

# SUPPLEMENTARY MATERIAL

## Two new sesquiterpenes from the rhizomes of *Atractylodes macrocephala* and their biological activities

Yi-Nan Zhao<sup>a,#</sup>, Gai Gao<sup>a,#</sup>, Jin-Lian Ma<sup>a</sup>, Rui-Zhu Xu<sup>a</sup>, Tao Guo<sup>c</sup>, Li-Min Wu<sup>a</sup>, Xin-Guang Liu<sup>a</sup>, Zhi-Shen Xie<sup>a</sup>, Jiang-Yan Xu<sup>a</sup>, Zhen-Qiang Zhang<sup>a,\*</sup> and Pan Wang<sup>a,b,\*</sup>

<sup>a</sup> Academy of Chinese Medical Sciences, Henan University of Chinese Medicine, Zhengzhou, China; <sup>b</sup> Zhongjing Wanxi Pharmaceutical Co., Ltd, Zhengzhou, China; <sup>c</sup> College of Pharmacy, Henan University of Chinese Medicine, Zhengzhou, China

\*Zhen-Qiang Zhang [zhang\\_zhenqiang@126.com](mailto:zhang_zhenqiang@126.com); \*Pan Wang [w.p1008@hotmail.com](mailto:w.p1008@hotmail.com)

# Yi-Nan Zhao and Gai Gao contributed equally to this work.

**Abstract:** Two new sesquiterpenes, named selina-4(14),7,11-trien-9-ol (**1**) and selina-4(14),11-dien-7-ol (**2**), along with two known compounds were isolated from rhizomes of *Atractylodes macrocephala* Koidz. All structures were assigned on the basis of detailed spectroscopic analyses. The absolute configuration of **1** was established by TDDFT-ECD calculations. Compound **1** was found to moderately inhibit LSD1 activity with IC<sub>50</sub> value of 34.0 μM. Compounds **1** and **4** exhibited a regulate effect on Keap1-Nrf2-ARE pathway.

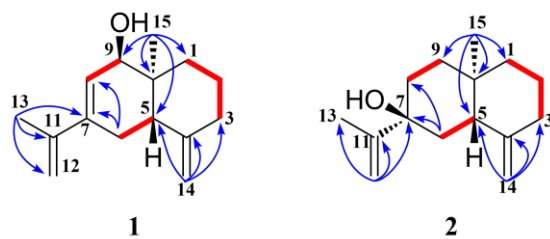
**Keywords:** *Atractylodes macrocephala*; sesquiterpenes; LSD1; Keap1-Nrf2-ARE

