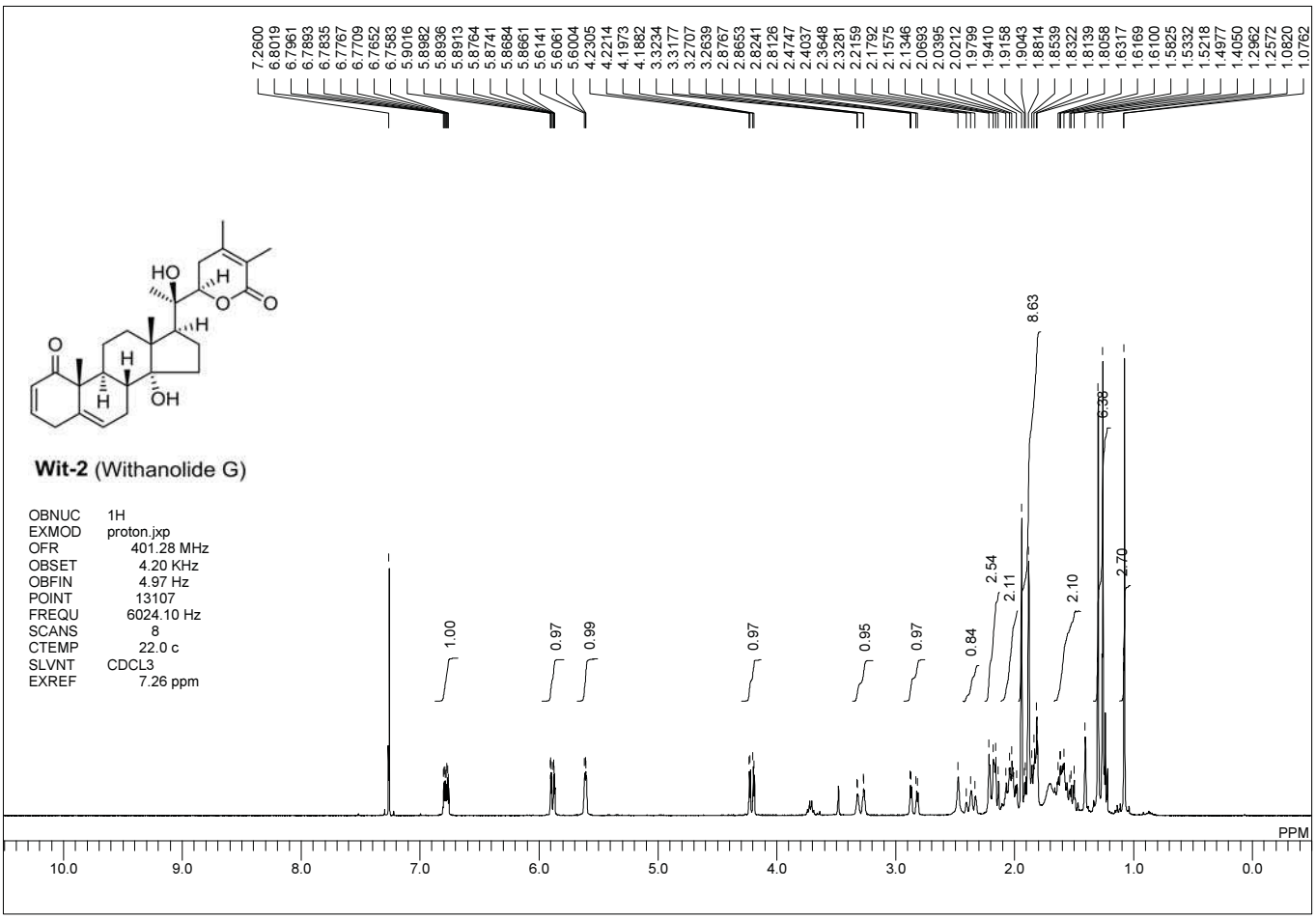

PC-1 (Physalin III)
OBNUC 13C
EXMOD carbon.jxp
OFR 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 26214
FREQU 31446.54 Hz
SCANS 20000
CTEMP 25.0 c
SLVNT DMSO
EXREF 39.52 ppm
PPM

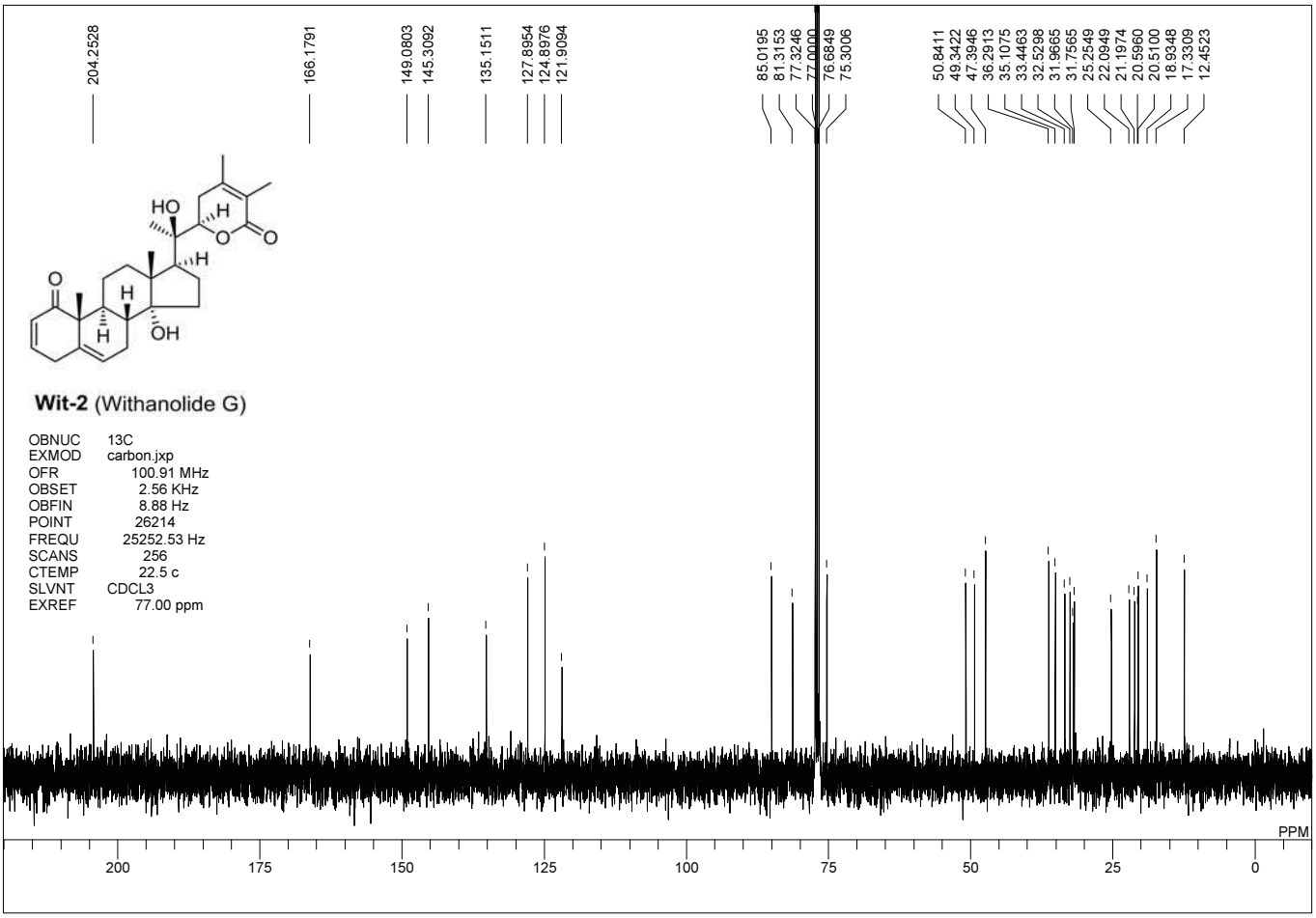

PC-2 (Physalin O)
OBNUC 1H
EXMOD proton.jxp
OFR 500.16 MHz
OBSET 2.41 KHz
OBFIN 6.01 Hz
POINT 13107
FREQU 7507.51 Hz
SCANS 64
CTEMP 20.8 c
SLVNT DMSO
EXREF 2.50 ppm
PPM

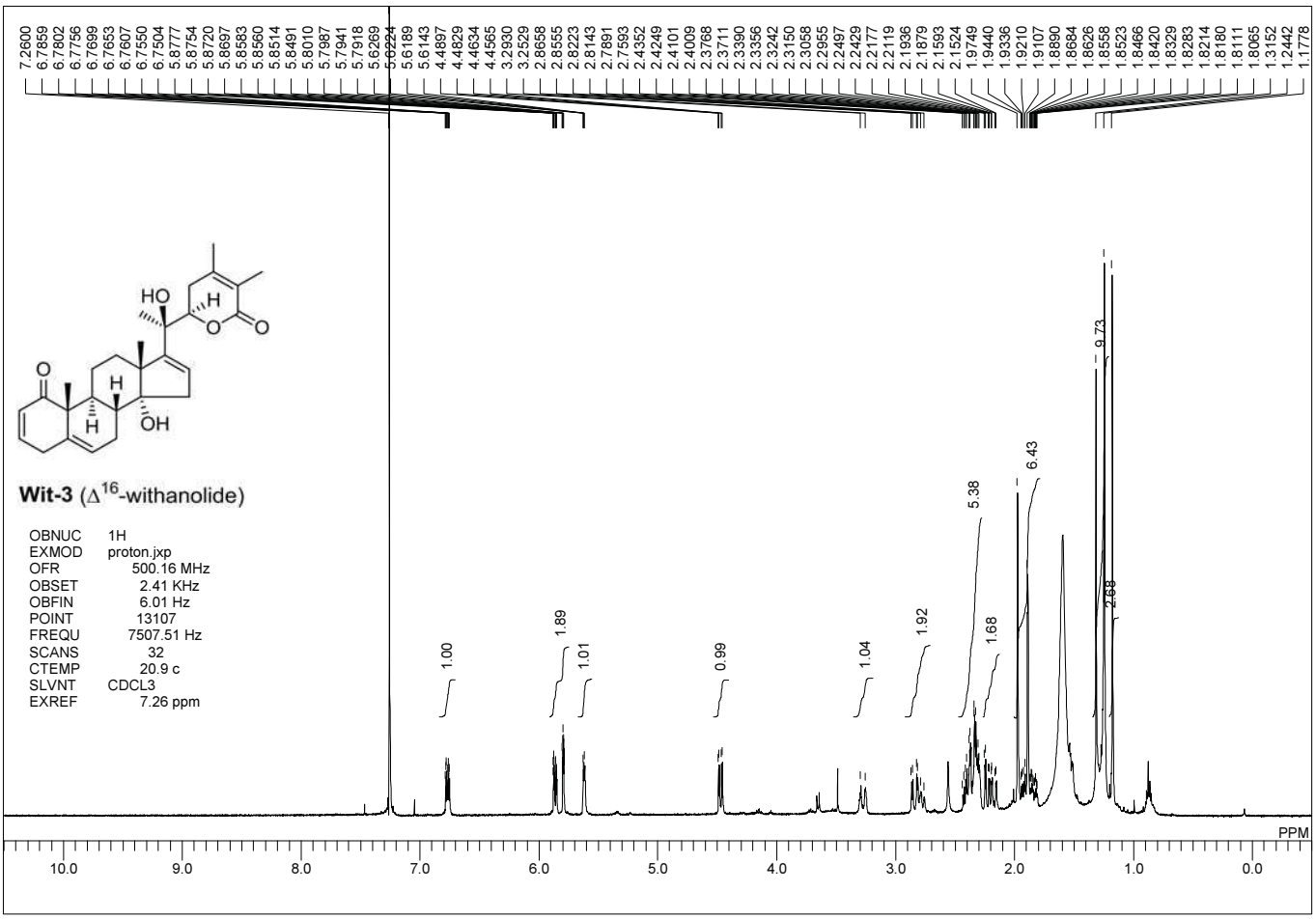

PC-2 (Physalin O)
OBNUC 13C
EXMOD carbon.jxp
OFR 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 26214
FREQU 31446.54 Hz
SCANS 20000
CTEMP 21.2 c
SLVNT DMSO
EXREF 39.52 ppm
PPM

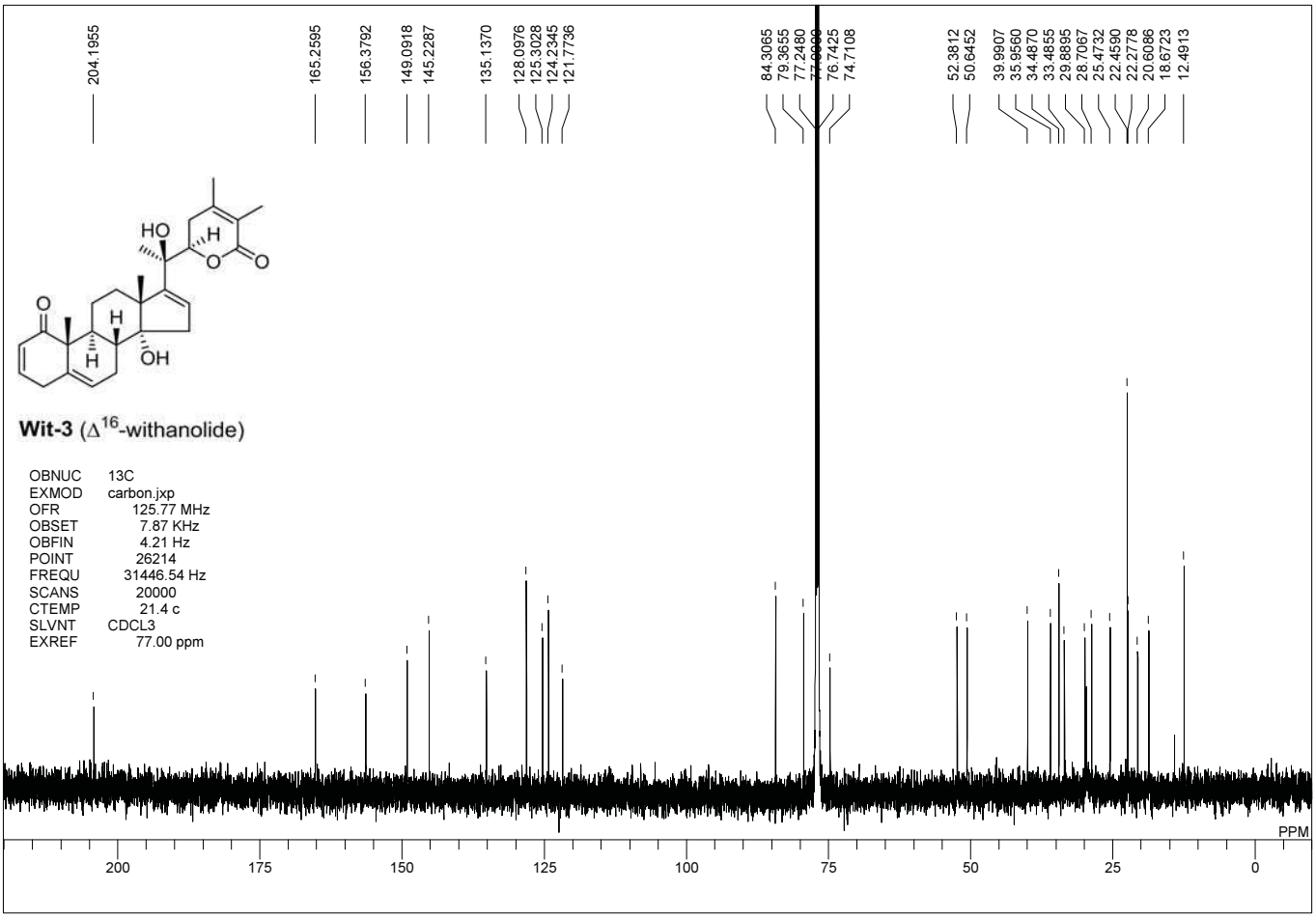

Wit-1 (Withanolide F)
OBNUC 1H
EXMOD proton.jxp
OFR 401.28 MHz
OBSET 4.20 KHz
OBFIN 4.97 Hz
POINT 13107
FREQU 6024.10 Hz
SCANS 16
CTEMP 21.5 c
SLVNT CDCL3
EXREF 7.26 ppm
PPM

