

## Supplementary material

### Phytochemical Study of *Seriphidium khorassanicum* (syn. *Artemisia khorassanica*) Aerial Parts: Sesquiterpene Lactones with Anti-Protozoal Activity

Maryam Fattahian<sup>a</sup>, Sara Abdeyazdan<sup>a</sup>, Mustafa Ghanadian<sup>a,c,\*</sup>, Behzad Zolfaghari<sup>a,\*</sup>, Sedigheh Saberi<sup>b</sup>, Fazila Zulfiqar<sup>d</sup>, Ikhlas A. Khan<sup>d</sup>, and Zulfiqar Ali<sup>d</sup>

<sup>a</sup> School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

<sup>b</sup> Department of Mycology and Parasitology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.

<sup>c</sup> Shahid Beheshti Phytochemistry Research center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

<sup>d</sup> National Center for Natural Products Research, University of Mississippi, MS 38677, USA

#### Abstract

Two new eudesmane-type sesquiterpene lactones, 1 $\beta$ ,3 $\alpha$ ,8 $\alpha$ -trihydroxy-11 $\beta$ ,13-dihydroeudesma-4(15)-en-12,6 $\alpha$ -olide (**1**) and 1 $\beta$ ,4 $\alpha$ ,8 $\alpha$ -trihydroxy-11 $\beta$ ,13-dihydroeudesma-12,6 $\alpha$ -olide (**2**), and an unprecedented elemene-type sesquiterpene lactone, 1 $\beta$ ,2 $\beta$ ,8 $\alpha$ -trihydroxy-11 $\beta$ ,13-dihydroelema-12,6 $\alpha$ -olide (**3**) along with a known eudesmanolide artapshin (**4**) were isolated from *Seriphidium khorassanicum*. Structures were elucidated by NMR, HR-ESI-MS, and ECD spectral data analysis. The anti-protozoal activity was evaluated against *Leishmania major* promastigotes and amastigote-infected macrophages. They showed dose- and time-dependent activity against *L. major* amastigotes with IC<sub>50</sub> values in the range of 4.9 to 25.3  $\mu$ M being favourably far below their toxicity against normal murine macrophages with CC<sub>50</sub> values ranging from 432.5 to 620.7  $\mu$ M after 48 h of treatment. Compound **3** exhibited the strongest activity and the highest selectivity index (SI) with IC<sub>50</sub> of 4.9  $\pm$  0.6  $\mu$ M and SI of 88.2 comparable with the standard drug, meglumine antimoniate (Glucantime), with IC<sub>50</sub> and SI values of 15.5  $\pm$  2.1  $\mu$ M and 40.0, respectively.

**Keywords:** *Seriphidium khorassanicum*, *Artemisia khorassanica*, Asteraceae, *Leishmania major*, Amastigote, Sesquiterpene Lactone, Eudesmanolide, Glucantime

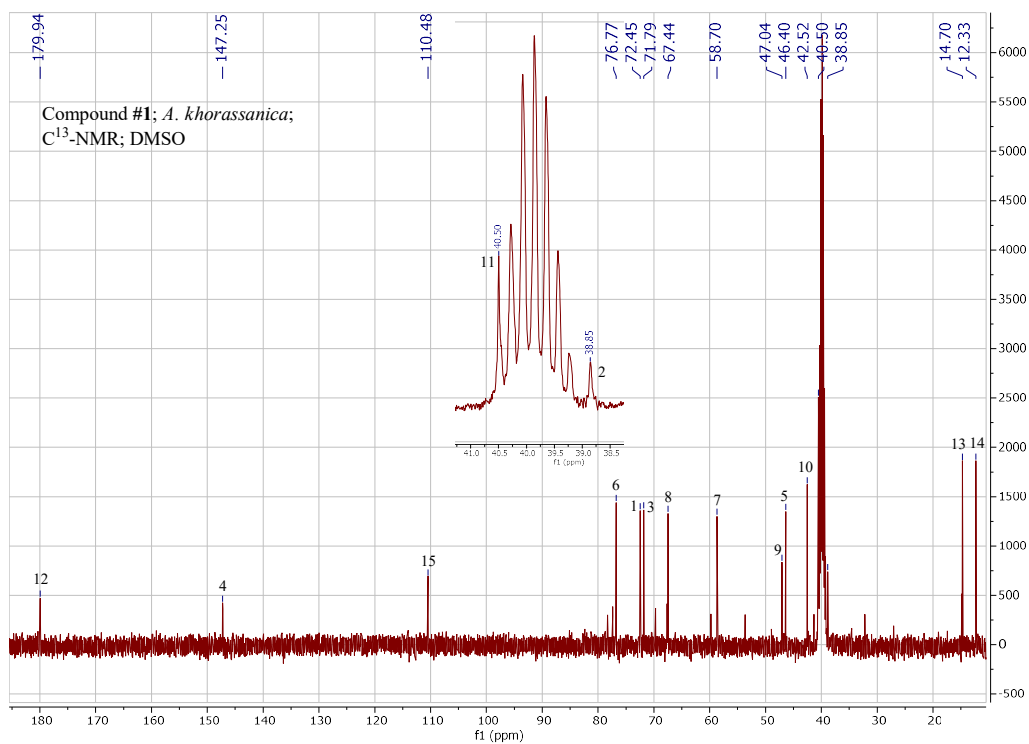
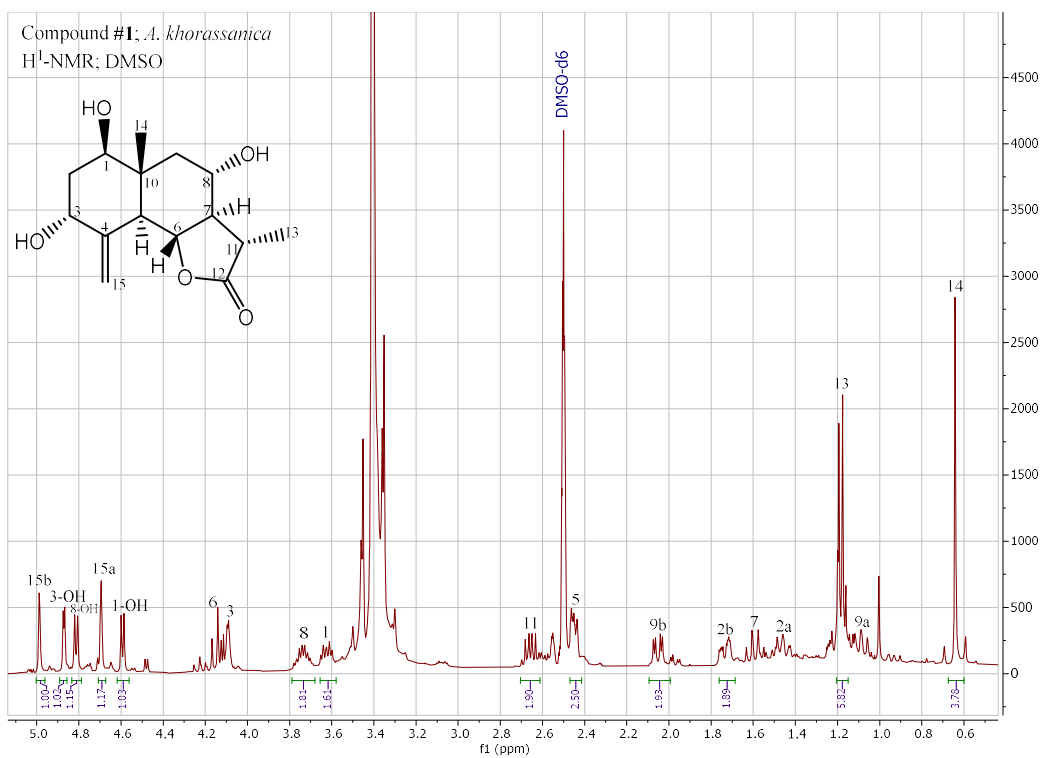
\* Authors for correspondence: Mustafa Ghanadian (Tel.: +98-313792-7131; E-mail: [ghannadian@gmail.com](mailto:ghannadian@gmail.com)); Behzad Zolfaghari (Tel.: +98-9133276201; E-mail: [behzadz@gmail.com](mailto:behzadz@gmail.com))

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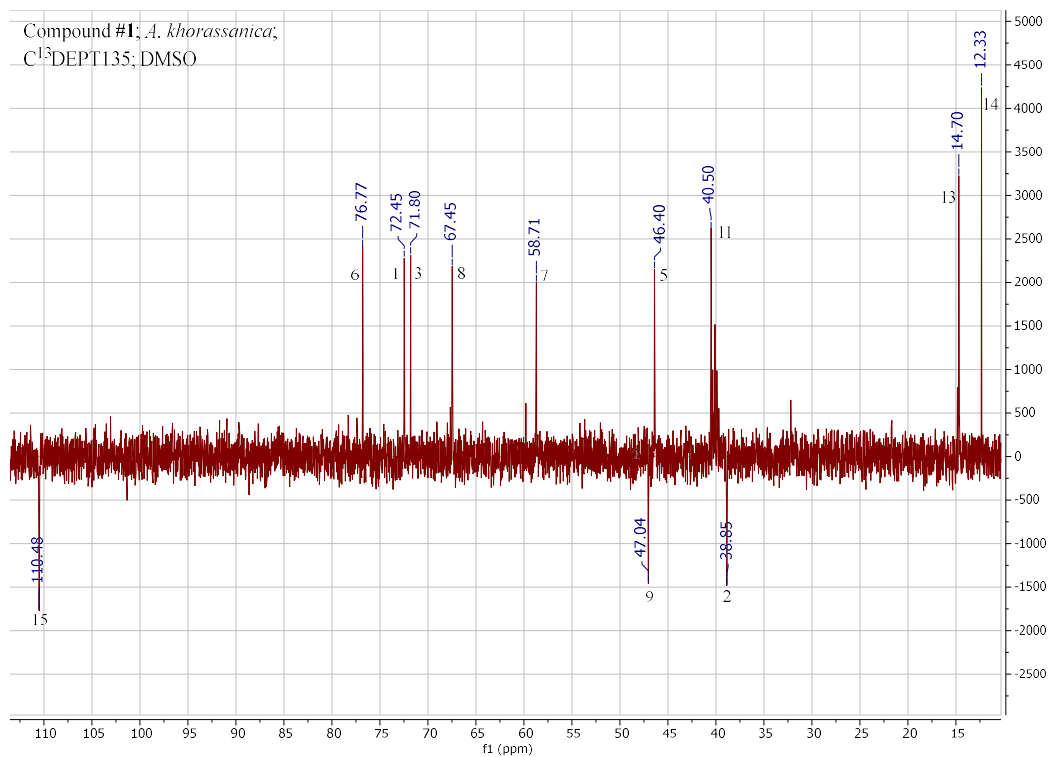