	<b>Table of contents</b>	1
<b>Table S1</b>	<sup>1</sup> H (500 MHz) and <sup>13</sup> C (125 MHz) NMR data for <b>1</b> in methanol- <i>d</i> <sub>4</sub> and <b>2</b> in CDCl <sub>3</sub> ( $\delta$ in ppm, <i>J</i> in Hz)	2
<b>Figure S1</b>	<sup>1</sup> H- <sup>1</sup> H COSY (bold red bonds) and key HMBC (blue arrows) correlations of <b>1</b> and <b>2</b>	3
<b>Figure S2</b>	Key NOESY correlations for <b>1</b> and <b>2</b>	3
<b>Figure S3</b>	Experimental and calculated ECD spectra of <b>1</b>	3
<b>Figure S4</b>	Compounds <b>1</b> and <b>4</b> activated Nrf2-ARE (n = 3)	4
<b>Figure S5</b>	Compounds <b>1</b> , <b>3</b> , and <b>4</b> docking with Keap1	4
<b>Scheme S1</b>	Proposed biosynthesis of the sesquiterpenes	4
<b>Figure S6</b>	UV spectrum of compound <b>1</b>	5
<b>Figure S7</b>	HRESIMS spectrum of compound <b>1</b>	5
<b>Figure S8</b>	<sup>1</sup> H NMR (500 MHz) spectrum of compound <b>1</b> in methanol- <i>d</i> <sub>4</sub>	6
<b>Figure S9</b>	<sup>13</sup> C NMR (125 MHz) spectrum of compound <b>1</b> in methanol- <i>d</i> <sub>4</sub>	6
<b>Figure S10</b>	DEPT135 (125 MHz) spectrum of compound <b>1</b> in methanol- <i>d</i> <sub>4</sub>	7
<b>Figure S11</b>	<sup>1</sup> H- <sup>1</sup> H COSY (500 MHz) spectrum of compound <b>1</b> in methanol- <i>d</i> <sub>4</sub>	7
<b>Figure S12</b>	HSQC (500 MHz) spectrum of compound <b>1</b> in methanol- <i>d</i> <sub>4</sub>	8
<b>Figure S13</b>	HMBC (500 MHz) spectrum of compound <b>1</b> in methanol- <i>d</i> <sub>4</sub>	8
<b>Figure S14</b>	NOESY (500 MHz) spectrum of compound <b>1</b> in methanol- <i>d</i> <sub>4</sub>	9
<b>Figure S15</b>	UV spectrum of compound <b>2</b>	10
<b>Figure S16</b>	HRESIMS spectrum of compound <b>2</b>	10
<b>Figure S17</b>	<sup>1</sup> H NMR (500 MHz) spectrum of compound <b>2</b> in CDCl <sub>3</sub>	11
<b>Figure S18</b>	<sup>13</sup> C NMR (125 MHz) spectrum of compound <b>2</b> in CDCl <sub>3</sub>	11
<b>Figure S19</b>	DEPT135 (125 MHz) spectrum of compound <b>2</b> in CDCl <sub>3</sub>	12
<b>Figure S20</b>	<sup>1</sup> H- <sup>1</sup> H COSY (500 MHz) spectrum of compound <b>2</b> in CDCl <sub>3</sub>	12
<b>Figure S21</b>	HSQC (500 MHz) spectrum of compound <b>2</b> in CDCl <sub>3</sub>	13
<b>Figure S22</b>	HMBC (500 MHz) spectrum of compound <b>2</b> in CDCl <sub>3</sub>	13
<b>Figure S23</b>	NOESY (500 MHz) spectrum of compound <b>2</b> in CDCl <sub>3</sub>	14

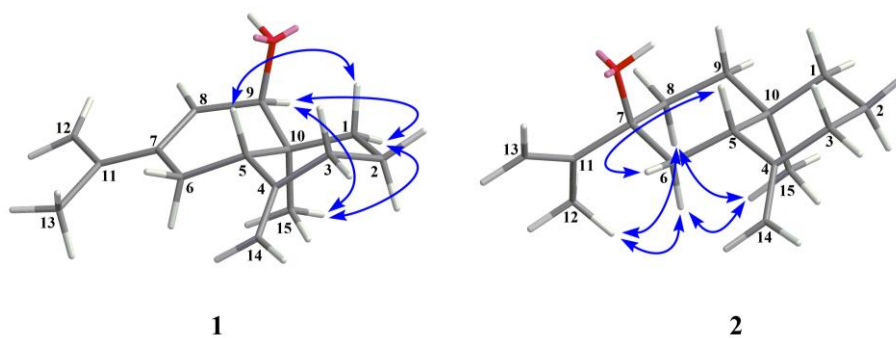
**Table S1.**  $^1\text{H}$  (500 MHz) and  $^{13}\text{C}$  (125 MHz) NMR data for **1** in methanol- $d_4$  and **2** in  $\text{CDCl}_3$  ( $\delta$  in ppm,  $J$  in Hz)<sup>a</sup>