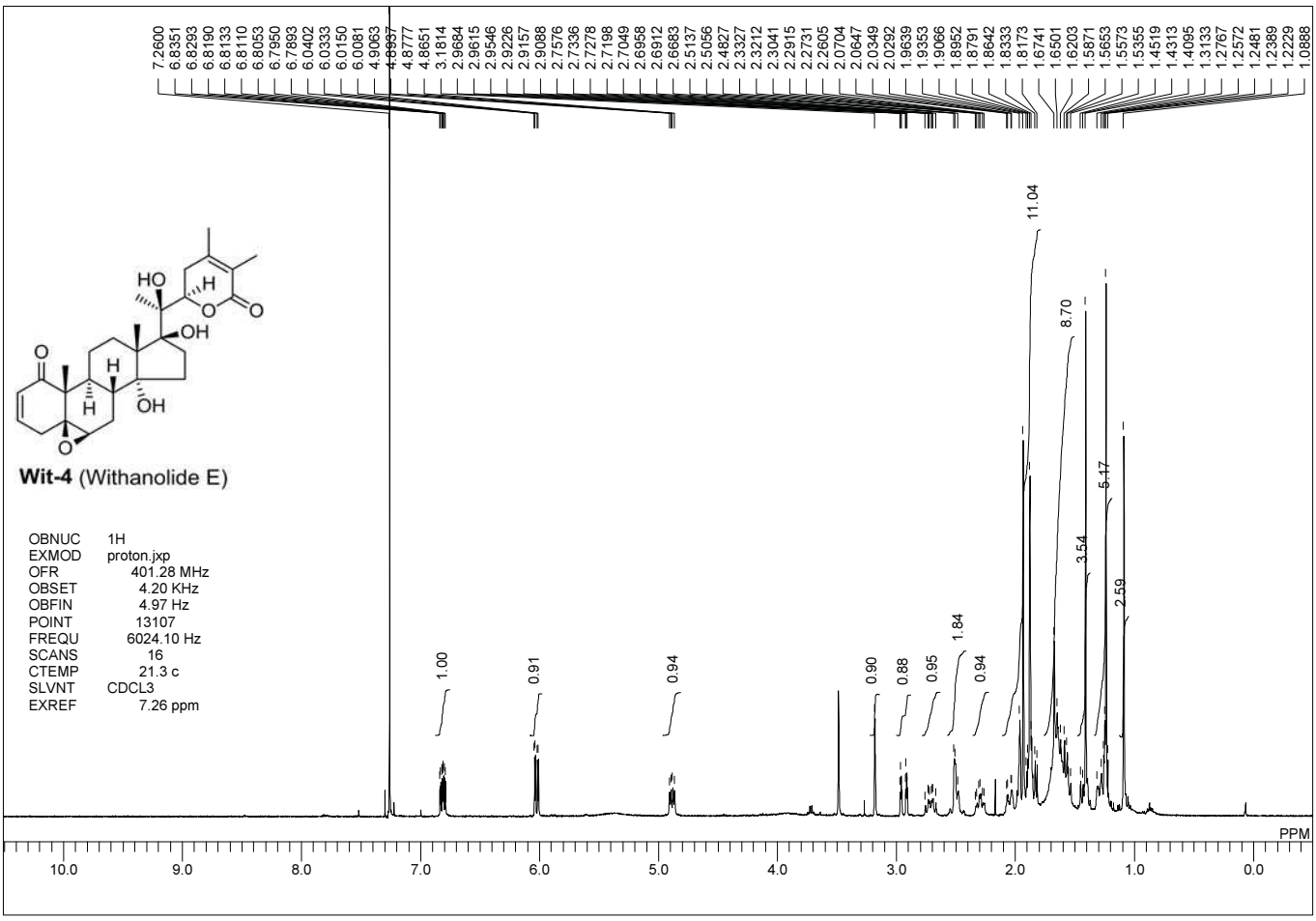

Wit-1 (Withanolide F)
OBNUC 13C
EXMOD carbon.jxp
OFR 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 26214
FREQU 31446.54 Hz
SCANS 3000
CTEMP 21.7 c
SLVNT DMSO
EXREF 39.52 ppm
PPM

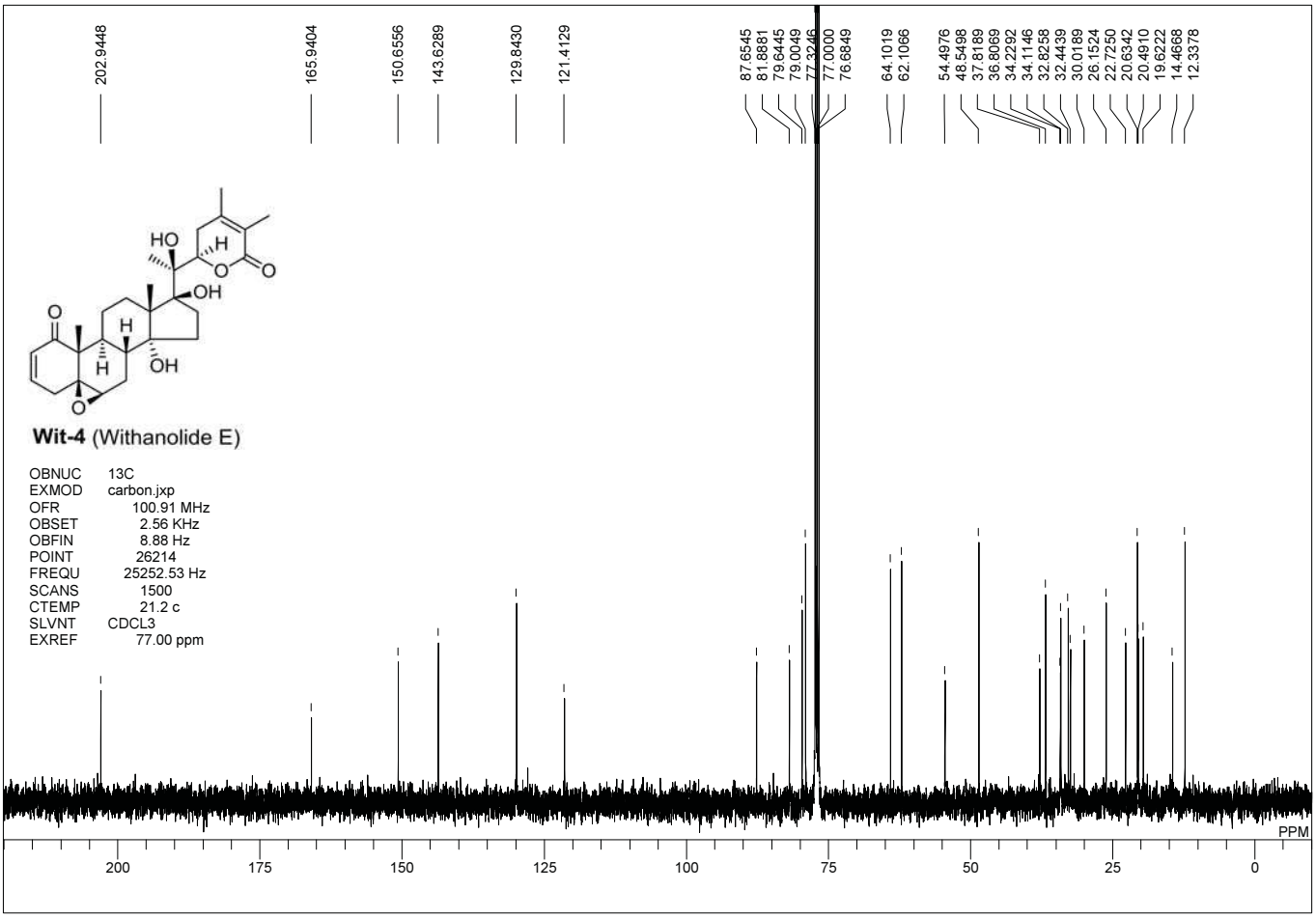

7.2600 6.8019 6.7961 6.7893 6.7835 6.7767 6.7709 6.7652 6.7583 5.9016 5.8982 5.8936 5.8913 5.8764 5.8741 5.8684 5.8661 5.6141 5.6061 5.6004 4.2305 4.2214 4.1973 4.1882 3.3234 3.3177 3.2707 3.2639 2.8767 2.8653 2.8241 2.8126 2.4747 2.4037 2.3648 2.3281 2.2159 2.1792 2.1575 2.1346 2.0693 2.0395 2.0212 1.9799 1.9410 1.9158 1.9043 1.8814 1.8539 1.8322 1.8139 1.8058 1.6317 1.6169 1.6100 1.5825 1.5332 1.5218 1.4977 1.4050 1.2962 1.2572 1.0820 1.0762
HO
H
O
O
H
H
H
OH
Wit-2 (Withanolide G)
OBNUC 1H
EXMOD proton.jxp
OFR 401.28 MHz
OBSET 4.20 KHz
OBFIN 4.97 Hz
POINT 13107
FREQU 6024.10 Hz
SCANS 8
CTEMP 22.0 c
SLVNT CDCL3
EXREF 7.26 ppm
1.00
0.97
0.99
0.97
0.95
0.97
0.84
2.54
2.11
2.10
8.63
6.38
2.70
PPM
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

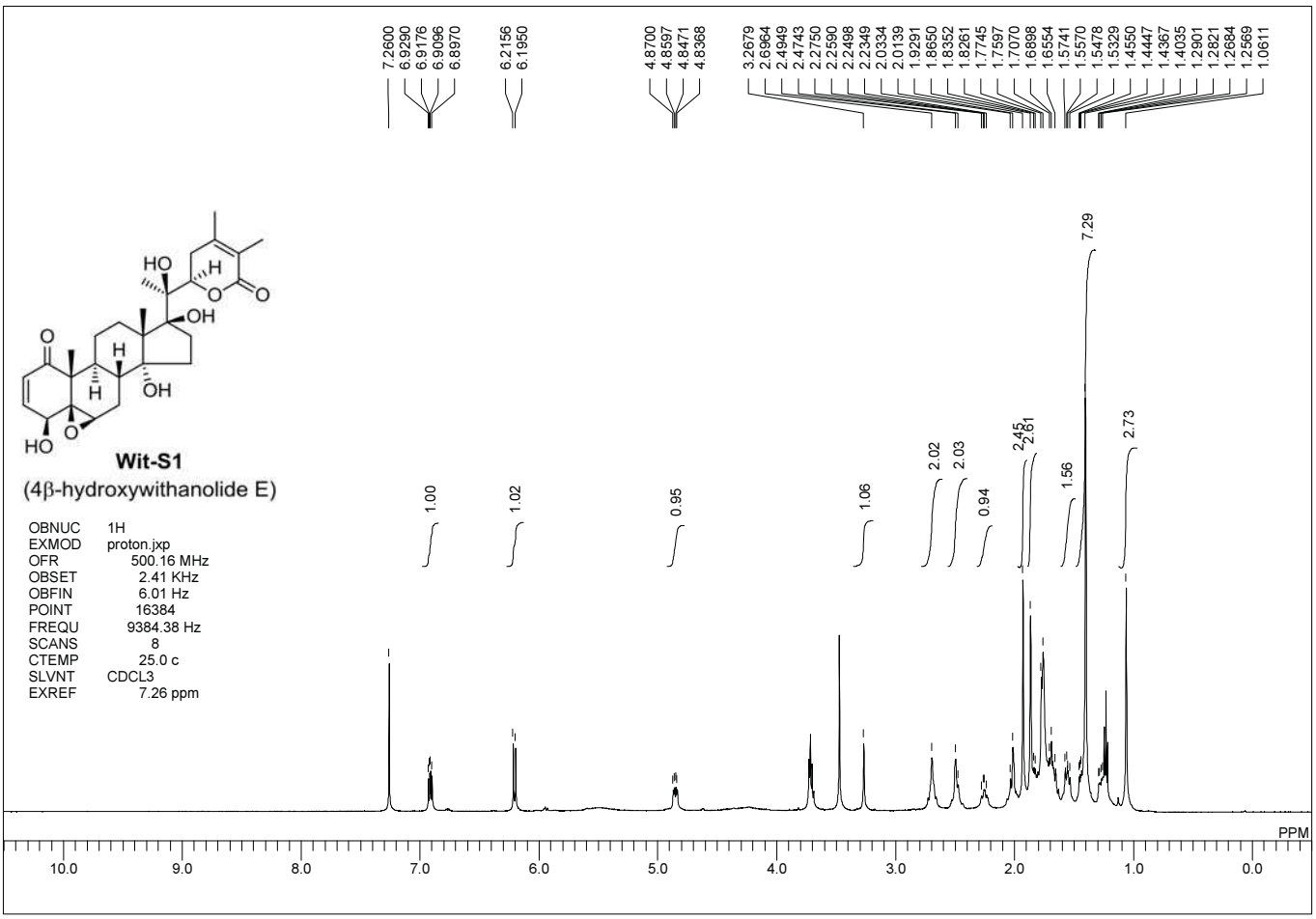

204.2528 166.1791 149.0803 145.3092 135.1511 127.8954 124.8976 121.9094
85.0195 81.3153 77.3246 77.0000 76.6849 75.3006
50.8411 49.3422 47.3946 36.2913 35.1075 33.4463 32.5298 31.9665 31.7565 25.2549 22.0949 21.1974 20.5960 20.5100 18.9348 17.3309 12.4523
HO
H
O
O
H
H
H
OH
Wit-2 (Withanolide G)
OBNUC 13C
EXMOD carbon.jxp
OFR 100.91 MHz
OBSET 2.56 KHz
OBFIN 8.88 Hz
POINT 26214
FREQU 25252.53 Hz
SCANS 256
CTEMP 22.5 c
SLVNT CDCL3
EXREF 77.00 ppm
PPM
200
175
150
125
100
75
50
25
0