Figure S.3 DEPT135 spectrum of Compound 1

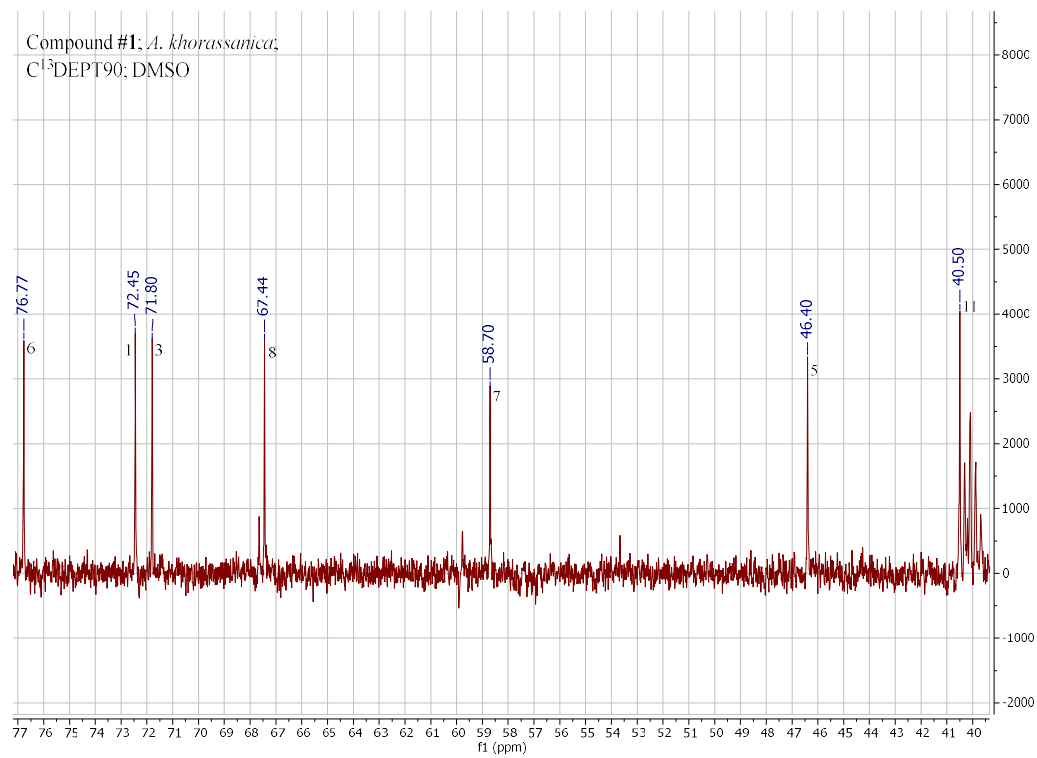


Figure S.4 DEPT90 spectrum of Compound 1

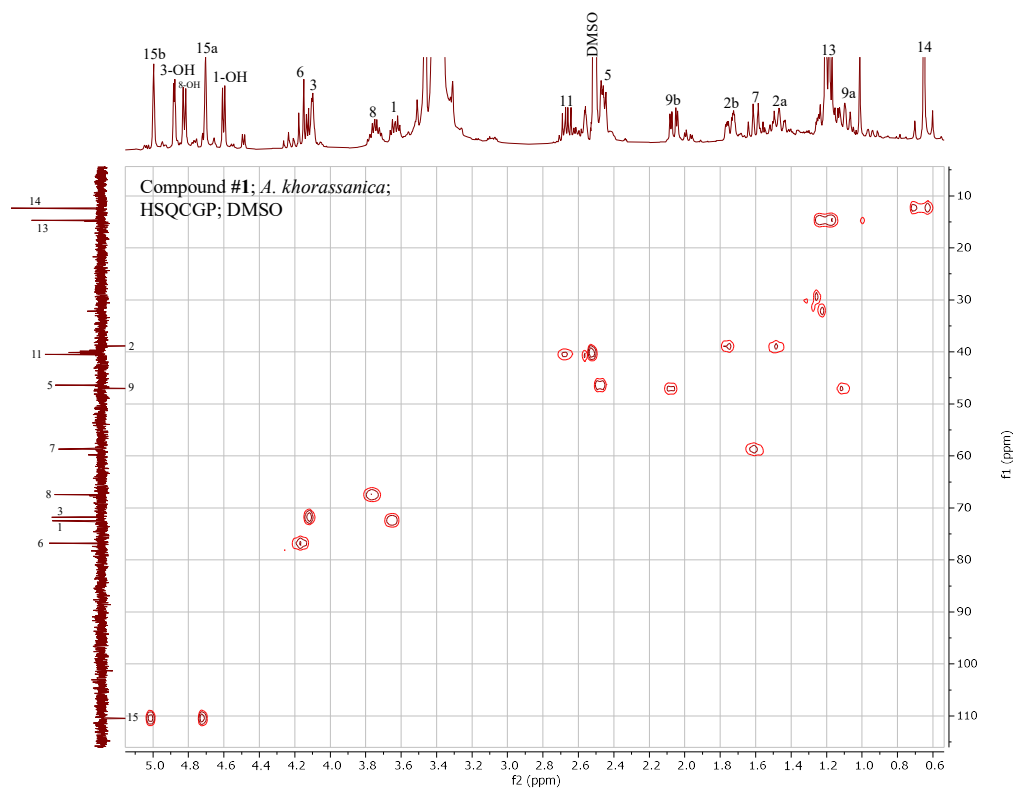


Figure S.5 HSQC spectrum of Compound 1

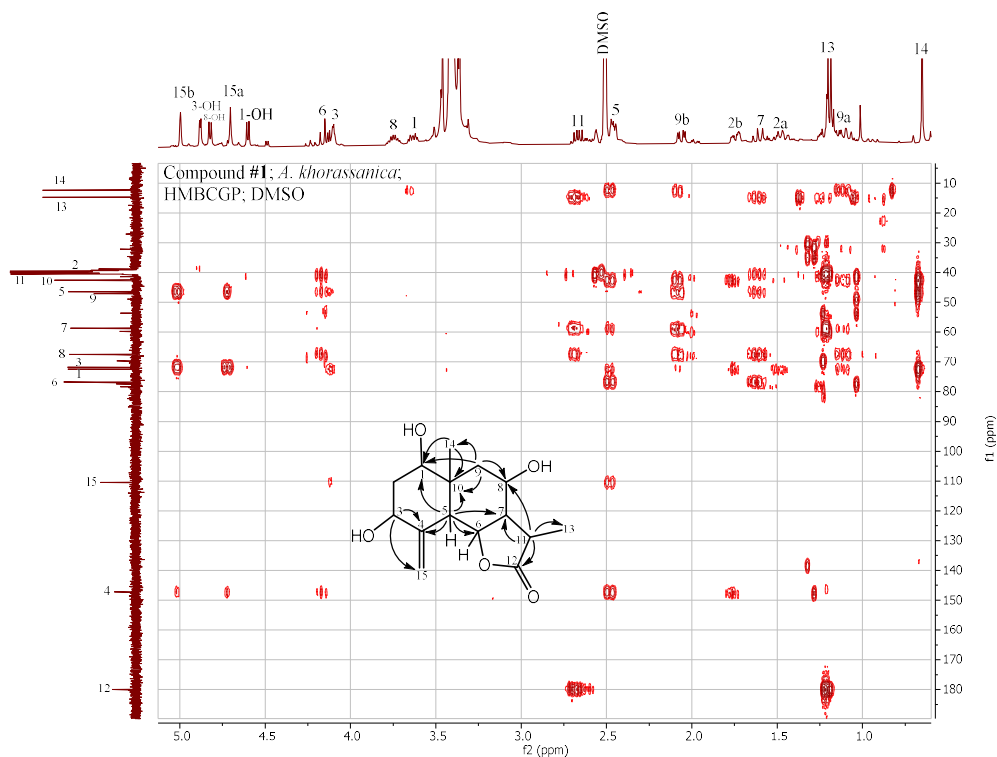


Figure S.6 HMBC spectrum of Compound 1

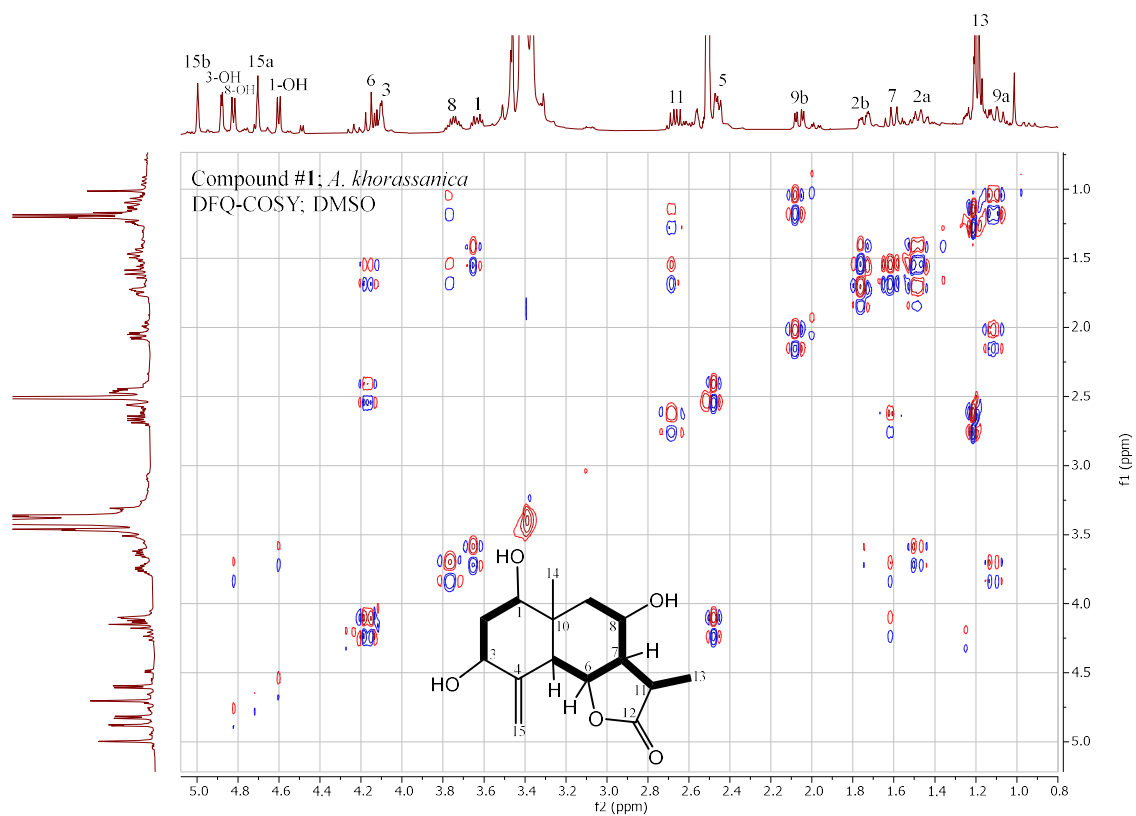