Position	<b>1</b>			<b>2</b>			
		$\delta_{\text{H}}$	$\delta_{\text{C}}$	mult	$\delta_{\text{H}}$	$\delta_{\text{C}}$	mult
1	$\beta$	2.01 m	34.0	$\text{CH}_2$	1.46 m	41.5	$\text{CH}_2$
	$\alpha$	1.29 br d (13.0)			1.40 dd (12.0, 6.0)		
2	$\beta$	1.74 m	23.3	$\text{CH}_2$	1.63 m	23.5	$\text{CH}_2$
	$\alpha$	1.60 m			1.63 m		
3	$\beta$	2.38 m	36.8	$\text{CH}_2$	2.34 m	36.9	$\text{CH}_2$
	$\alpha$	2.05 m			2.07 m		
4			150.2	C		150.8	C
5		2.36 m	38.1	CH	2.32 m	44.1	CH
6	$\beta$	2.32 dd (16.9, 12.0)	25.8	$\text{CH}_2$	1.71 dd (13.0, 4.8)	34.2	$\text{CH}_2$
	$\alpha$	2.15 dd (16.9, 4.8)			1.49 m		
7			138.0	C		74.5	C
8	$\beta$	5.95 d (5.0)	123.4	CH	1.85 m	31.1	$\text{CH}_2$
	$\alpha$				1.47 m		
9	$\beta$	3.62 d (5.0)	72.5	CH	1.67 m	36.2	$\text{CH}_2$
	$\alpha$				1.34 m		
10			38.2	C		35.6	C
11			143.1	C		152.6	C
12	a	5.19 s	111.2	$\text{CH}_2$	5.08 s	108.8	$\text{CH}_2$
	b	5.00 s			4.84 s		
13		1.96 s	19.5	$\text{CH}_3$	1.86 s	19.1	$\text{CH}_3$
14	a	4.87 s	105.9	$\text{CH}_2$	4.73 s	105.1	$\text{CH}_2$
	b	4.70 s			4.41 s		
15		0.68 s	15.7	$\text{CH}_3$	0.74 s	15.3	$\text{CH}_3$

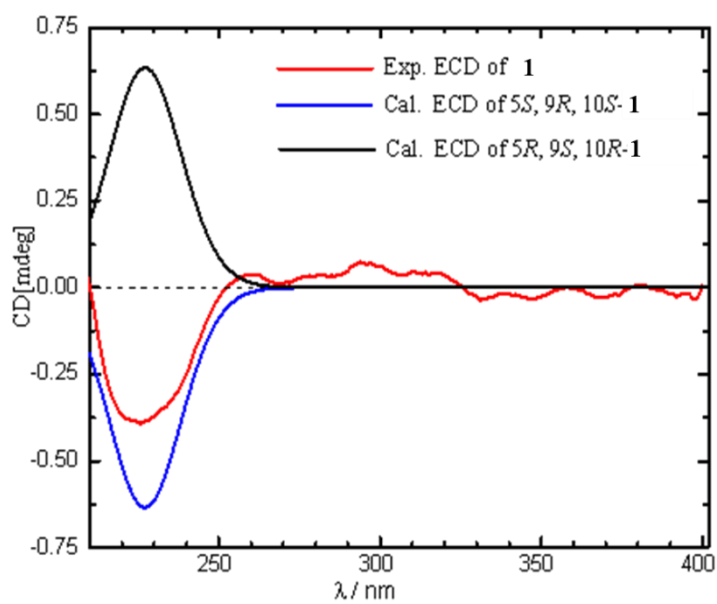
<sup>a</sup> overlapped signals assigned by  $^1\text{H}$ - $^1\text{H}$  COSY, HSQC, and HMBC spectra without designating multiplicity.



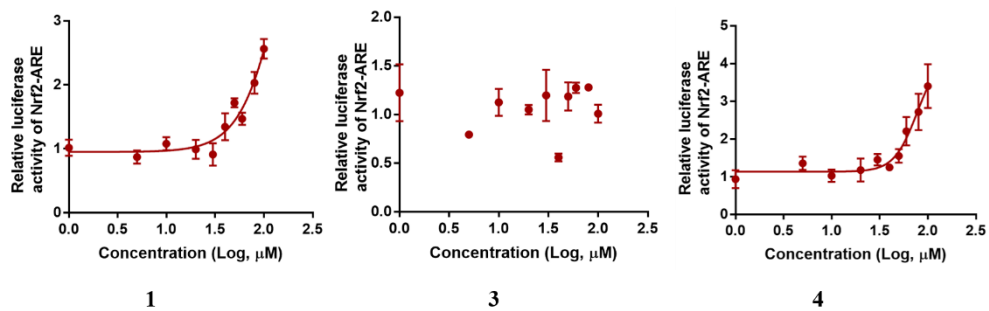
**Figure S1.**  $^1\text{H}$ - $^1\text{H}$  COSY (bold red bonds) and key HMBC (blue arrows) correlations of **1** and **2**.