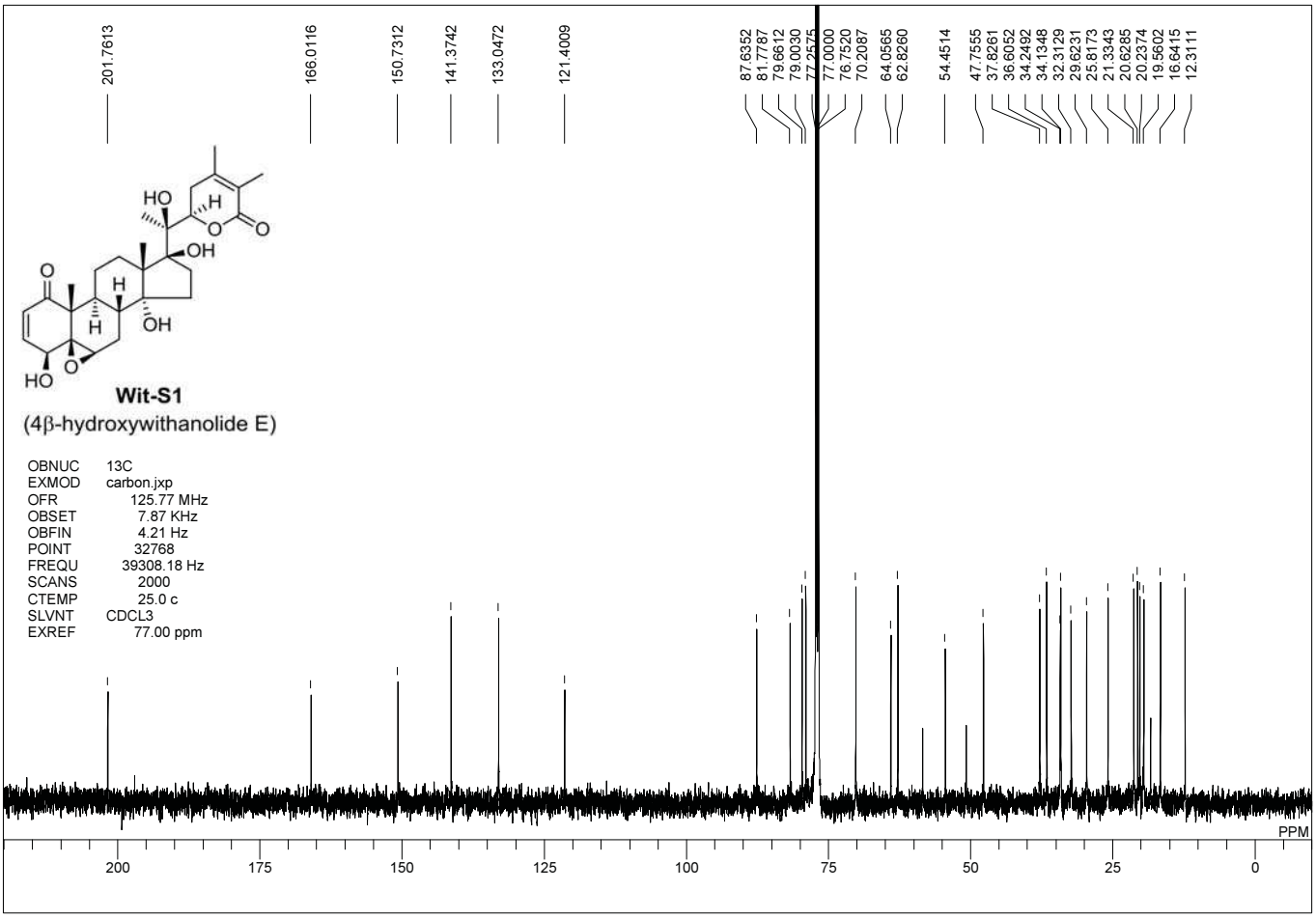

Wit-3 (Δ16-withanolide)
OBNUC 1H
EXMOD proton.jxp
OFR 500.16 MHz
OBSET 2.41 KHz
OBFIN 6.01 Hz
POINT 13107
FREQU 7507.51 Hz
SCANS 32
CTEMP 20.9 c
SLVNT CDCL3
EXREF 7.26 ppm
1.00
1.89
1.01
0.99
1.04
1.92
1.68
5.38
6.43
9.73
2.88
PPM

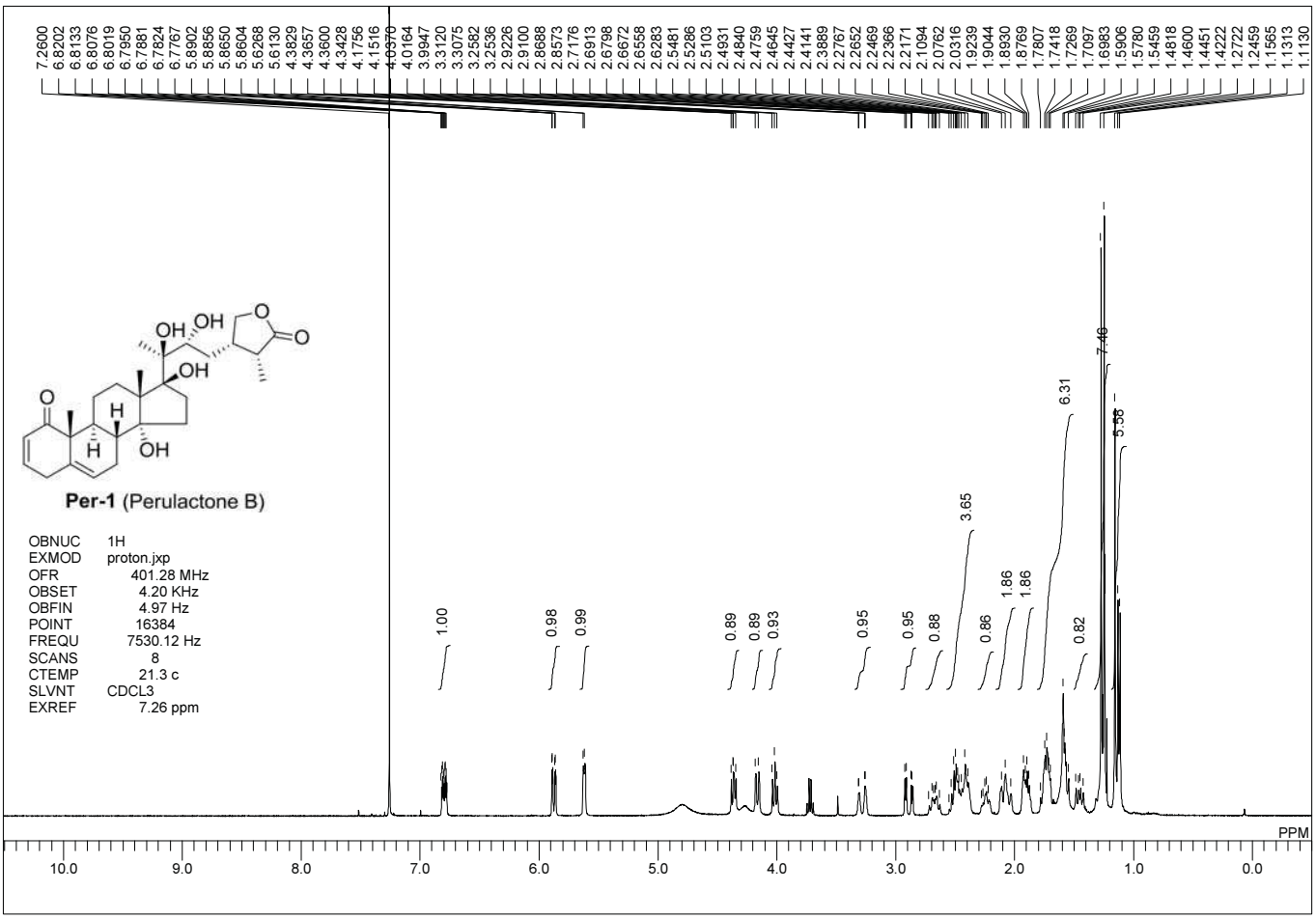

Wit-3 (Δ16-withanolide)
OBNUC 13C
EXMOD carbon.jxp
OFR 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 26214
FREQU 31446.54 Hz
SCANS 20000
CTEMP 21.4 c
SLVNT CDCL3
EXREF 77.00 ppm
204.1955
165.2595
156.3792
149.0918
145.2287
135.1370
128.0976
125.3028
124.2345
121.7736
84.3065
79.3655
77.2480
76.7425
74.7108
52.3812
50.6452
39.9907
35.9560
34.4870
33.4855
29.8895
28.7067
25.4732
22.4590
22.2778
20.6086
18.6723
12.4913
PPM

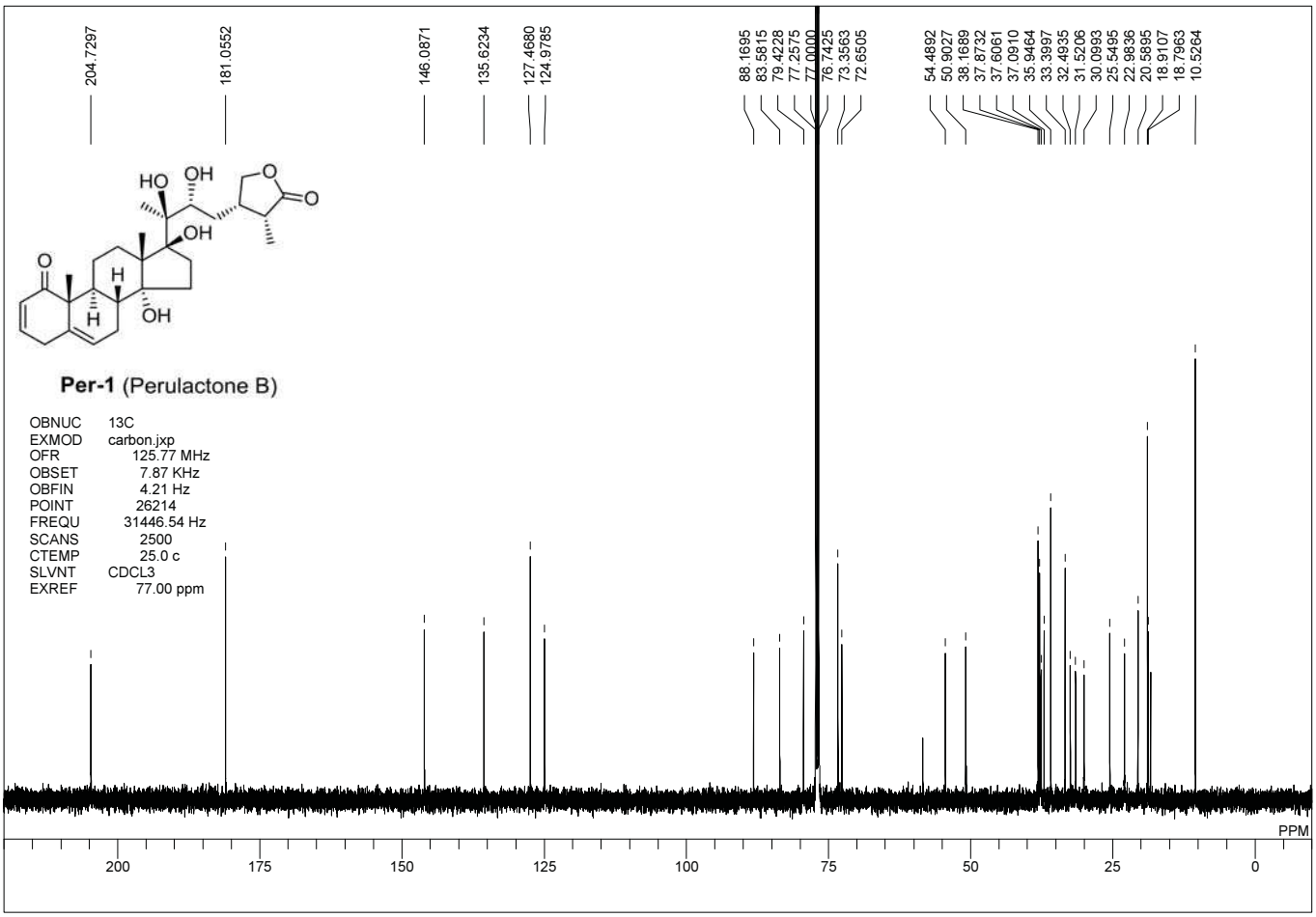

Wit-4 (Withanolide E)
OBNUC 1H
EXMOD proton.jxp
OFR 401.28 MHz
OBSET 4.20 KHz
OBFIN 4.97 Hz
POINT 13107
FREQU 6024.10 Hz
SCANS 16
CTEMP 21.3 c
SLVNT CDCL3
EXREF 7.26 ppm
PPM