Figure S.7 DQF-COSY spectrum of Compound **1**

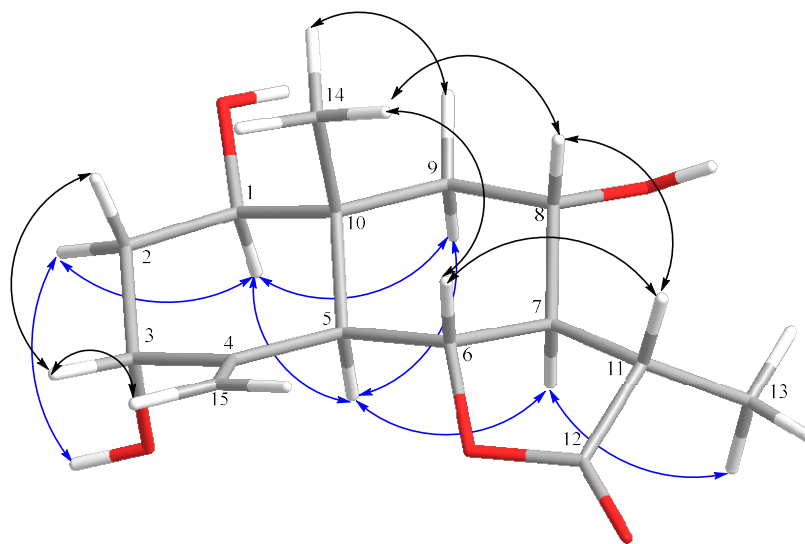


Figure S.8 Representation of key NOESY correlations of Compound **1**

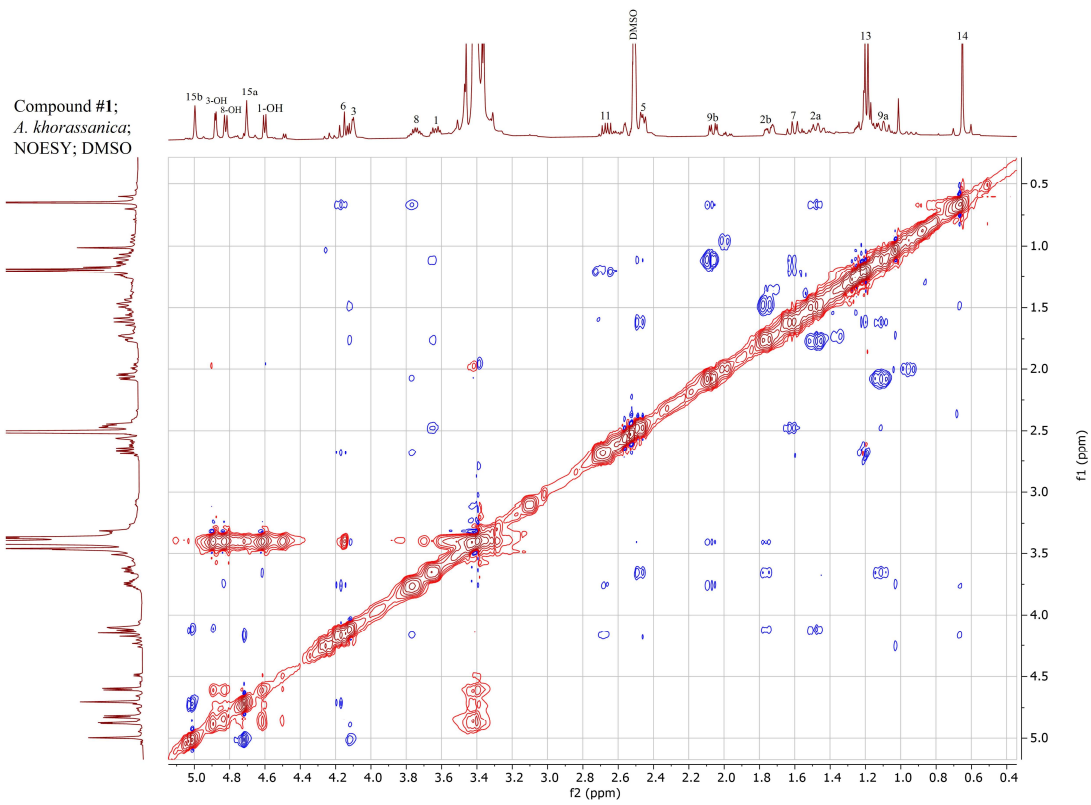


Figure S.9 NOESY spectrum of Compound 1

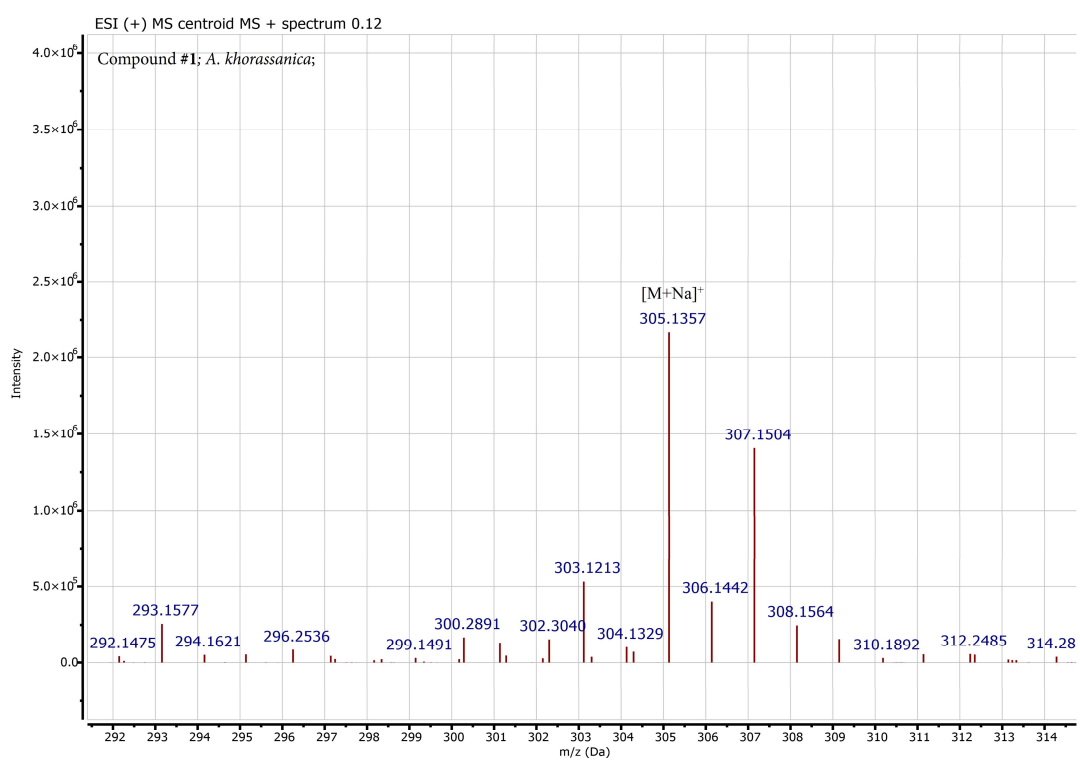
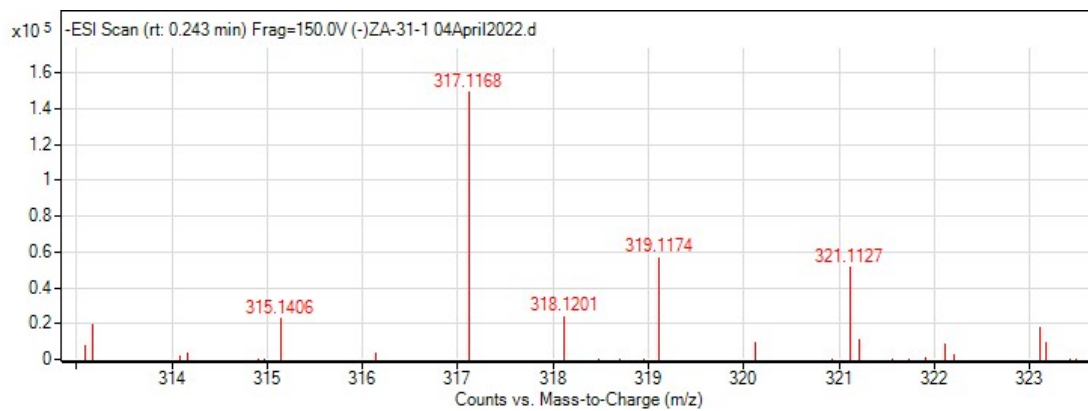