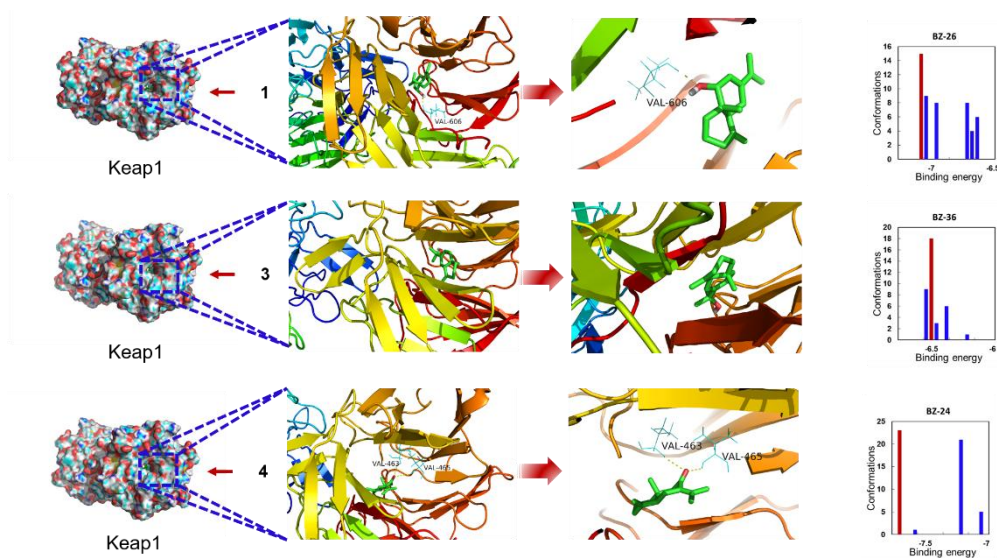
**Figure S2.** Key NOESY correlations for **1** and **2**.



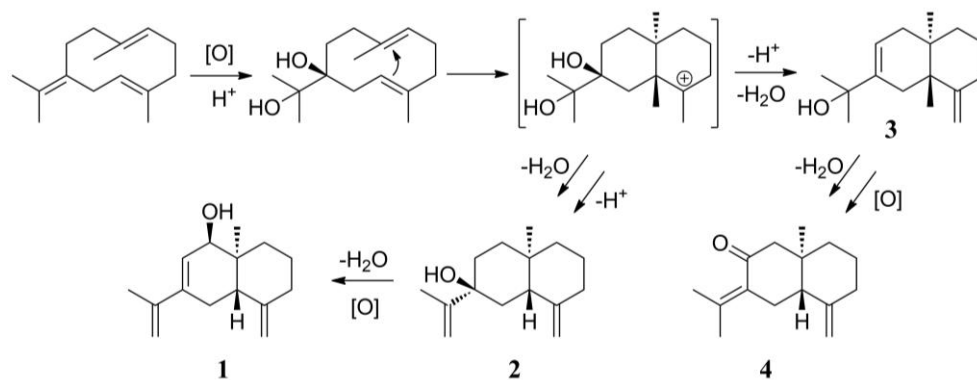
**Figure S3.** Experimental and calculated ECD spectra of **1**.