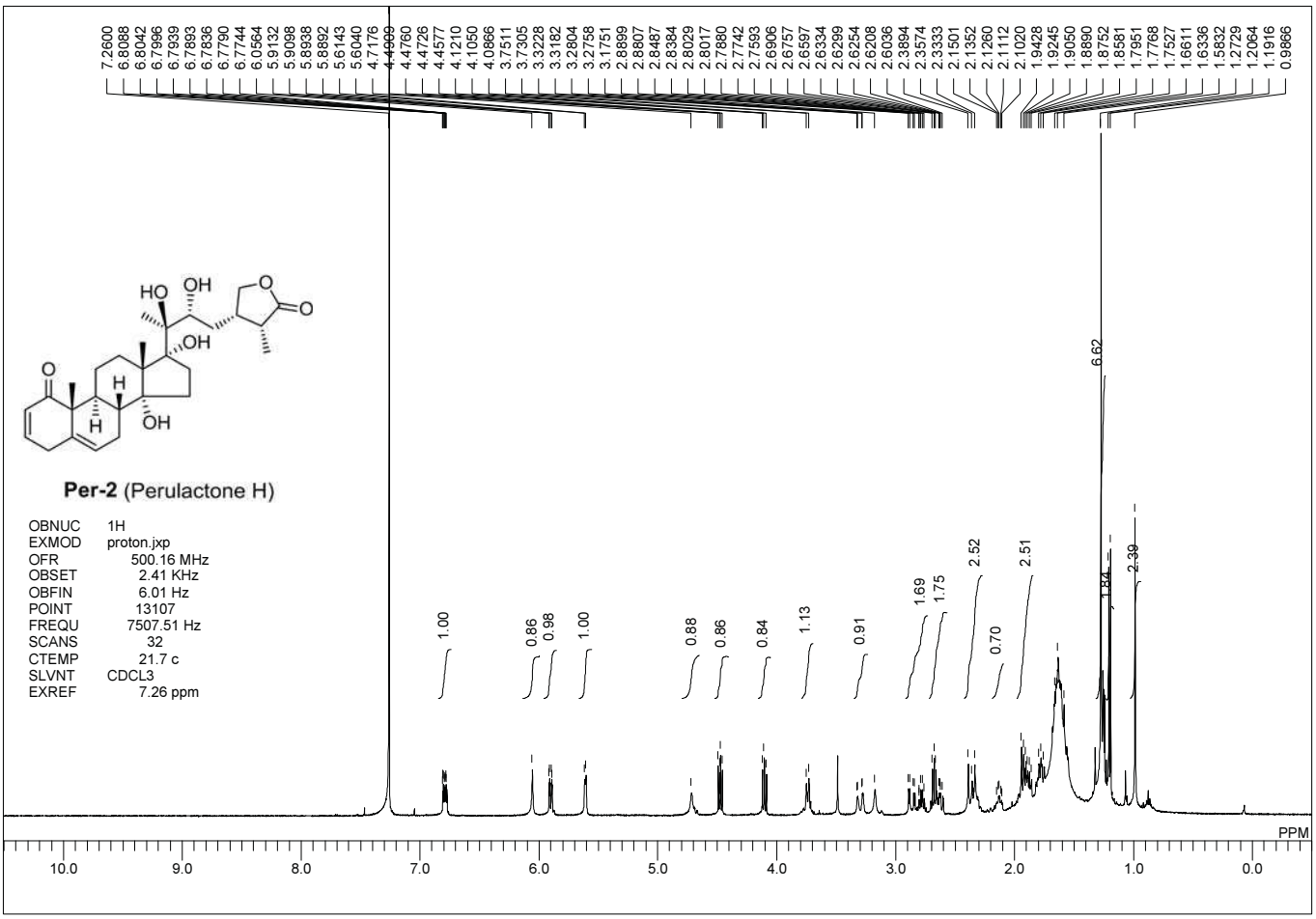

Wit-4 (Withanolide E)
OBNUC 13C
EXMOD carbon.jxp
OFR 100.91 MHz
OBSET 2.56 KHz
OBFIN 8.88 Hz
POINT 26214
FREQU 25252.53 Hz
SCANS 1500
CTEMP 21.2 c
SLVNT CDCL3
EXREF 77.00 ppm
PPM

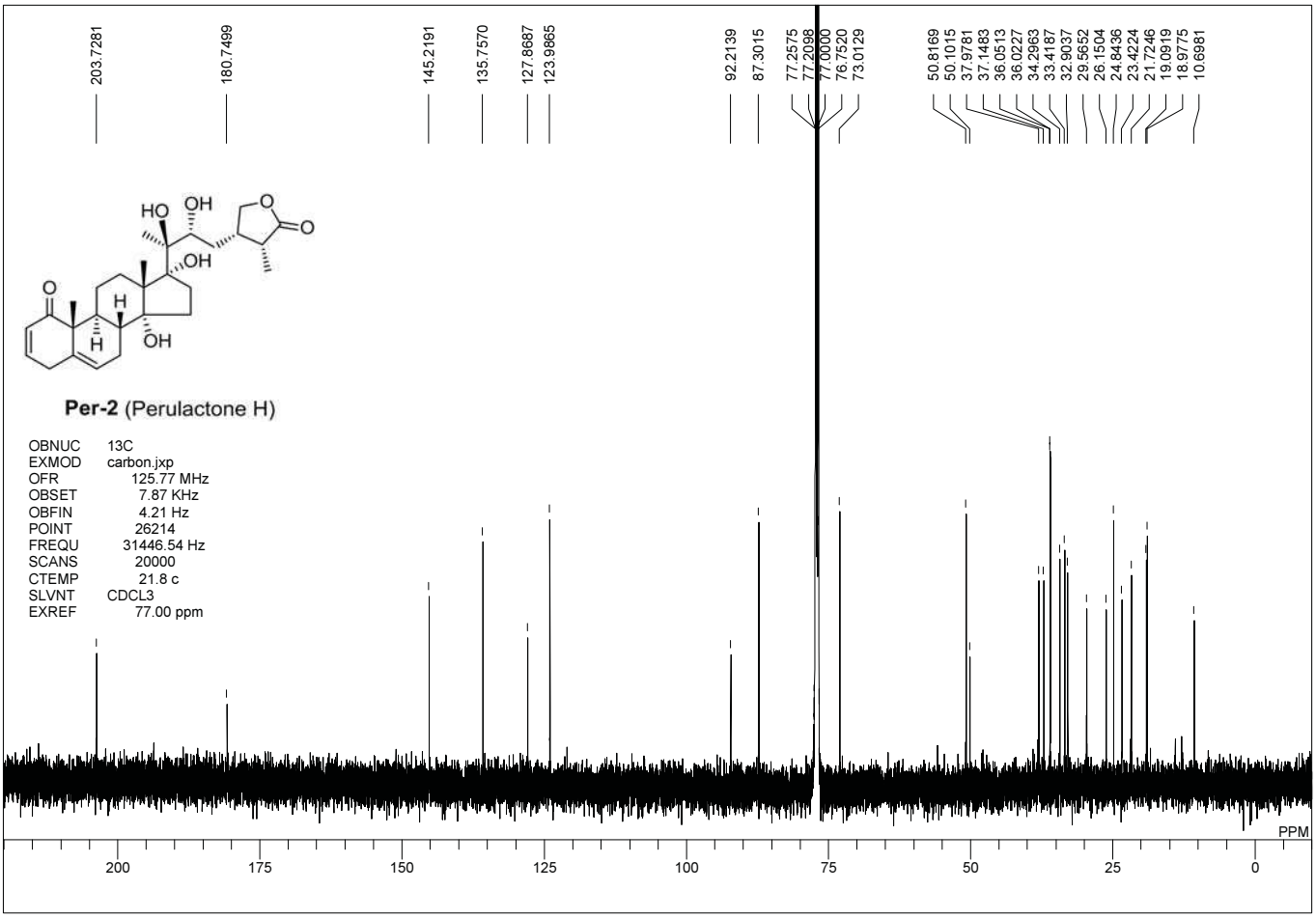

7.2600
6.9290
6.9176
6.9096
6.8970
6.2156
6.1950
4.8700
4.8597
4.8471
4.8368
3.2679
2.6964
2.4949
2.4743
2.2750
2.2590
2.2498
2.2349
2.0334
2.0139
1.9291
1.8650
1.8352
1.8261
1.7745
1.7597
1.7070
1.6898
1.6554
1.5741
1.5570
1.5478
1.5329
1.4550
1.4447
1.4367
1.4035
1.2901
1.2821
1.2684
1.2569
1.0611
7.29
2.73
2.45
2.81
2.02
2.03
1.56
0.94
1.06
0.95
1.02
1.00
Wit-S1
(4β-hydroxywithanolide E)
OBNUC 1H
EXMOD proton.jxp
OFR 500.16 MHz
OBSET 2.41 KHz
OBFIN 6.01 Hz
POINT 16384
FREQU 9384.38 Hz
SCANS 8
CTEMP 25.0 c
SLVNT CDCL3
EXREF 7.26 ppm
PPM
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

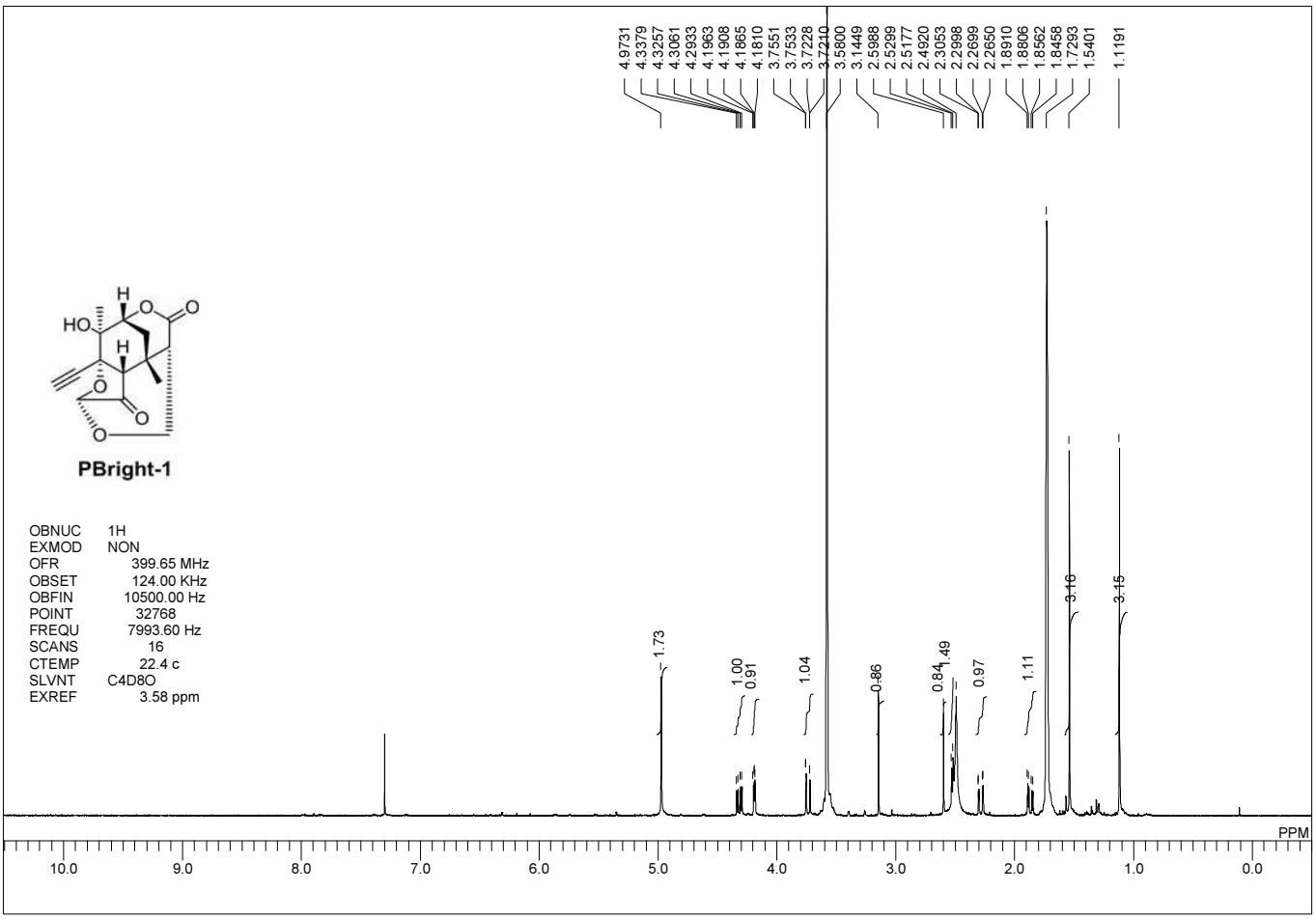

201.7613
166.0116
150.7312
141.3742
133.0472
121.4009
87.6352
81.7787
79.8612
79.0030
77.2575
77.0000
76.7520
70.2087
64.0565
62.8260
54.4514
47.7555
37.8261
36.6052
34.2492
34.1348
32.3129
29.6231
25.8173
21.3343
20.6285
20.2374
19.5602
16.6415
12.3111
Wit-S1
(4β-hydroxywithanolide E)
OBNUC 13C
EXMOD carbon.jxp
OFR 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 32768
FREQU 39308.18 Hz
SCANS 2000
CTEMP 25.0 c
SLVNT CDCL3
EXREF 77.00 ppm
PPM
200
175
150
125
100
75
50
25
0