Figure S.10 HR-ESI-Mass spectrum of Compound 1



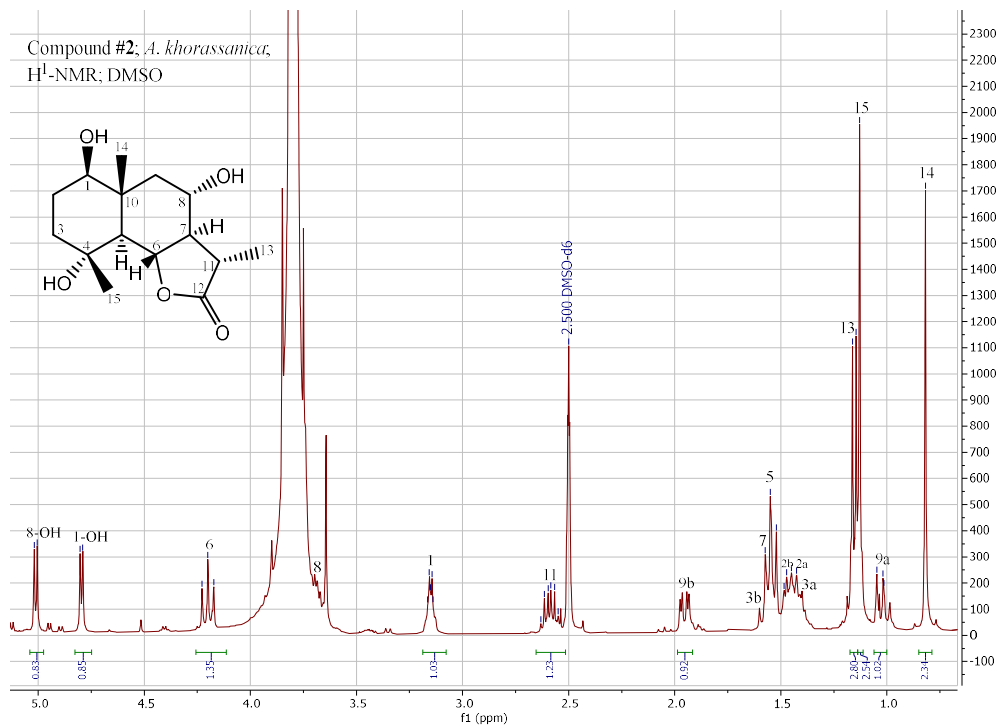


Figure S.11  $^1\text{H-NMR}$  spectrum of Compound 2

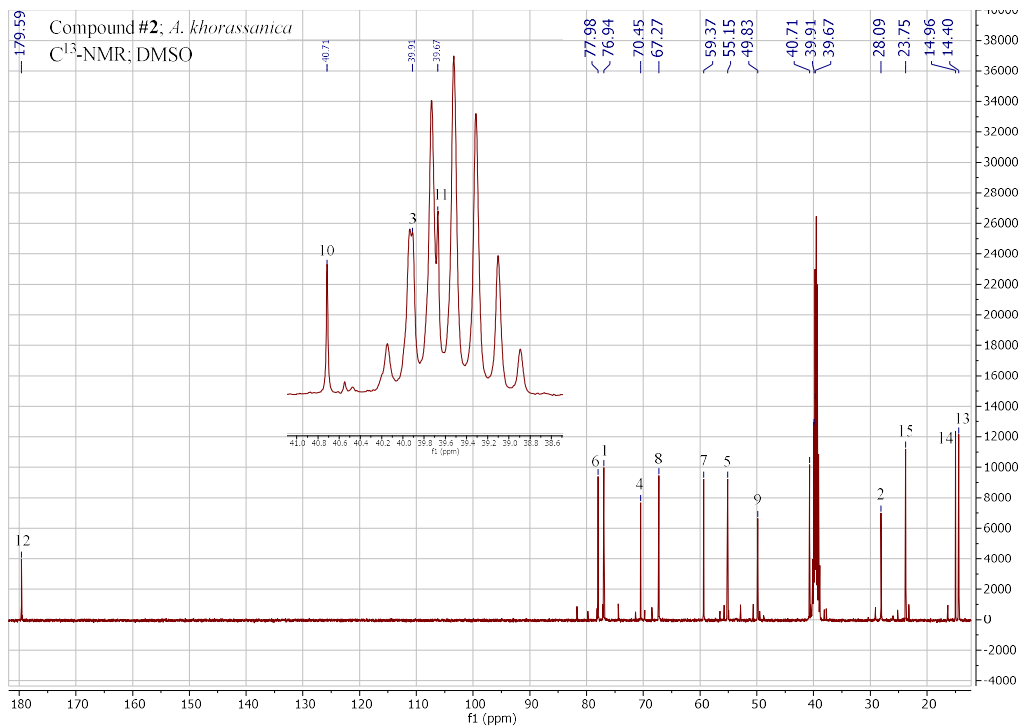


Figure S.12  $^{13}\text{C-NMR}$  spectrum of Compound 2

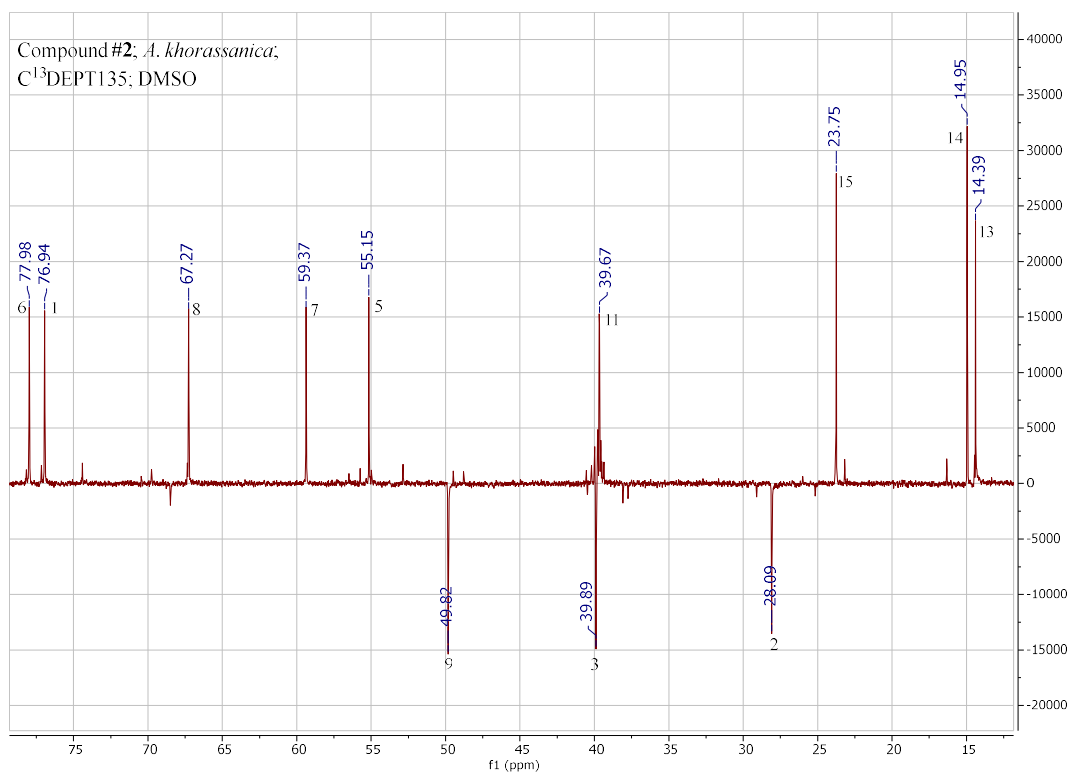


Figure S.13 DEPT135 spectrum of Compound 2

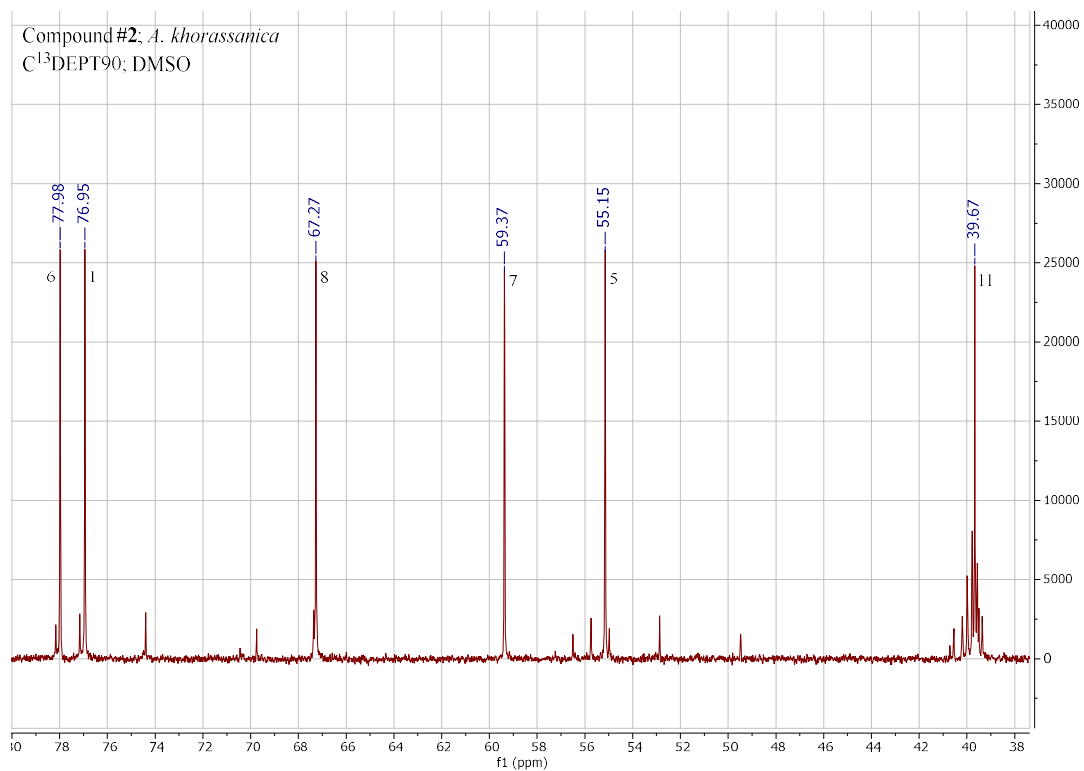


Figure S.14 DEPT90 spectrum of Compound 2

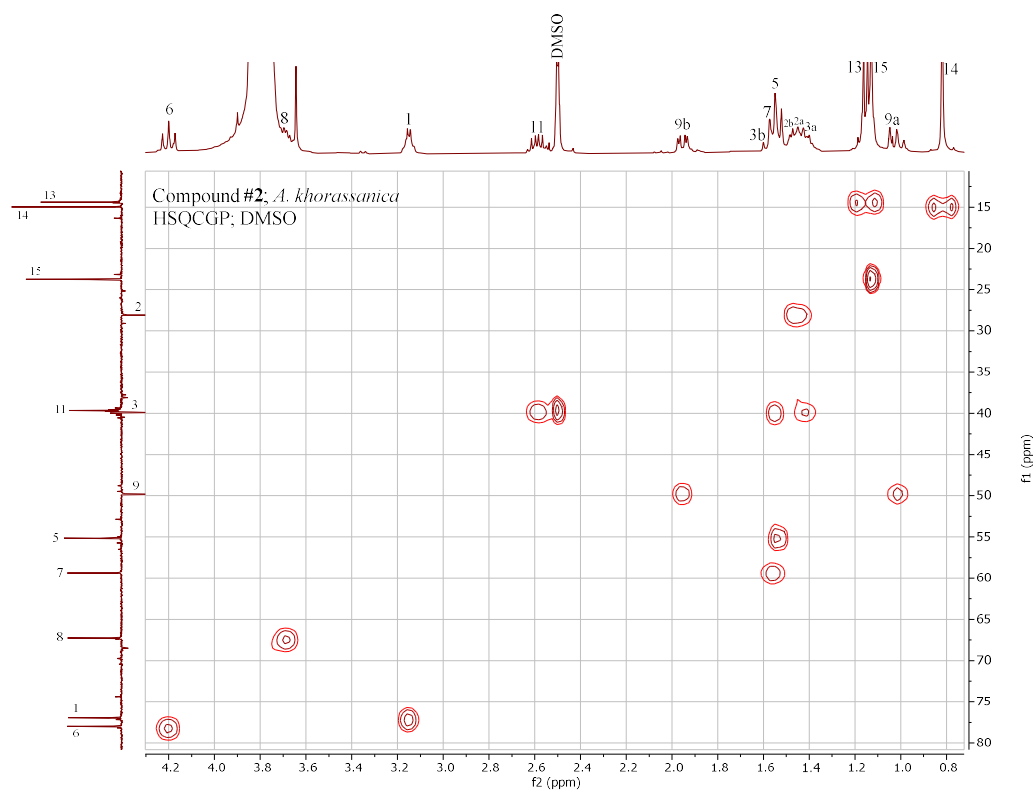


Figure S.15 HSQC spectrum of Compound 2