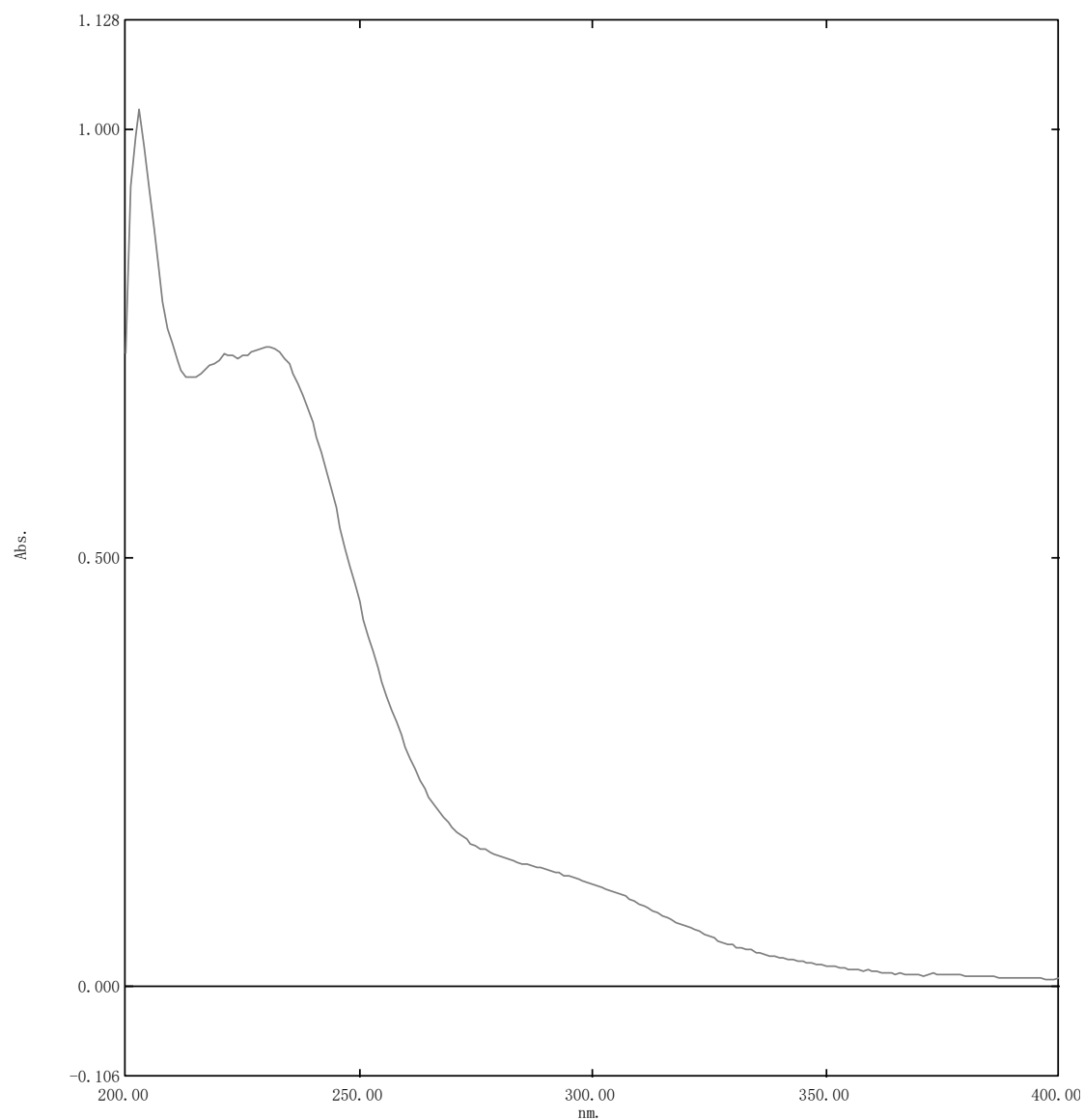
**Figure S4.** Compounds **1** and **4** activated Nrf2-ARE (n = 3).



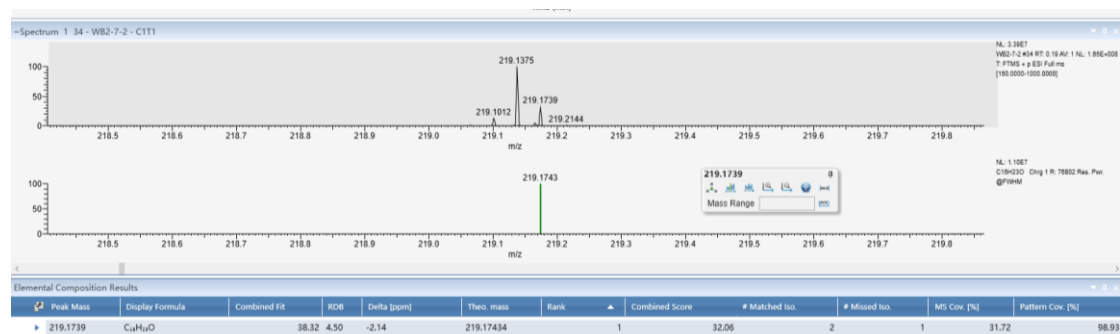
**Figure S5.** Compounds **1**, **3**, and **4** docking with Keap1.