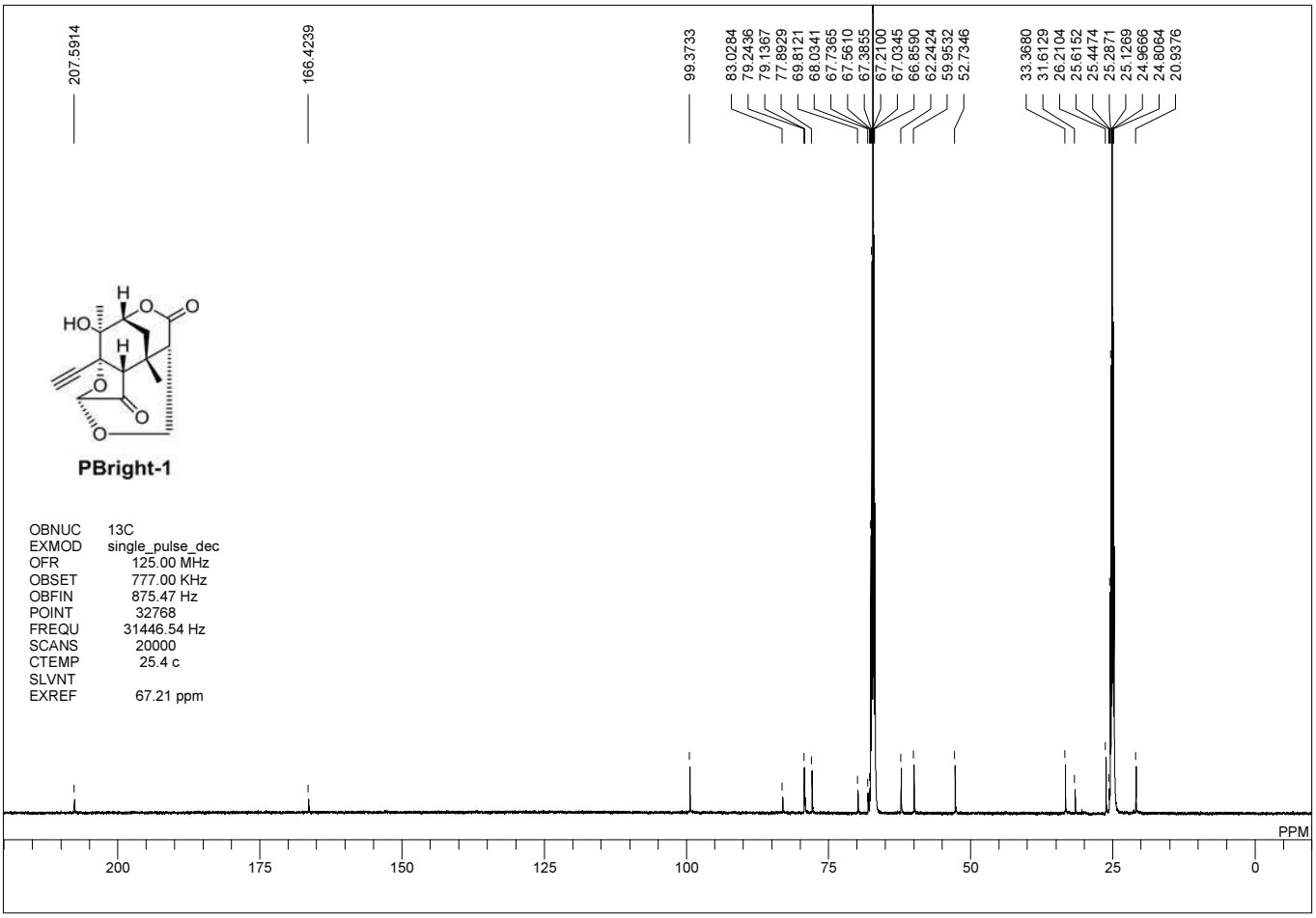

Per-1 (Perulactone B)
OBNUC 1H
EXMOD proton.jxp
OFR 401.28 MHz
OBSET 4.20 KHz
OBFIN 4.97 Hz
POINT 16384
FREQU 7530.12 Hz
SCANS 8
CTEMP 21.3 c
SLVNT CDCL3
EXREF 7.26 ppm
PPM

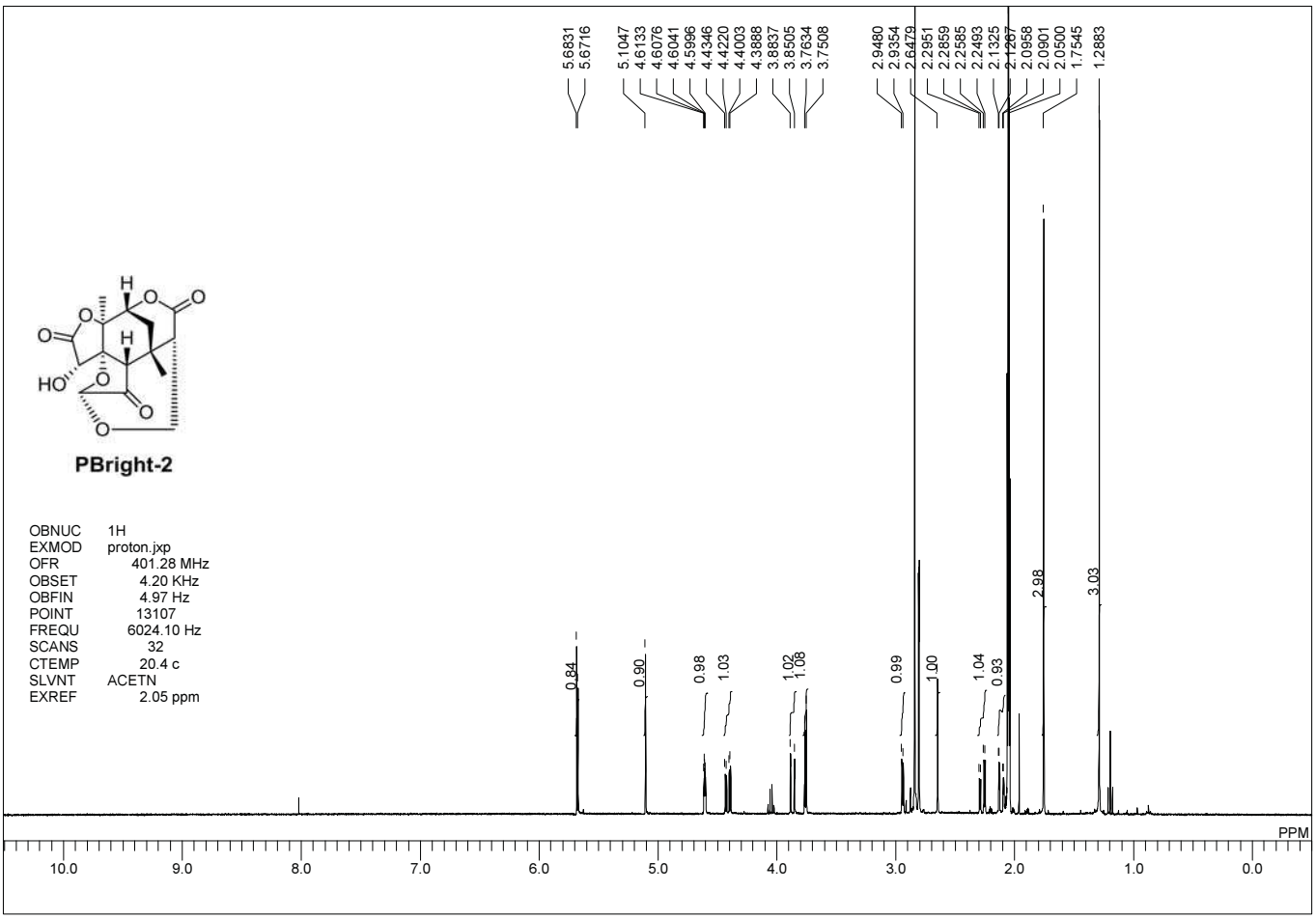

Per-1 (Perulactone B)
OBNUC 13C
EXMOD carbon.jxp
OFR 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 26214
FREQU 31446.54 Hz
SCANS 2500
CTEMP 25.0 c
SLVNT CDCL3
EXREF 77.00 ppm
PPM

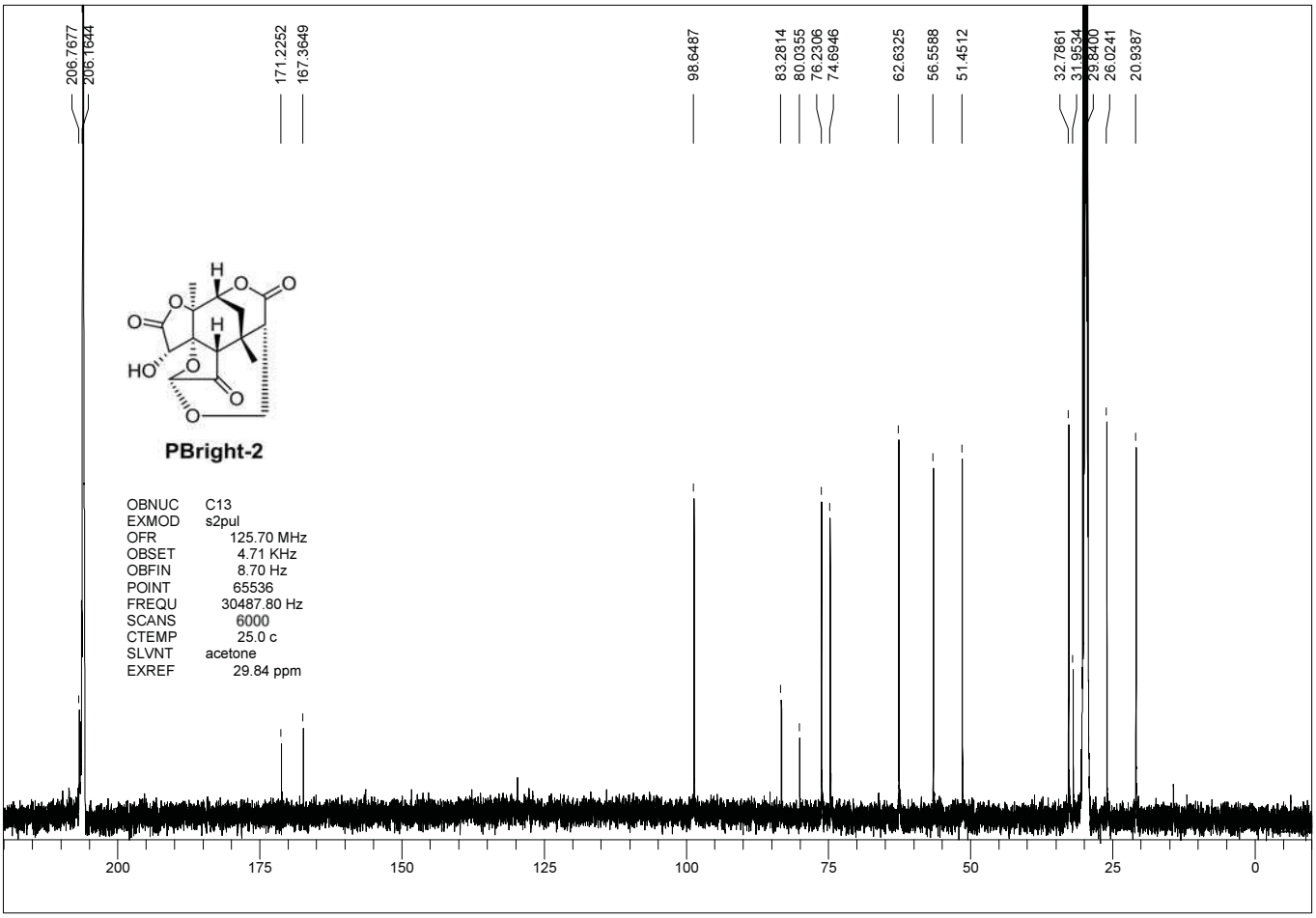

Per-2 (Perulactone H)
OBNUC 1H
EXMOD proton.jxp
OFR 500.16 MHz
OBSET 2.41 KHz
OBFIN 6.01 Hz
POINT 13107
FREQU 7507.51 Hz
SCANS 32
CTEMP 21.7 c
SLVNT CDCL3
EXREF 7.26 ppm
PPM

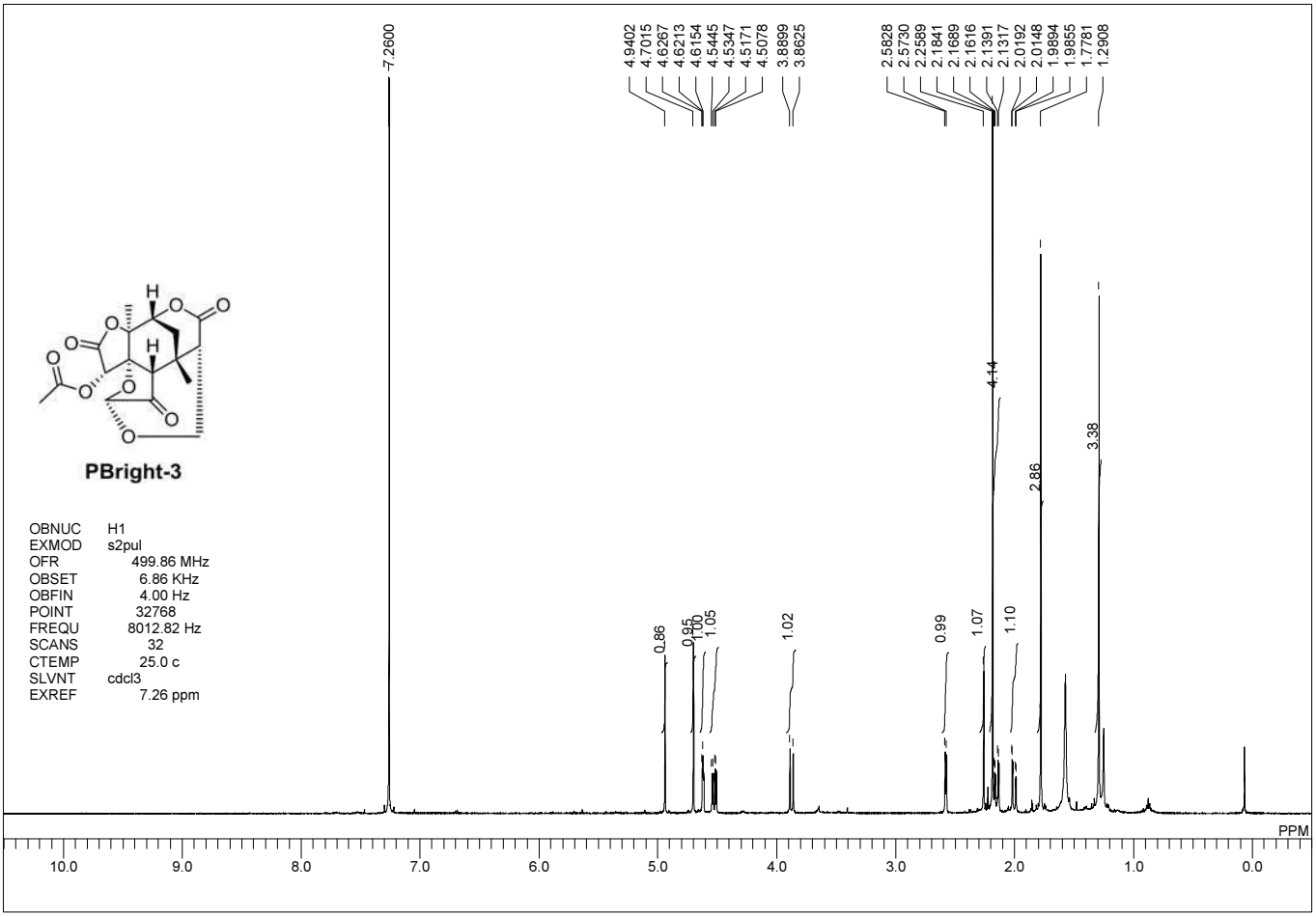

Per-2 (Perulactone H)
OBNUC 13C
EXMOD carbon.jxp
OFR 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 26214
FREQU 31446.54 Hz
SCANS 20000
CTEMP 21.8 c
SLVNT CDCL3
EXREF 77.00 ppm
PPM