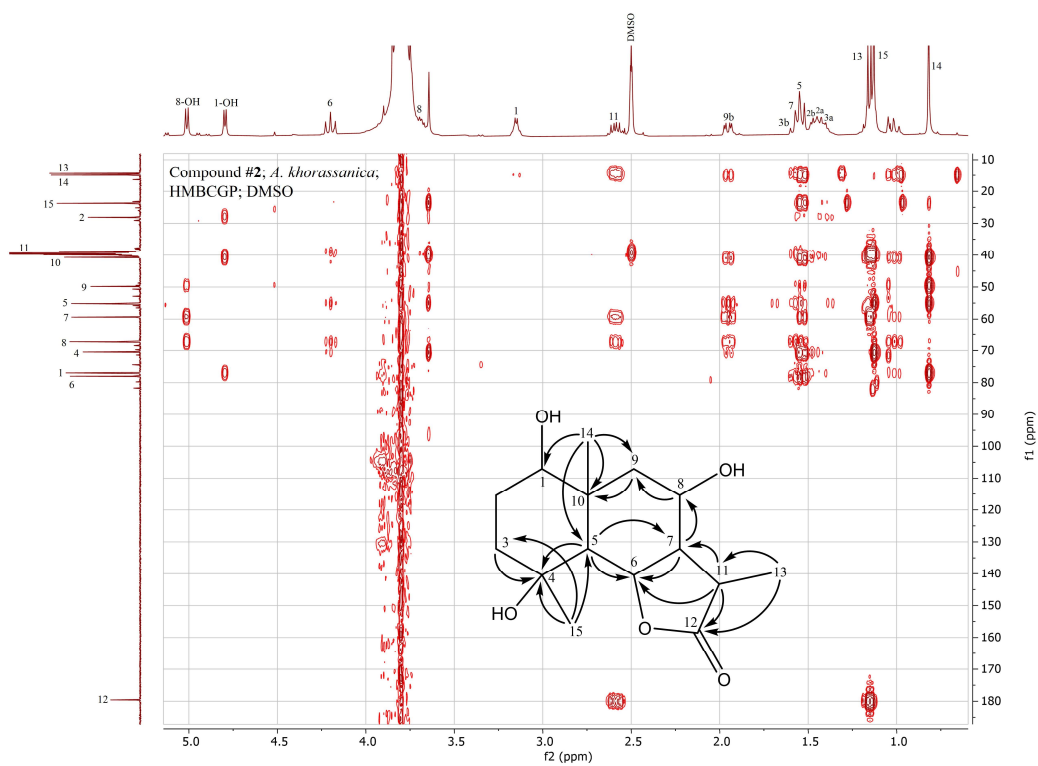


Figure S.16 HMBC spectrum of Compound 2

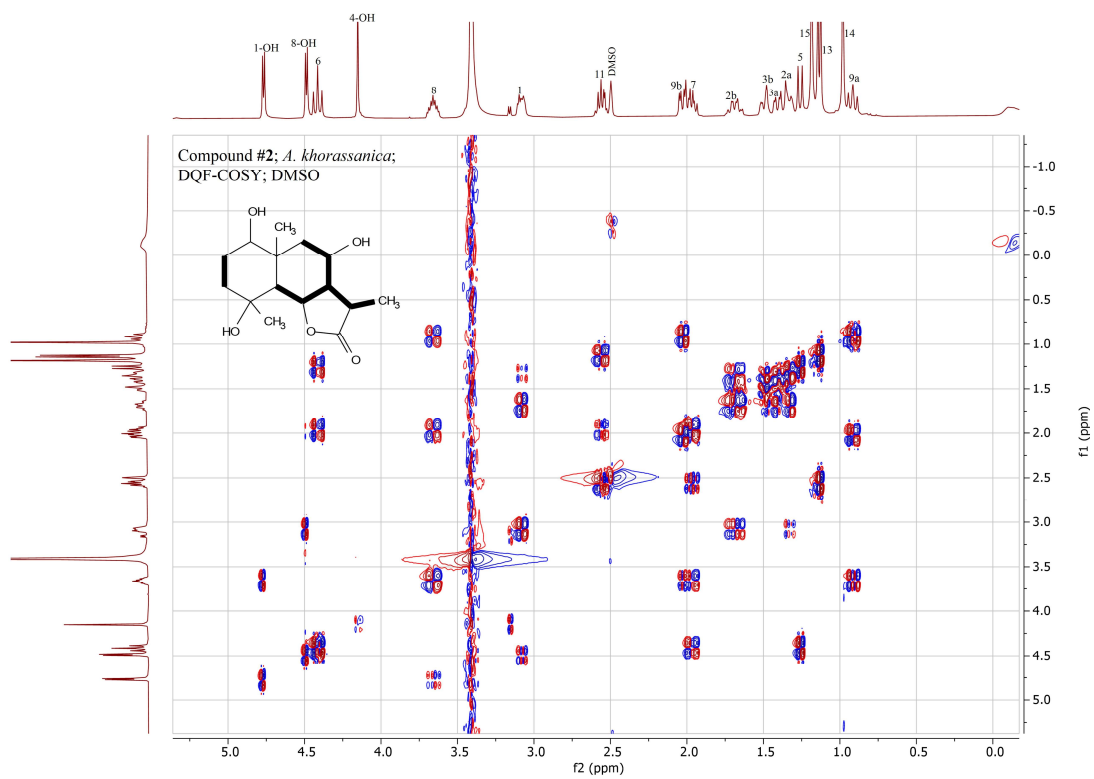


Figure S.17 DQF-COSY spectrum of Compound 2

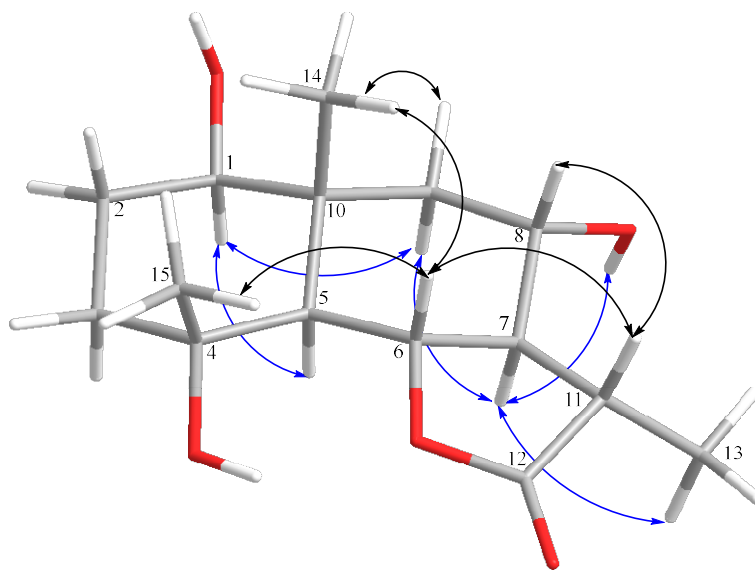


Figure S.18 Representation of key NOESY correlations of Compound 2

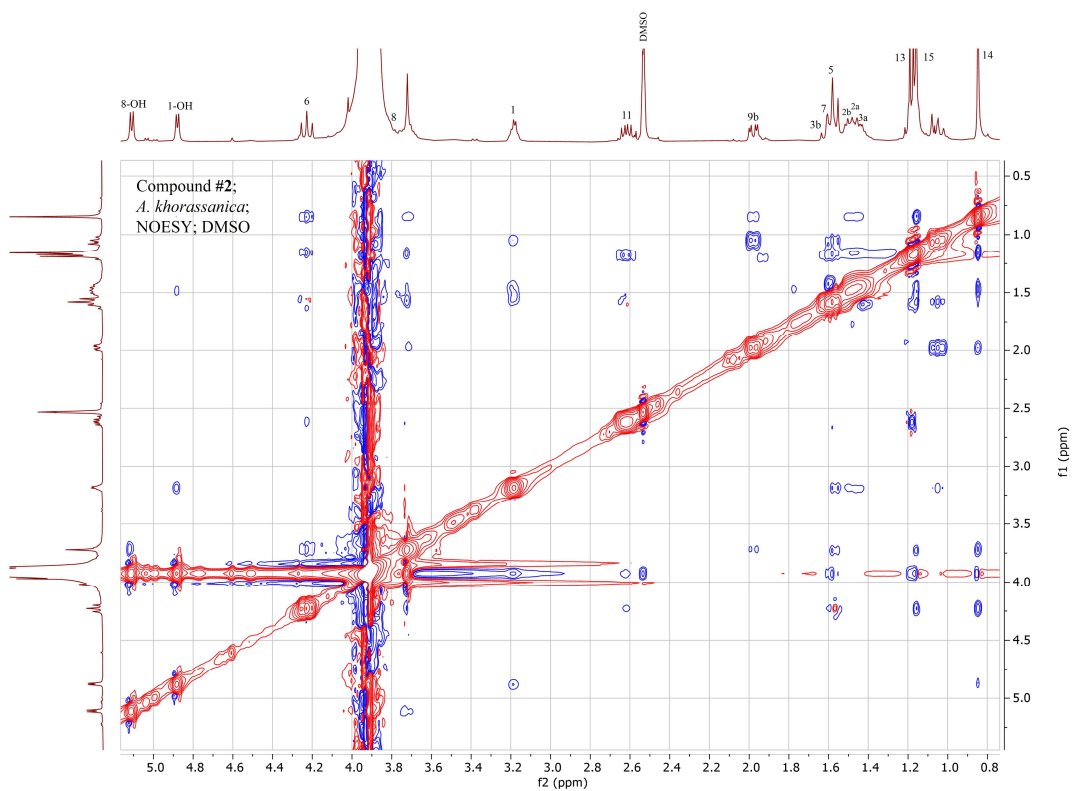


Figure S.19 NOESY spectrum of Compound 2

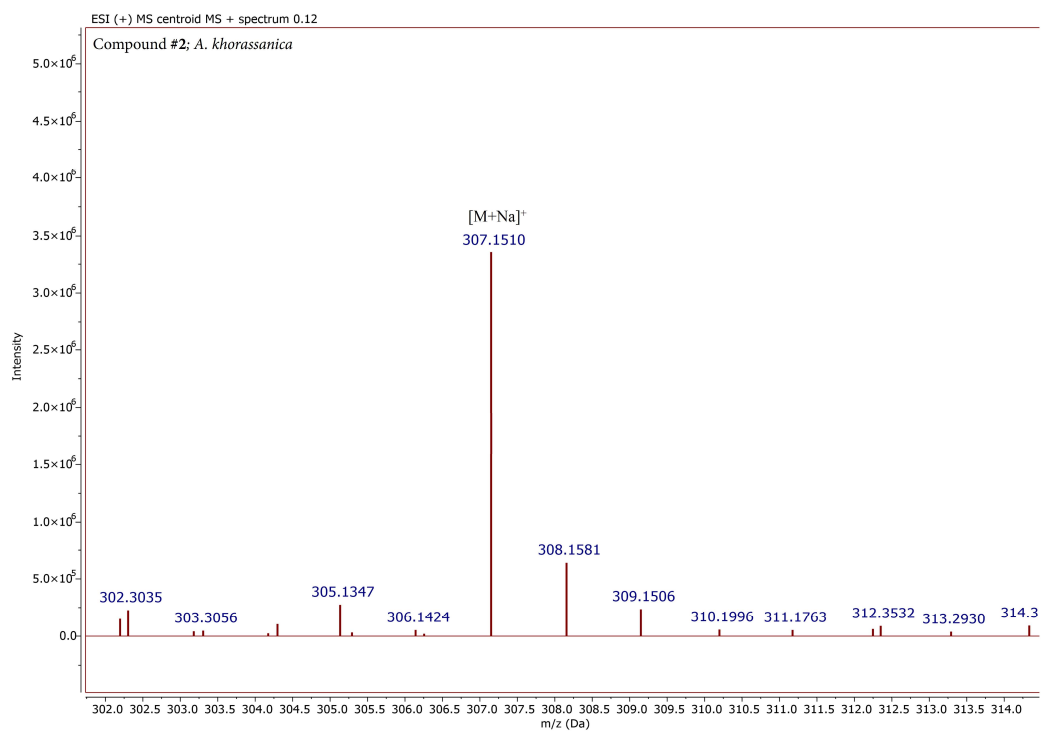
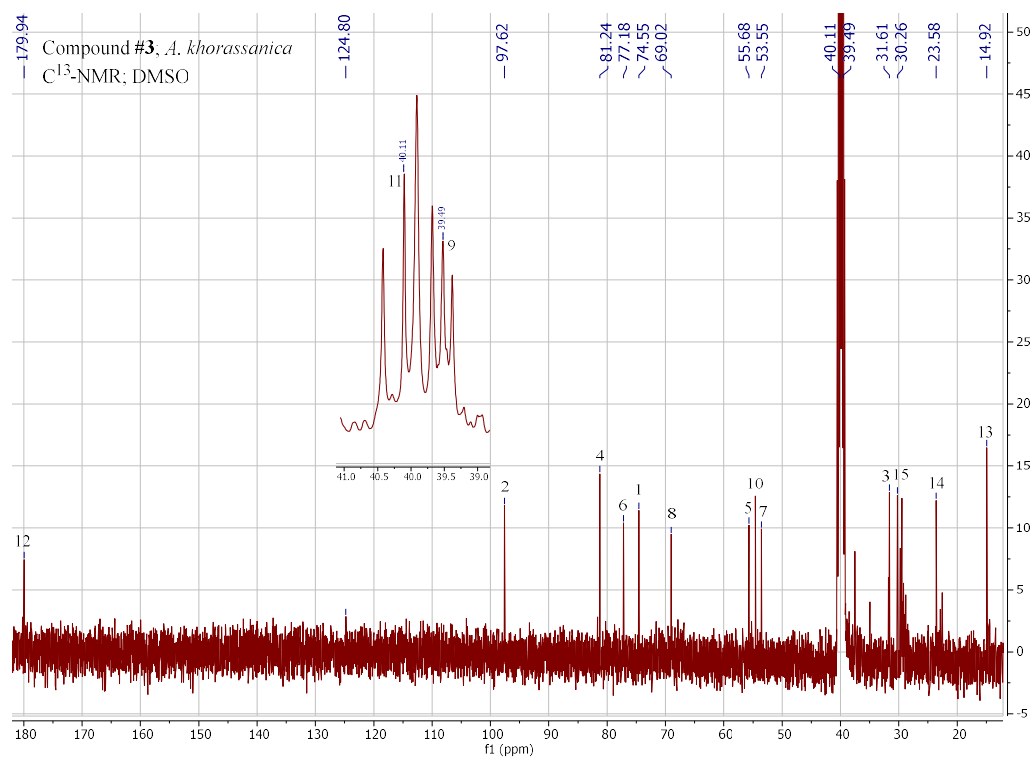
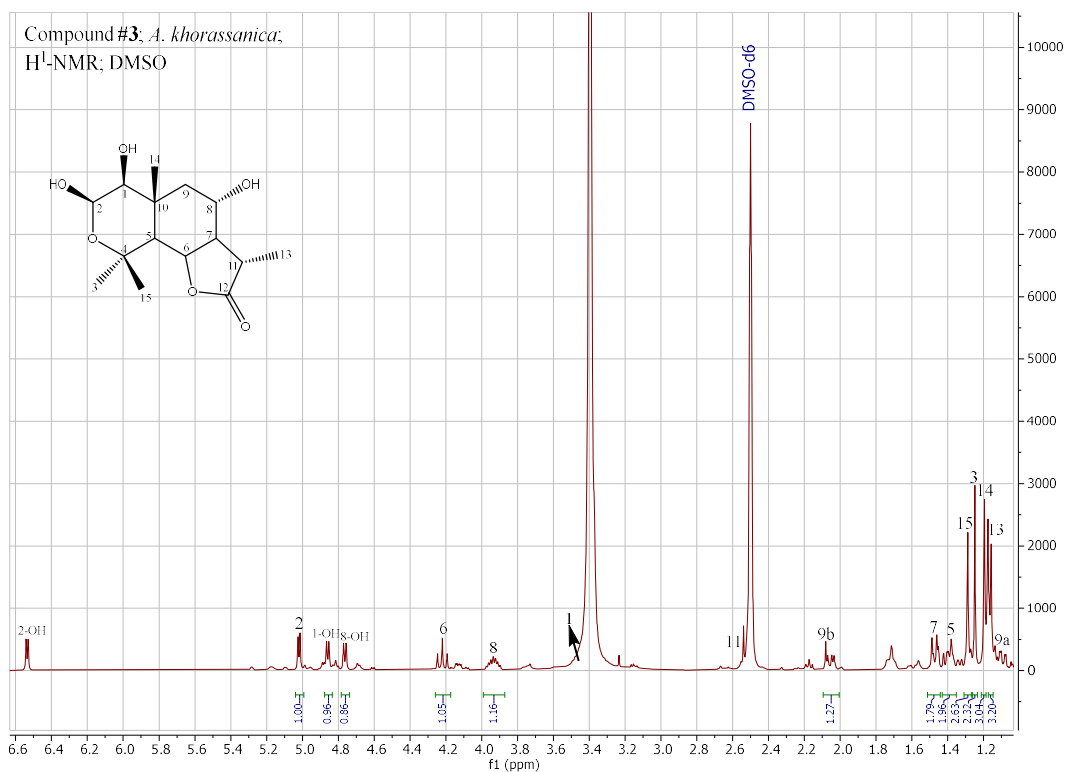