**Scheme S1.** Proposed biosynthesis of the sesquiterpenes.

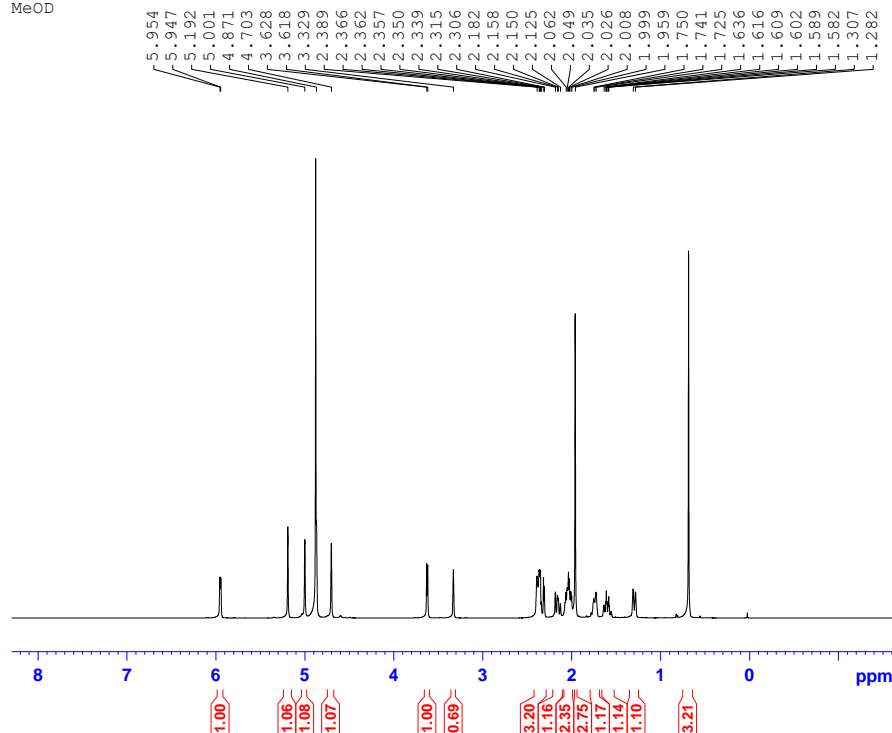


**Figure S6** UV spectrum of compound **1**



**Figure S7** HRESIMS spectrum of compound **1**

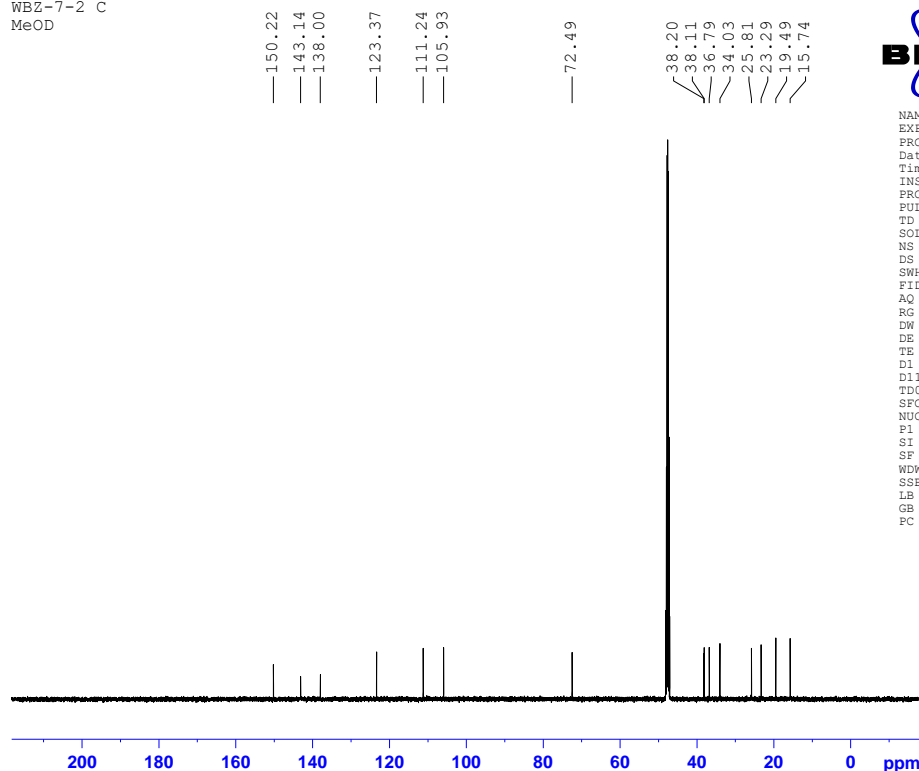
WBZ-7-2 H  
MeOD



```
NAME WBZ-7-2
EXPNO 1
PROCNO 1
Date_ 20190911
Time_ 10.41 h
INSTRUM spect
PROBHD Z119470_0014 (
PULPROG zg30
TD 65536
SOLVENT MeOD
NS 16
DS 2
SWH 10000.000 Hz
FIDRES 0.305176 Hz
AQ 3.2768500 sec
RG 71.37
DW 50.000 usec
DE 6.50 usec
TE 298.1 K
D1 1.00000000 sec
TDO 1
SFO1 500.1930887 MHz
NUC1 1H
P1 11.86 usec
SI 65536
SF 500.1900000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
```