4.9731
4.3379
4.3257
4.3061
4.2933
4.1963
4.1908
4.1865
4.1810
3.7551
3.7533
3.7228
3.7210
3.5800
3.1449
2.5988
2.5299
2.5177
2.4920
2.3053
2.2998
2.2699
2.2650
1.8910
1.8806
1.8562
1.8458
1.7293
1.5401
1.1191
PBright-1
OBNUC 1H
EXMOD NON
OFR 399.65 MHz
OBSET 124.00 KHz
OBFIN 10500.00 Hz
POINT 32768
FREQU 7993.60 Hz
SCANS 16
CTEMP 22.4 c
SLVNT C4D8O
EXREF 3.58 ppm
1.73
1.00
0.91
1.04
0.86
0.84
1.49
0.97
1.11
3.16
3.15
PPM
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0


207.5914
166.4239
99.3733
83.0284
79.2436
79.1367
77.8929
69.8121
68.0341
67.7365
67.5610
67.3855
67.2100
67.0345
66.8590
62.2424
59.9532
52.7346
33.3680
31.6129
26.2104
25.6152
25.4474
25.2871
25.1269
24.9666
24.8064
20.9376
PBright-1
OBNUC 13C
EXMOD single_pulse_dec
OFR 125.00 MHz
OBSET 777.00 KHz
OBFIN 875.47 Hz
POINT 32768
FREQU 31446.54 Hz
SCANS 20000
CTEMP 25.4 c
SLVNT
EXREF 67.21 ppm
PPM
200
175
150
125
100
75
50
25
0

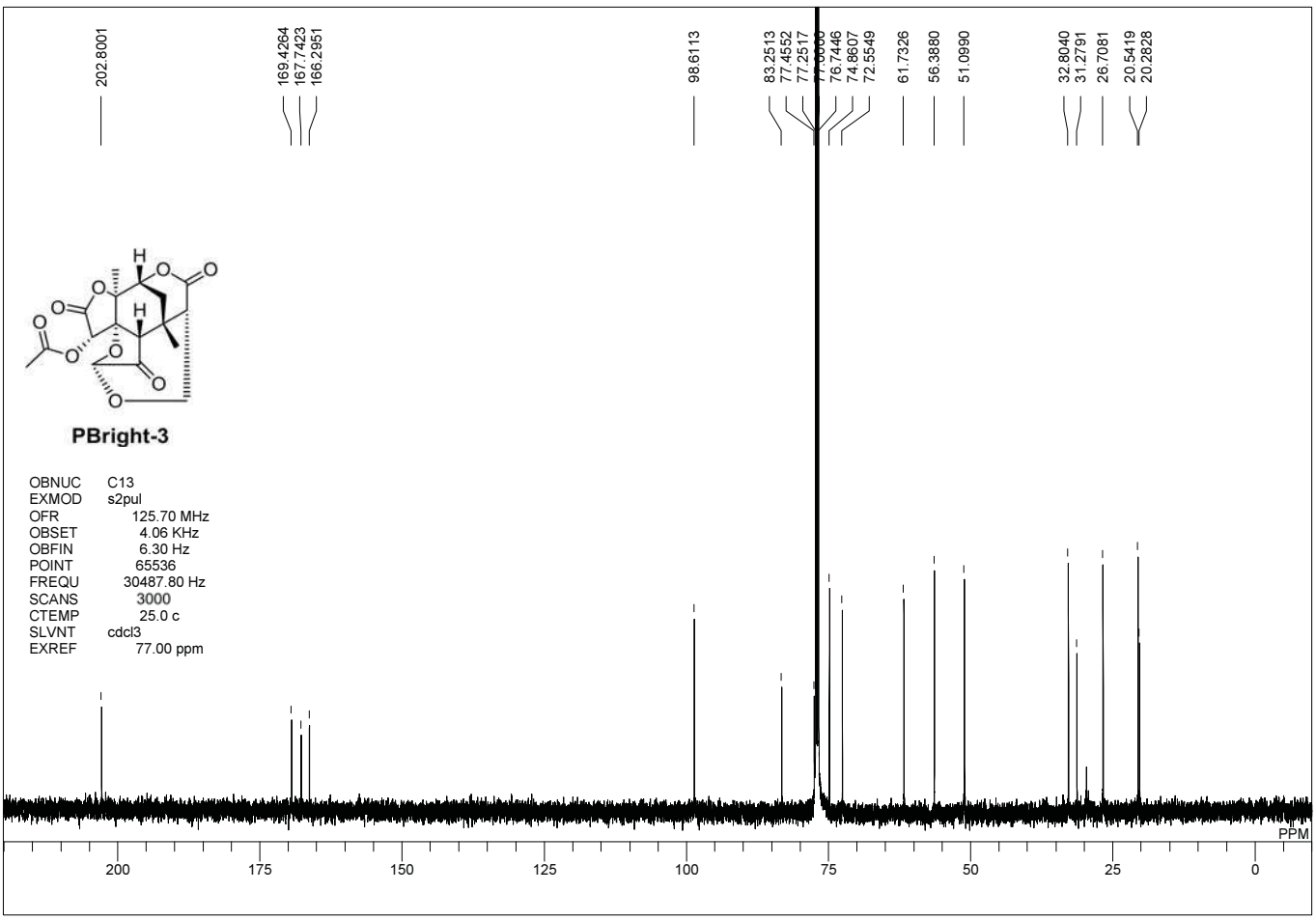

5.6831
5.6716
5.1047
4.6133
4.6076
4.6041
4.5996
4.4346
4.4220
4.4003
4.3888
3.8837
3.8505
3.7634
3.7508
2.9480
2.9354
2.6479
2.2951
2.2859
2.2585
2.2493
2.1325
2.1287
2.0958
2.0901
2.0500
1.7545
1.2883
PBright-2
OBNUC 1H
EXMOD proton.jxp
OFR 401.28 MHz
OBSET 4.20 KHz
OBFIN 4.97 Hz
POINT 13107
FREQU 6024.10 Hz
SCANS 32
CTEMP 20.4 c
SLVNT ACETN
EXREF 2.05 ppm
0.84
0.90
0.98
1.03
1.02
1.08
0.99
1.00
1.04
0.93
2.98
3.03
PPM
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0


206.7677
206.1644
171.2252
167.3649
98.6487
83.2814
80.0355
76.2306
74.6946
62.6325
56.5588
51.4512
32.7861
31.9534
29.8400
26.0241
20.9387
PBright-2
OBNUC C13
EXMOD s2pul
OFR 125.70 MHz
OBSET 4.71 KHz
OBFIN 8.70 Hz
POINT 65536
FREQU 30487.80 Hz
SCANS 6000
CTEMP 25.0 c
SLVNT acetone
EXREF 29.84 ppm
200
175
150
125
100
75
50
25
0



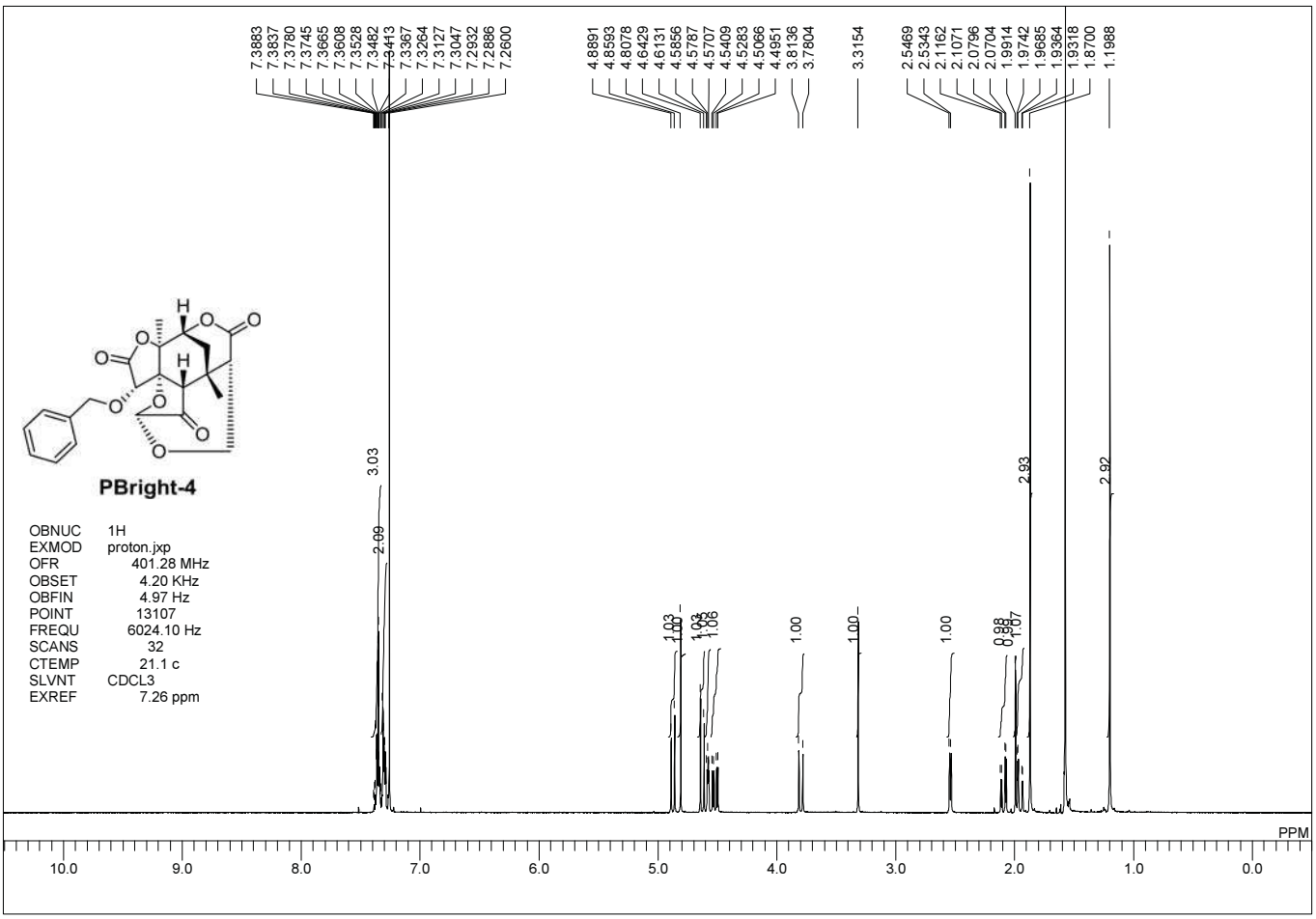
PBright-3

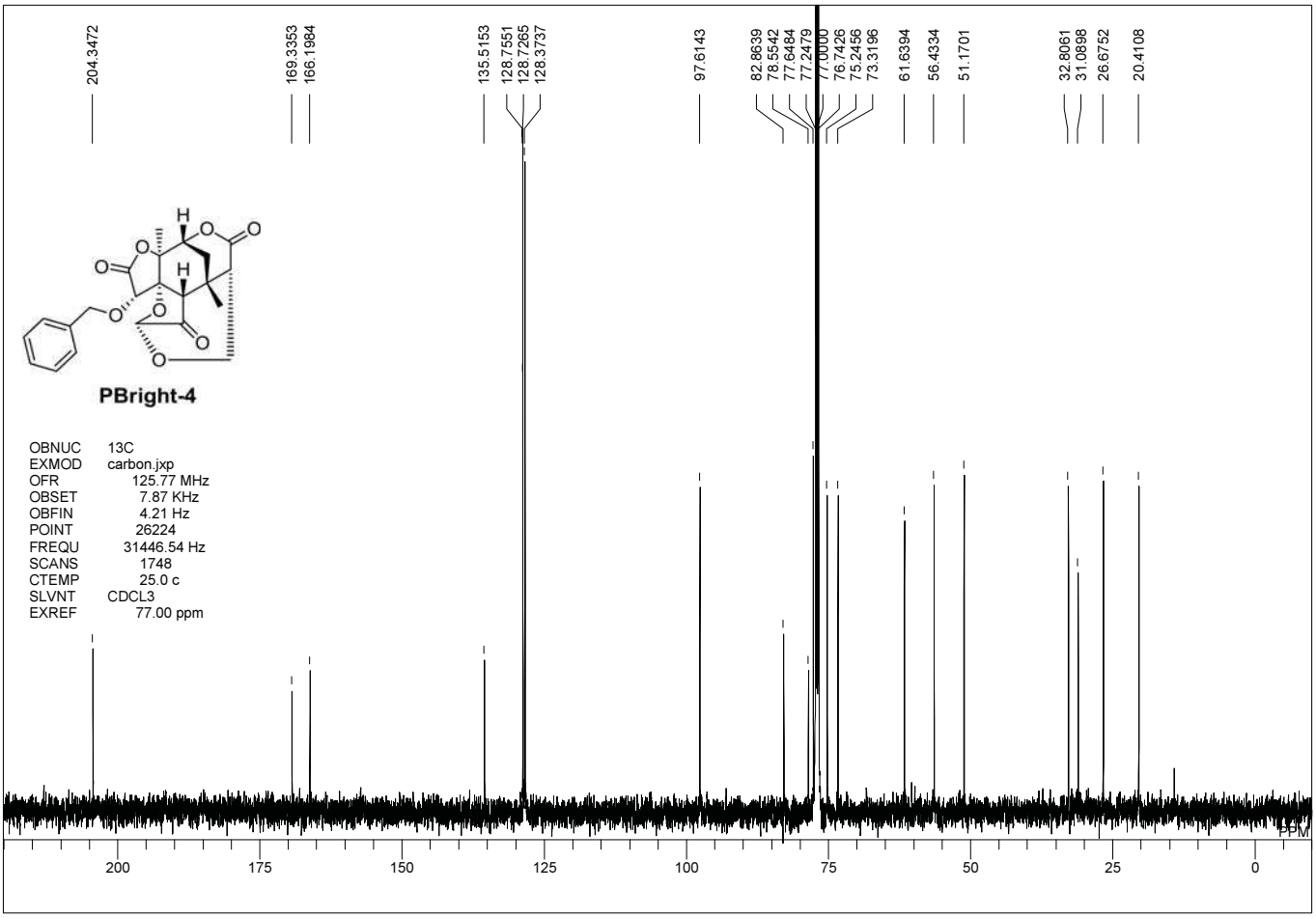
OBNUC	H1
EXMOD	s2pul
OFR	499.86 MHz
OBSET	6.86 KHz
OBFIN	4.00 Hz
POINT	32768
FREQU	8012.82 Hz
SCANS	32
CTEMP	25.0 c
SLVNT	cdcl3
EXREF	7.26 ppm