Figure S.20 HR-ESI-Mass spectrum of Compound 2



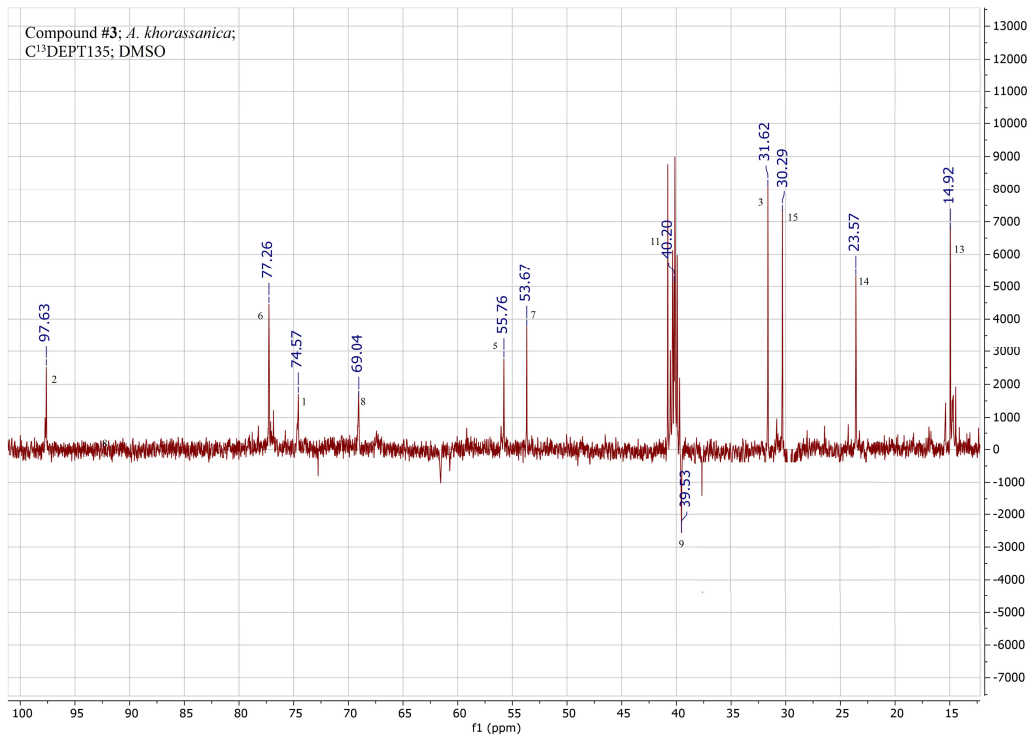


Figure S.23 DEPT135 spectrum of Compound 3

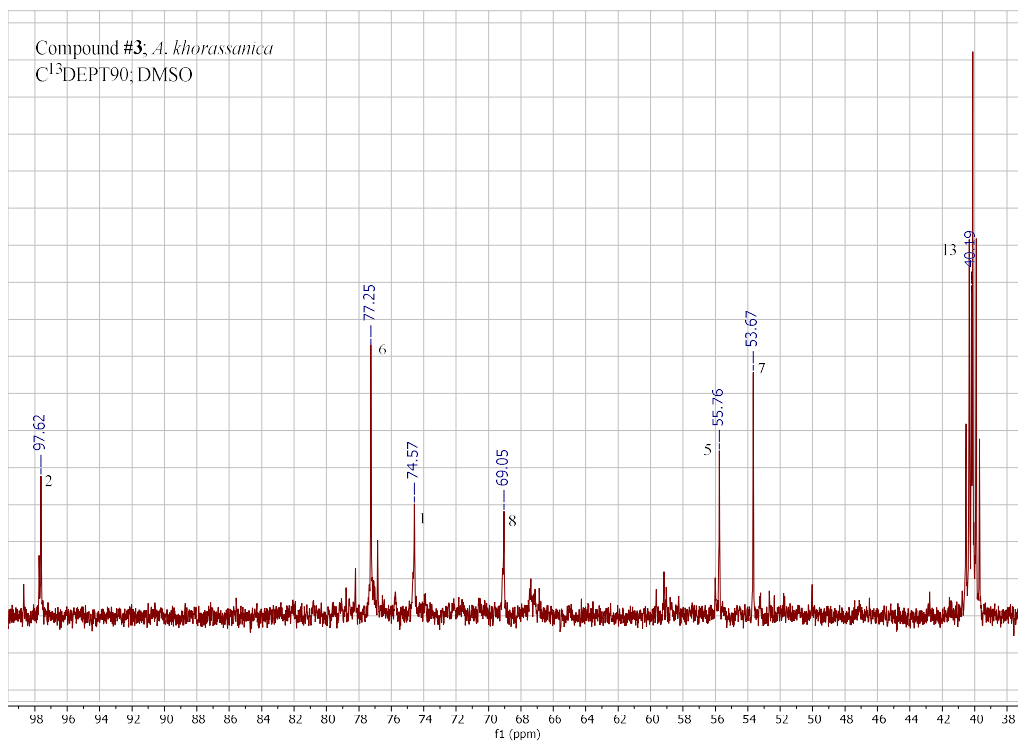


Figure S.24 DEPT90 spectrum of Compound 3

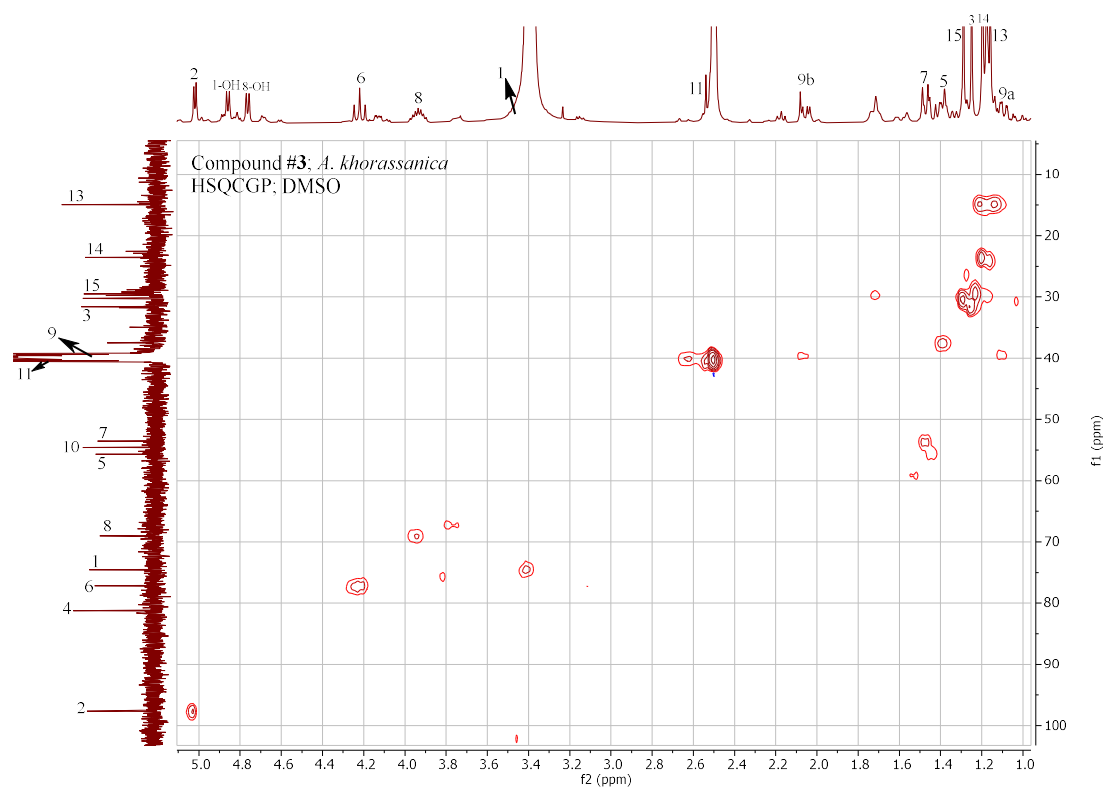


Figure S.25 HSQC spectrum of Compound 3

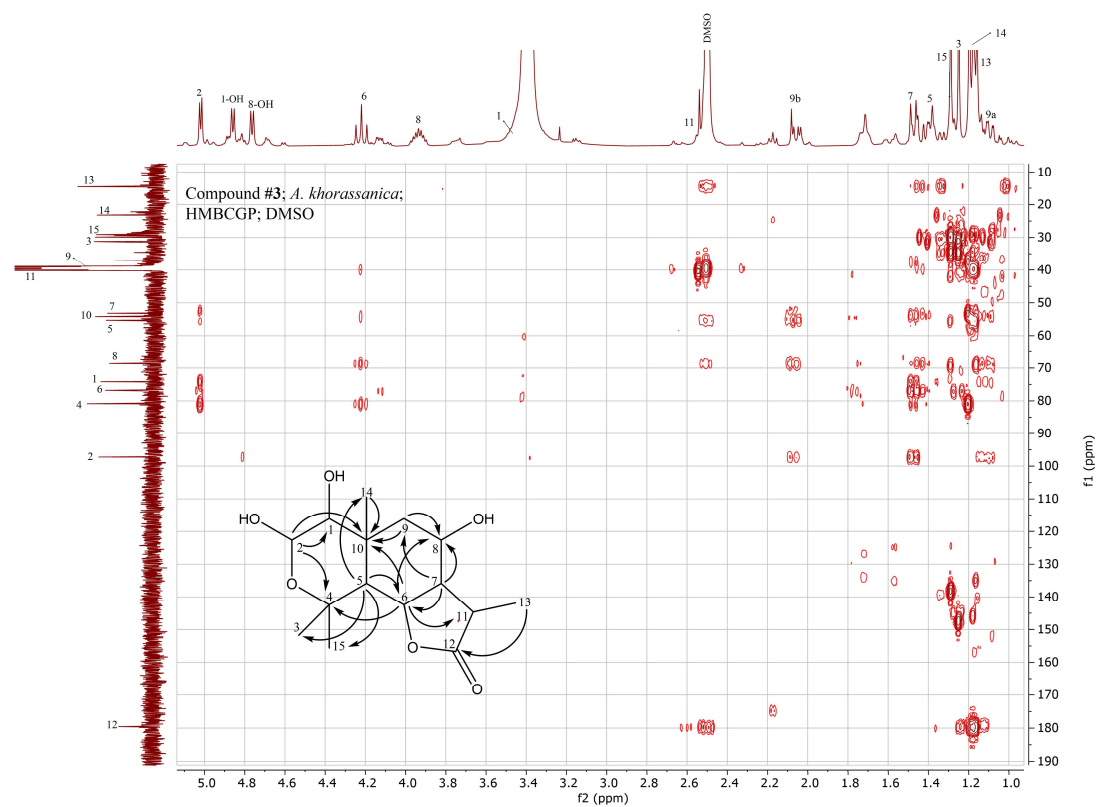


Figure S.26 HMBC spectrum of Compound 3



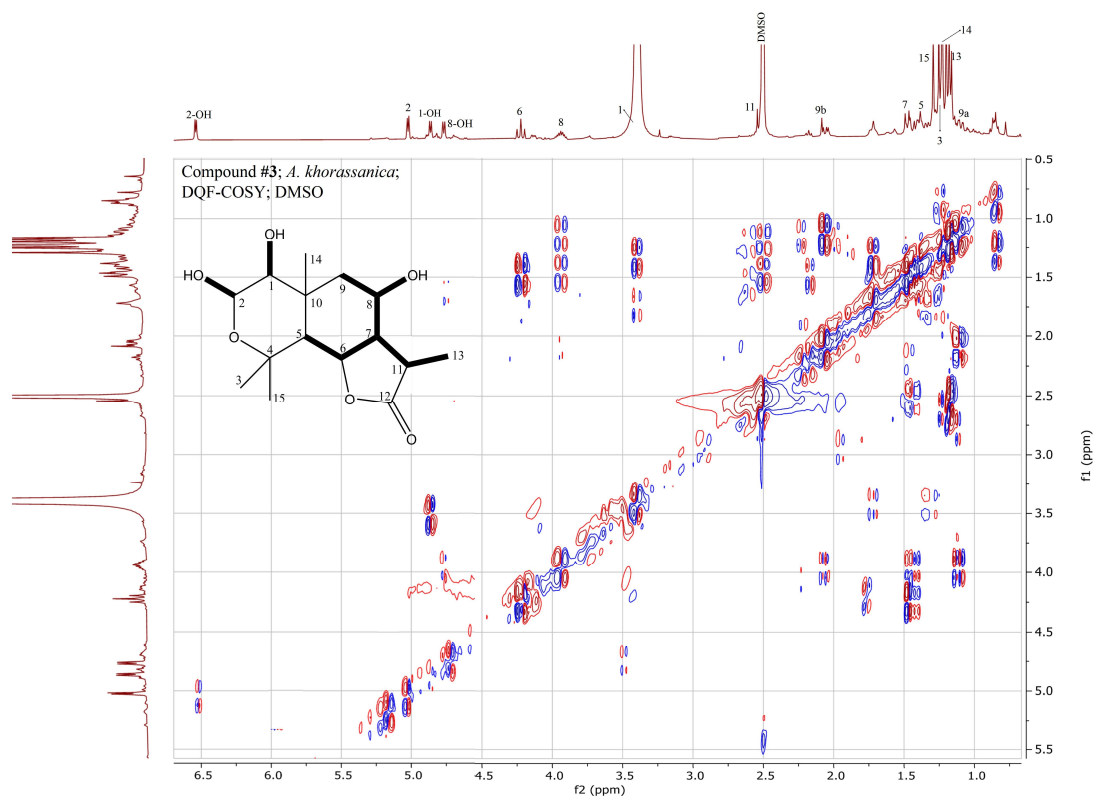


Figure S.27 DQF-COSY spectrum of Compound 3

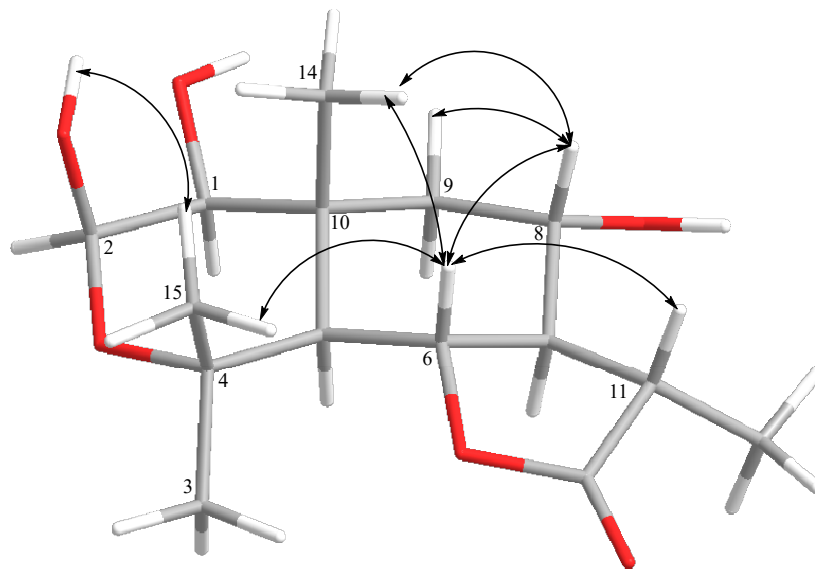


Figure S.28 Representation of key NOESY correlations of Compound 3

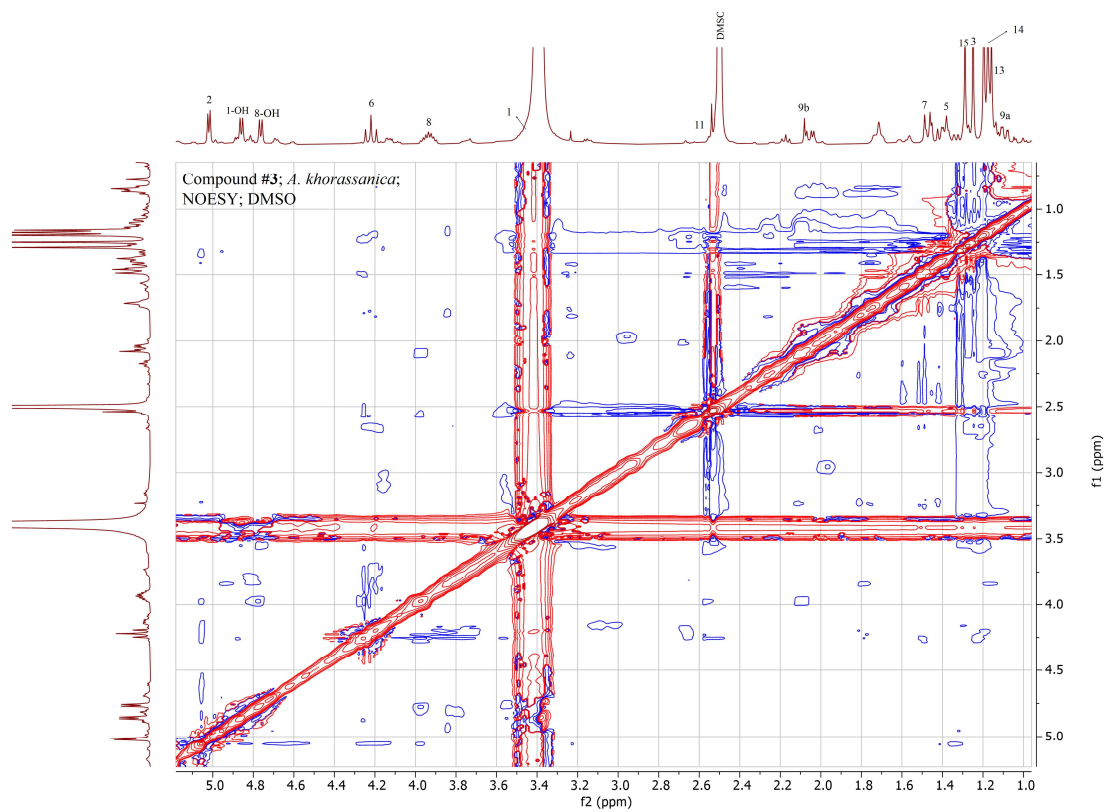


Figure S.29 NOESY spectrum of Compound 3

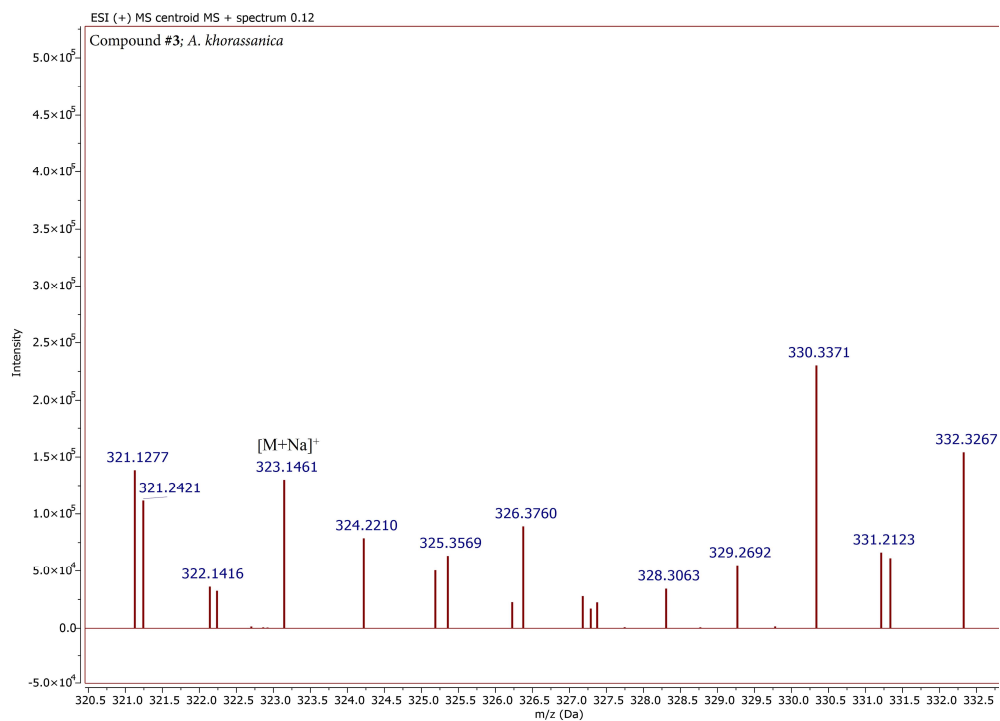


Figure S.30 HR-ESI-Mass spectrum of Compound 3

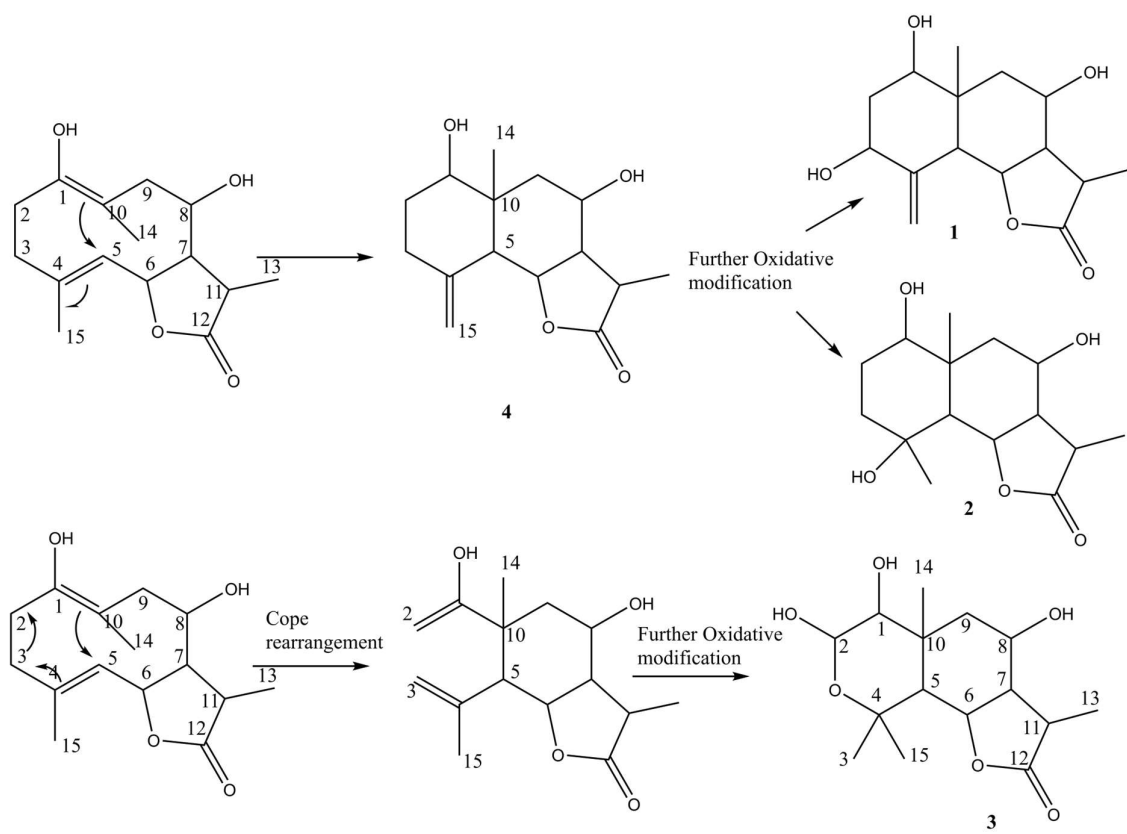
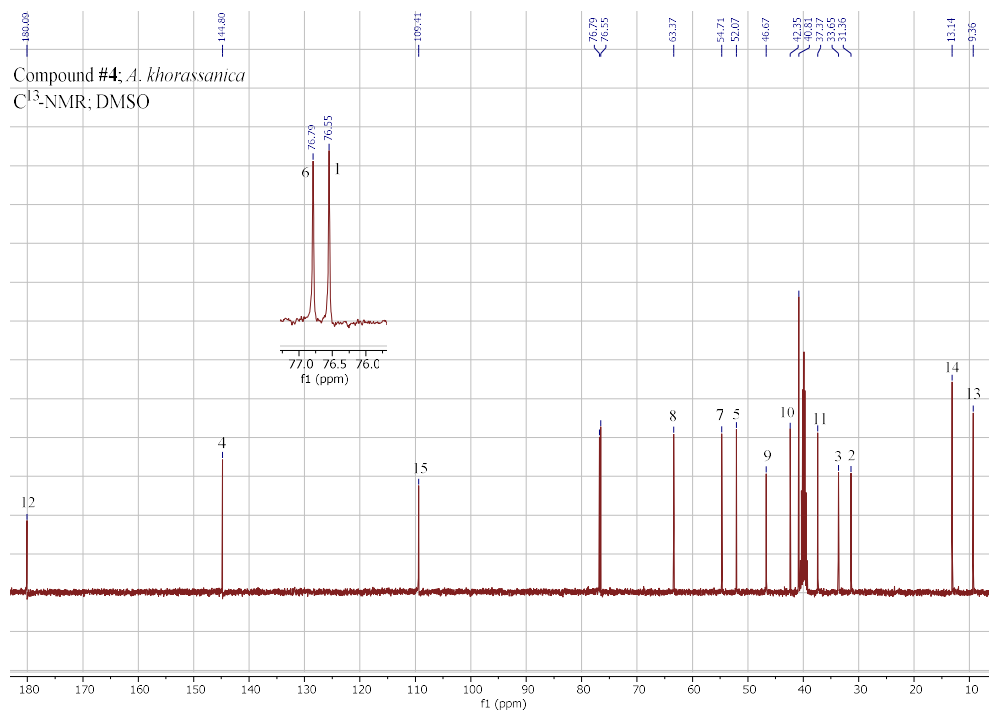
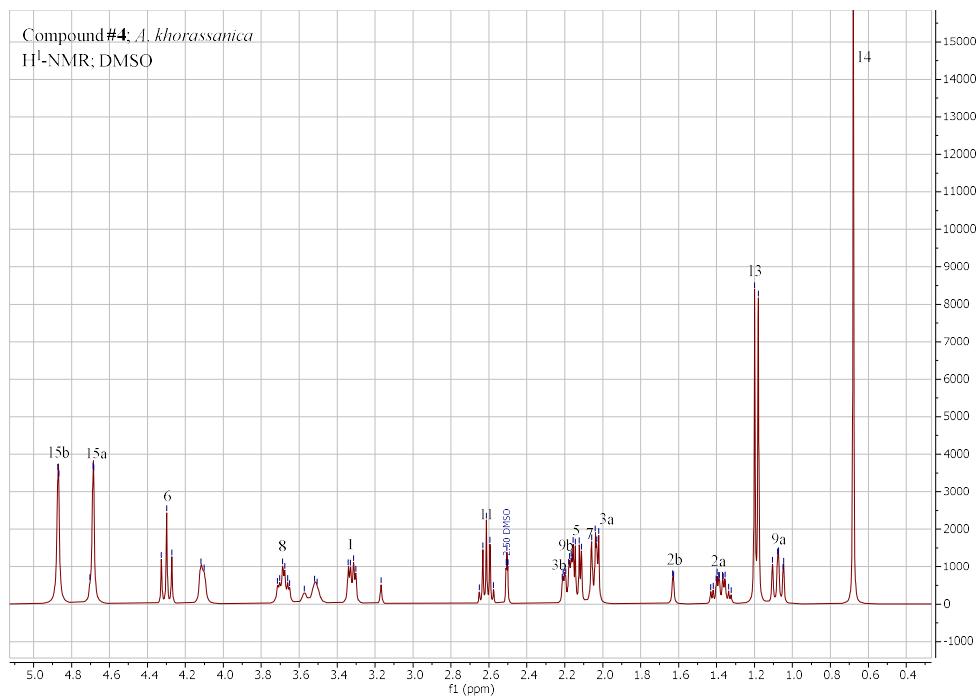


Figure S.31 Possible biosynthetic pathway of isolated compounds (1-4)



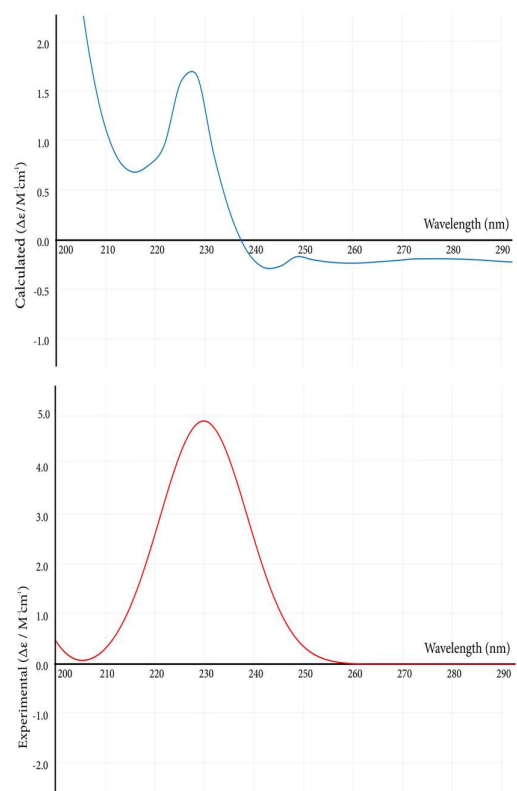