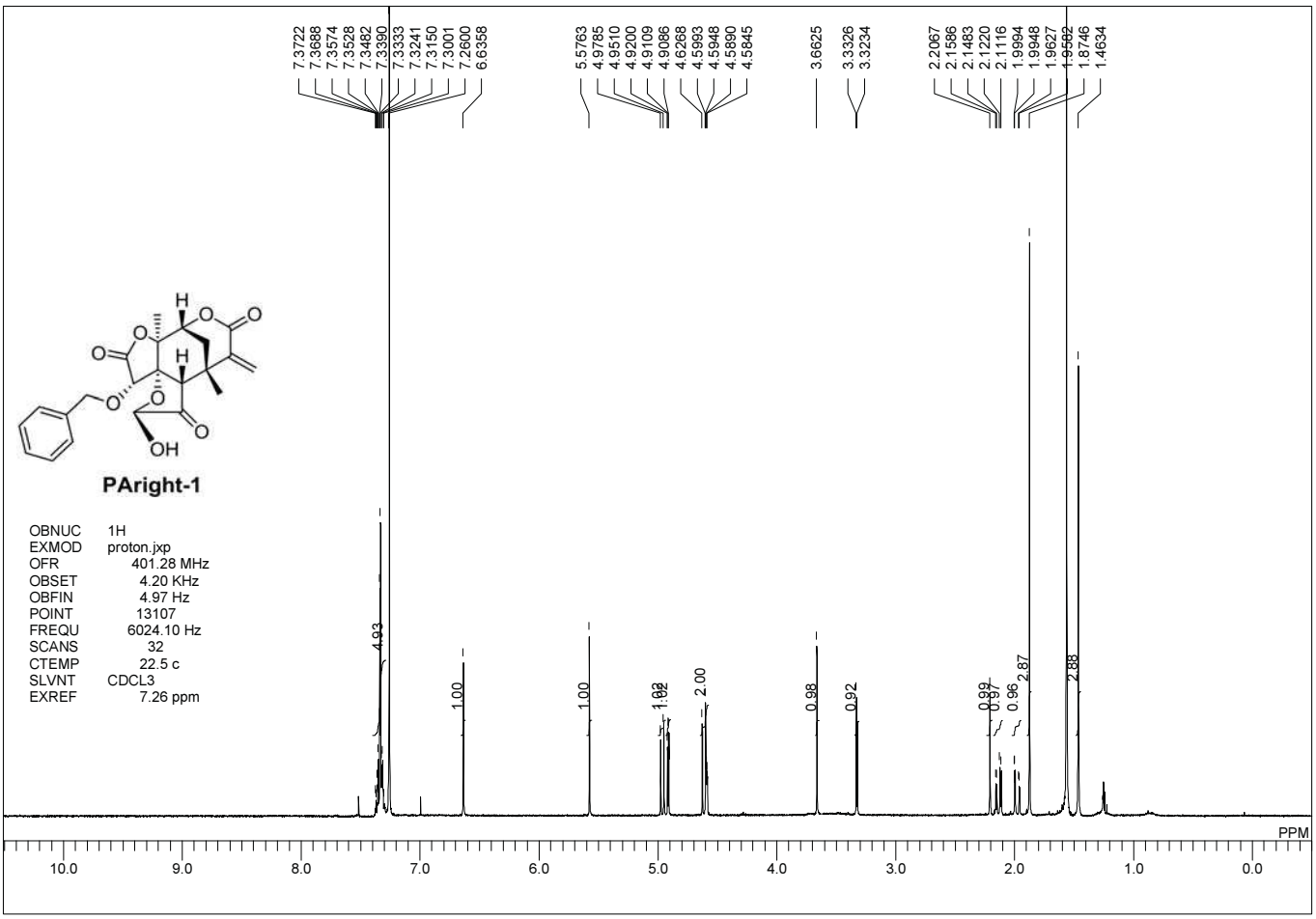


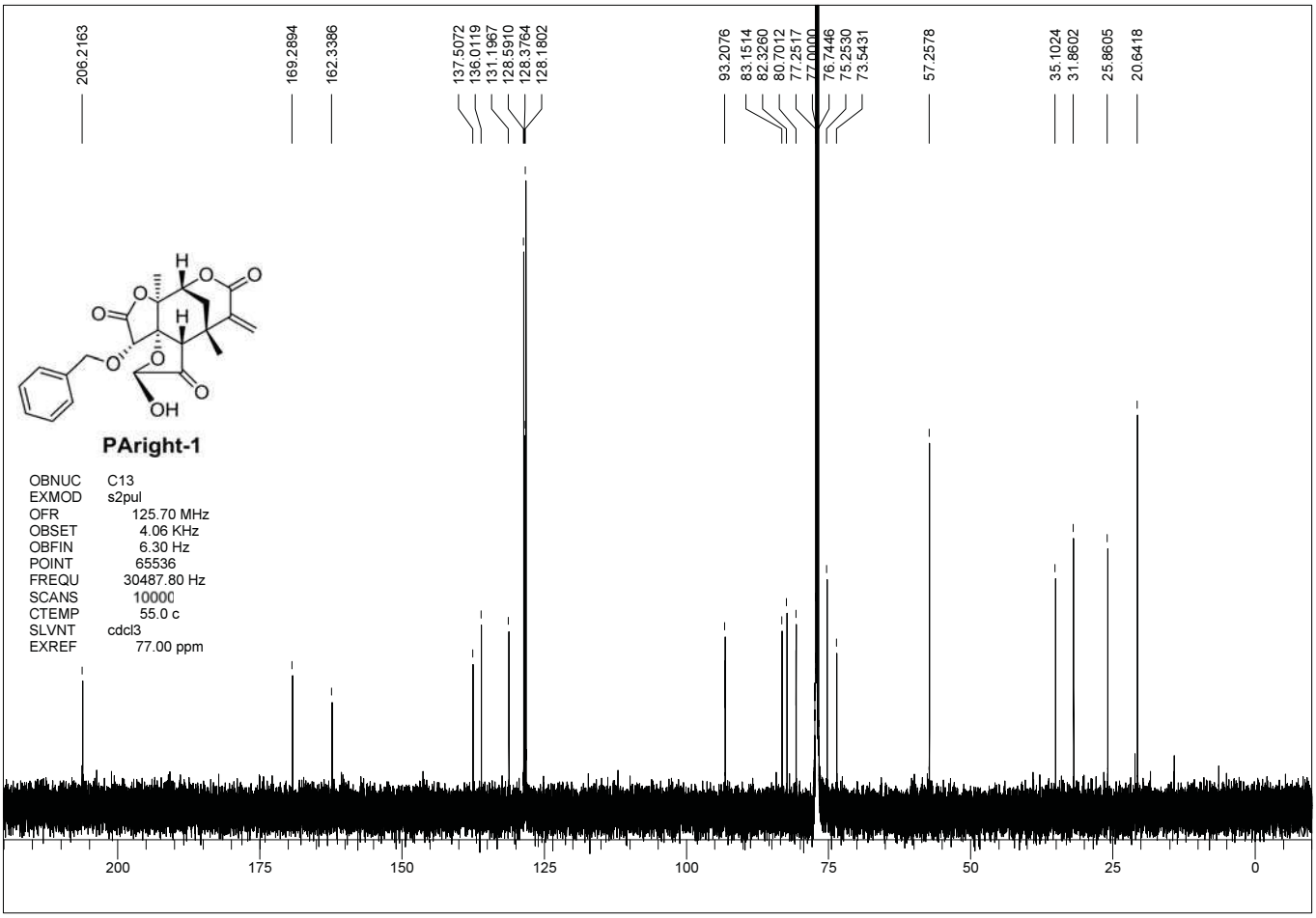
PBright-3

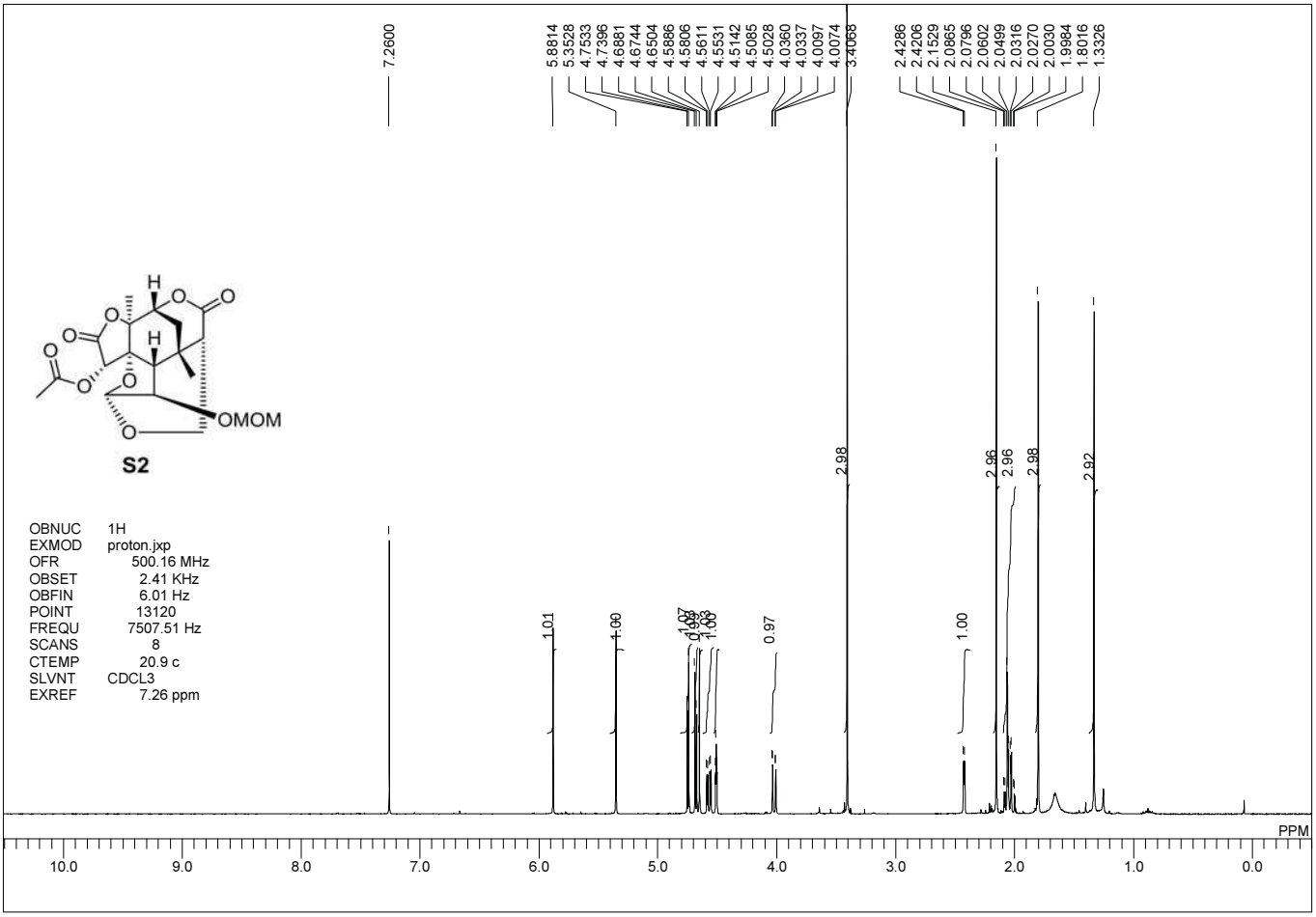
OBNUC	C13
EXMOD	s2pul
OFR	125.70 MHz
OBSET	4.06 KHz
OBFIN	6.30 Hz
POINT	65536
FREQU	30487.80 Hz
SCANS	3000
CTEMP	25.0 c
SLVNT	cdcl3
EXREF	77.00 ppm

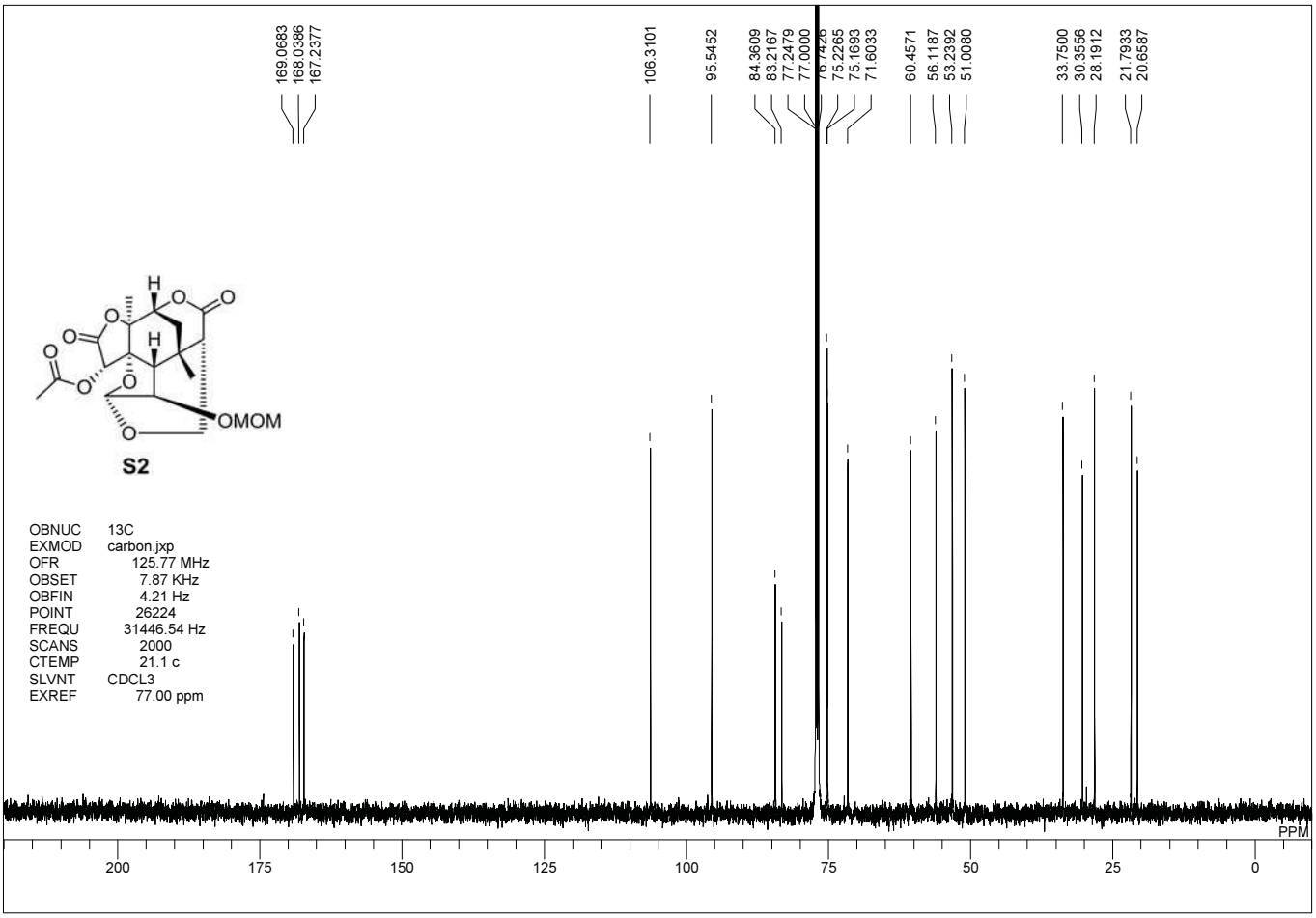

7.3883
7.3837
7.3780
7.3745
7.3665
7.3608
7.3528
7.3482
7.3413
7.3367
7.3264
7.3127
7.3047
7.2932
7.2886
7.2600
4.8891
4.8593
4.8078
4.6429
4.6131
4.5856
4.5787
4.5707
4.5409
4.5283
4.5066
4.4951
3.8136
3.7804
3.3154
2.5469
2.5343
2.1162
2.1071
2.0796
2.0704
1.9914
1.9742
1.9685
1.9364
1.9318
1.8700
1.1988
3.03
2.09
2.93
2.92
1.03
1.00
1.03
1.03
1.06
1.00
1.00
1.00
0.98
0.97
0.97
PBright-4
OBNUC 1H
EXMOD proton.jxp
OFR 401.28 MHz
OBSET 4.20 KHz
OBFIN 4.97 Hz
POINT 13107
FREQU 6024.10 Hz
SCANS 32
CTEMP 21.1 c
SLVNT CDCL3
EXREF 7.26 ppm
PPM
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

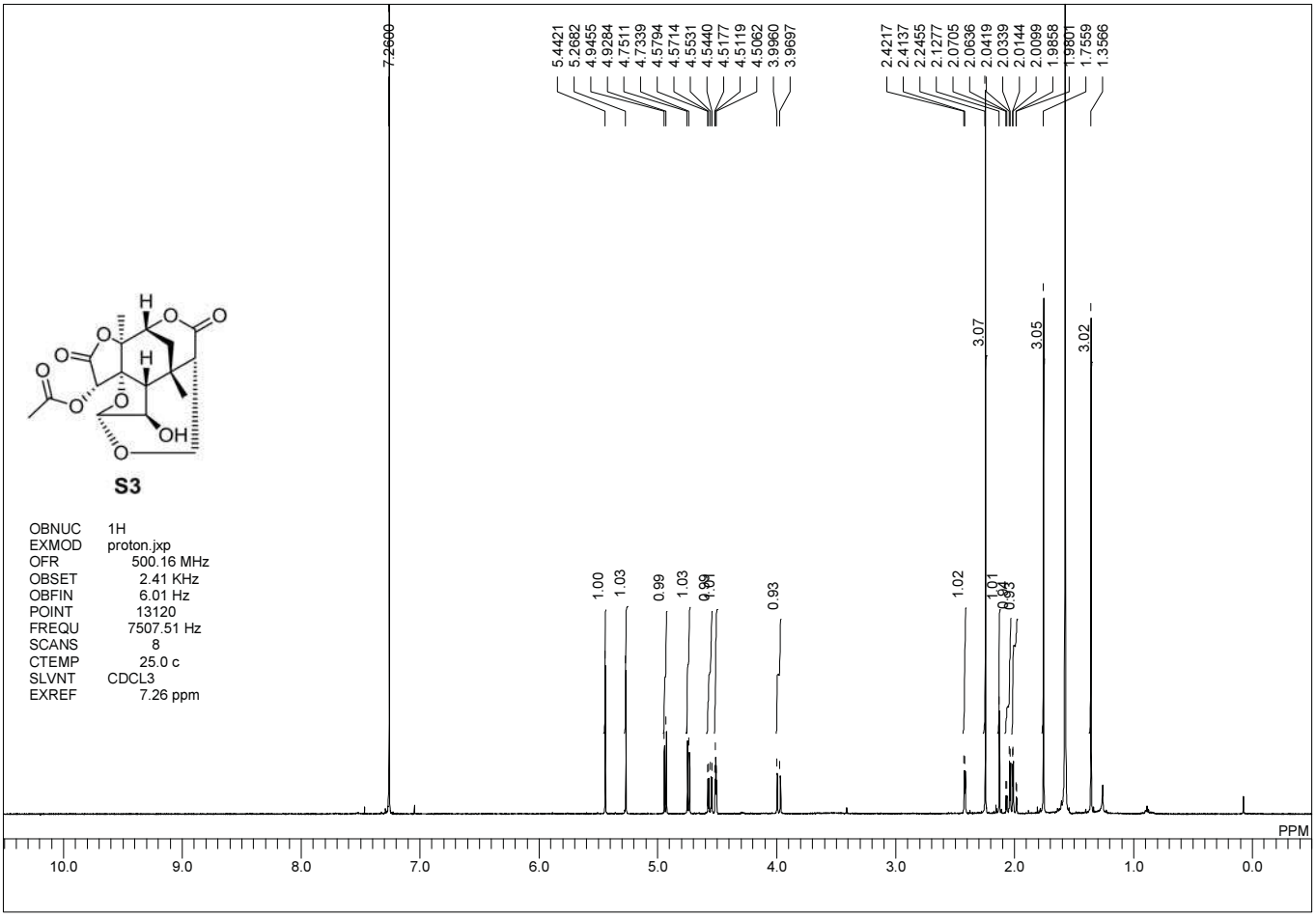

204.3472
169.3353
166.1984
135.5153
128.7551
128.7265
128.3737
97.6143
82.8639
78.5542
77.6484
77.2479
77.0000
76.7426
75.2456
73.3196
61.6394
56.4334
51.1701
32.8061
31.0898
26.6752
20.4108
PBright-4
OBNUC 13C
EXMOD carbon.jxp
OFR 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 26224
FREQU 31446.54 Hz
SCANS 1748
CTEMP 25.0 c
SLVNT CDCL3
EXREF 77.00 ppm
PPM
200
175
150
125
100
75
50
25
0


7.3722
7.3688
7.3574
7.3528
7.3482
7.3390
7.3333
7.3241
7.3150
7.3001
7.2600
6.6358
5.5763
4.9785
4.9510
4.9200
4.9109
4.9086
4.6268
4.5993
4.5948
4.5890
4.5845
3.6625
3.3326
3.3234
2.2067
2.1586
2.1483
2.1220
2.1116
1.9994
1.9948
1.9627
1.8746
1.4634
PAright-1
OBNUC 1H
EXMOD proton.jxp
OFR 401.28 MHz
OBSET 4.20 KHz
OBFIN 4.97 Hz
POINT 13107
FREQU 6024.10 Hz
SCANS 32
CTEMP 22.5 c
SLVNT CDCL3
EXREF 7.26 ppm
4.93
1.00
1.00
1.02
2.00
0.98
0.92
0.99
0.97
0.96
2.87
2.88
PPM
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0


206.2163
169.2894
162.3386
137.5072
136.0119
131.1967
128.5910
128.3764
128.1802
93.2076
83.1514
82.3260
80.7012
77.2517
77.0000
76.7446
75.2530
73.5431
57.2578
35.1024
31.8602
25.8605
20.6418
PAright-1
OBNUC C13
EXMOD s2pul
OFR 125.70 MHz
OBSET 4.06 KHz
OBFIN 6.30 Hz
POINT 65536
FREQU 30487.80 Hz
SCANS 10000
CTEMP 55.0 c
SLVNT cdcl3
EXREF 77.00 ppm
200
175
150
125
100
75
50
25
0

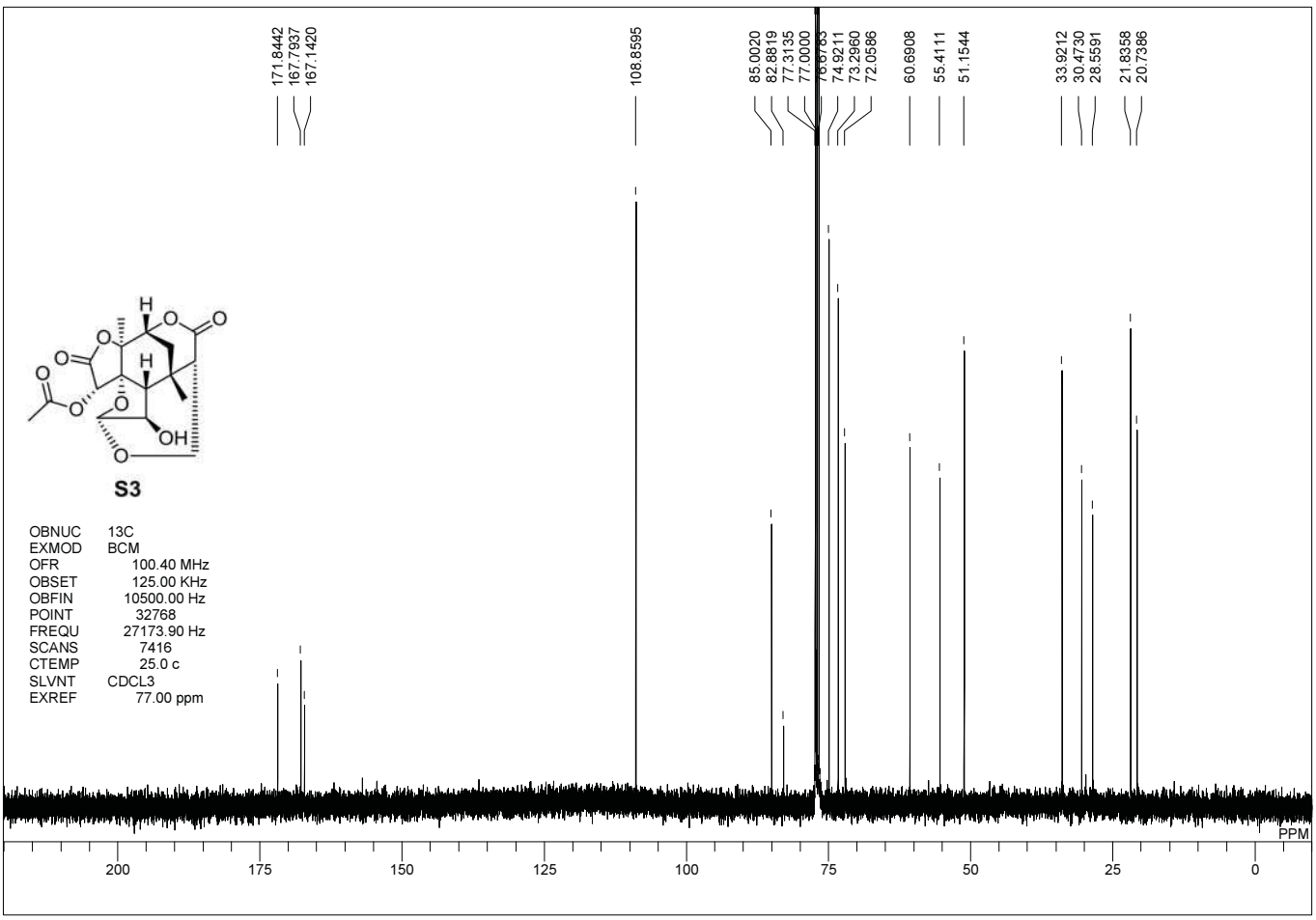

7.2600
5.8814
5.3528
4.7533
4.7396
4.6881
4.6744
4.6504
4.5886
4.5806
4.5611
4.5531
4.5142
4.5085
4.5028
4.0360
4.0337
4.0097
4.0074
3.4068
2.4286
2.4206
2.1529
2.0865
2.0796
2.0602
2.0499
2.0316
2.0270
2.0030
1.9984
1.8016
1.3326
OMOM
S2
OBNUC 1H
EXMOD proton.jxp
OFR 500.16 MHz
OBSET 2.41 KHz
OBFIN 6.01 Hz
POINT 13120
FREQU 7507.51 Hz
SCANS 8
CTEMP 20.9 c
SLVNT CDCL3
EXREF 7.26 ppm
1.01
1.00
0.97
1.00
2.98
2.96
2.96
2.98
2.92
PPM
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0


169.0683
168.0386
167.2377
106.3101
95.5452
84.3609
83.2167
77.2479
77.0000
76.7426
75.2265
75.1693
71.6033
60.4571
56.1187
53.2392
51.0080
33.7500
30.3556
28.1912
21.7933
20.6587
OMOM
S2
OBNUC 13C
EXMOD carbon.jxp
OFR 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 26224
FREQU 31446.54 Hz
SCANS 2000
CTEMP 21.1 c
SLVNT CDCL3
EXREF 77.00 ppm
PPM
200
175
150
125
100
75
50
25
0



S3

OBNUC 1H
EXMOD proton.jxp
OFR 500.16 MHz
OBSET 2.41 KHz
OBFIN 6.01 Hz
POINT 13120
FREQU 7507.51 Hz
SCANS 8
CTEMP 25.0 c
SLVNT CDCL3
EXREF 7.26 ppm



S3

OBNUC 13C
EXMOD BCM
OFR 100.40 MHz
OBSET 125.00 KHz
OBFIN 10500.00 Hz
POINT 32768
FREQU 27173.90 Hz
SCANS 7416
CTEMP 25.0 c
SLVNT CDCL3
EXREF 77.00 ppm