Figure S.344 Experimental (red) and calculated (blue) ECD spectra of **2**. Calculated spectrum was obtained using the time-dependent density function theory (TDDFT) method at B3LYP/3-21G\*\* in MeOH using the SCRf (self-consistent reaction field) method.

Table S.1  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of compounds **1-3** ( $\text{CDCl}_3$ , 400 MHz for  $\delta_{\text{H}}$ ; 100 MHz for  $\delta_{\text{C}}$ )<sup>a</sup>

Position	Compound 1		Compound 2		Compound 3	
	$\delta_{\text{H}}$ (mult., <i>J</i> in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (mult., <i>J</i> in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (mult., <i>J</i> in Hz)	$\delta_{\text{C}}$
1	3.65 (dt, 9.8, 4.4)	72.45	3.15 (dt, 9.3, 5.0)	76.94	3.4 (s)	74.55
2a	1.48 (ddd, 13.5, 9.8, 4.4)	38.85	1.45 (m) <sup>b</sup>	28.09	5.02 (d, 4.5)	97.62
2b	1.75 (ddd, 13.6, 4.4, 2.5)	38.85	1.47 (m)	28.09	-	-
3a	4.10 (bdd, 5.6, 2.5)	71.79	1.41 (m)	39.91	1.24 (s)	31.61
3b	-	-	1.60 (m)	39.91	-	-
4	-	147.25	-	70.45	-	81.00
5	2.46 (bd, 10.9)	46.40	1.54 (d, 11.0)	55.15	1.43 (m)	55.68
6	4.16 (t, 10.9)	76.77	4.21 (t, 11.0)	77.98	4.21 (dd, 10.8, 10.8)	77.18
7	1.61 (dt, 12.1, 10.9)	58.70	1.57 (m)	59.37	1.47 (m)	53.55
8	3.76 (tt, 10.9, 4.5)	64.44	3.69 (tt, 10.9, 4.8)	67.27	3.93 (tt, 10.4, 5.3)	69.02
9a	1.11 (dd, 13.0, 10.8)	47.04	1.01 (dd, 12.1, 4.1)	49.82	1.08 (dd, 13.2, 11.6)	39.50
9b	2.07 (dd, 13.0, 4.2)	47.04	1.97 (dd, 12.1, 4.1)	49.82	2.08 (dd, 13.2, 4.8)	39.50
10	-	42.52	-	40.71	-	54.58
11	2.66 (dq, 12.1, 6.8)	40.50	2.58 (dq, 13.8, 7.0)	39.67	2.60 (m)	40.12
12	-	179.94	-	179.59	-	180.00
13	1.19 (d, 7.60)	14.70	1.14 (d, 7.0)	14.40	1.17 (d, 7.0)	14.92
14	0.65 (s)	12.33	0.81 (s)	14.96	1.19 (s)	23.58
15a	4.70 (s)	110.48	1.13 (s)	23.75	1.28 (s)	30.26
15b	4.99 (s)	110.48	-	-	-	-
1-OH	4.60 (d, 5.3)	-	4.62 (d, 4.6)	-	4.86 (d, 5.2)	-
2-OH	-	-	-	-	6.54 (d, 4.3)	-
3-OH	4.88 (bdd, 5.6, 2.5)	-	-	-	-	-
4-OH	-	-	3.16 (s)	-	-	-
8-OH	4.82 (d, 5.6)	-	4.79 (d, 4.8)	-	4.76 (d, 5.6)	-

<sup>a</sup>  $\delta$  values were established from HMBC, COSY, and HSQC.<sup>b</sup> overlapped with other signals.