Figure S8  $^1\text{H}$  NMR (500 MHz) spectrum of compound **1** in methanol- $d_4$

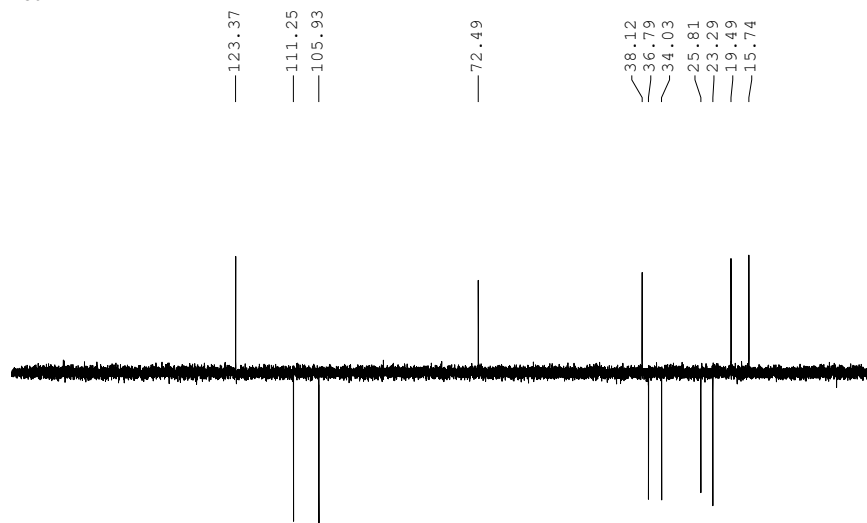
WBZ-7-2 C  
MeOD



```
NAME WBZ-7-2
EXPNO 2
PROCNO 1
Date_ 20190920
Time_ 11.04 h
INSTRUM spect
PROBHD Z119470_0014 (
PULPROG zgpg30
TD 65536
SOLVENT MeOD
NS 97
DS 4
SWH 29761.904 Hz
FIDRES 0.908261 Hz
AQ 1.1010548 sec
RG 202.63
DW 16.800 usec
DE 6.50 usec
TE 298.2 K
D1 2.00000000 sec
D11 0.03000000 sec
TDO 1
SFO1 125.7854528 MHz
NUC1 13C
P1 9.45 usec
SI 32768
SF 125.7728760 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
```

Figure S9  $^{13}\text{C}$  NMR (125 MHz) spectrum of compound **1** in methanol- $d_4$

WBZ-7-2 DEPT135  
MeOD



```
NAME WBZ-7-2
EXPNO 3
PROCNO 1
Date_ 20190920
Time 11.06 h
INSTRUM spect
PROBHD Z119470 0014 (
PULPROG deptsp135
TD 65536
SOLVENT MeOD
NS 14
DS 8
SWH 22727.273 Hz
FIDRES 0.693581 Hz
AQ 1.4418420 sec
RG 202.63
DW 22.000 usec
DE 6.50 usec
TE 298.2 K
CNST2 145.0000000
D1 2.000000000 sec
D2 0.00344828 sec
D12 0.00002000 sec
TD0 1
SFO1 125.7829374 MHz
NUC1 13C
PI 9.45 usec
PI3 2000.00 usec
SI 32768
SF 125.7728760 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
```

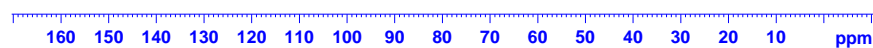
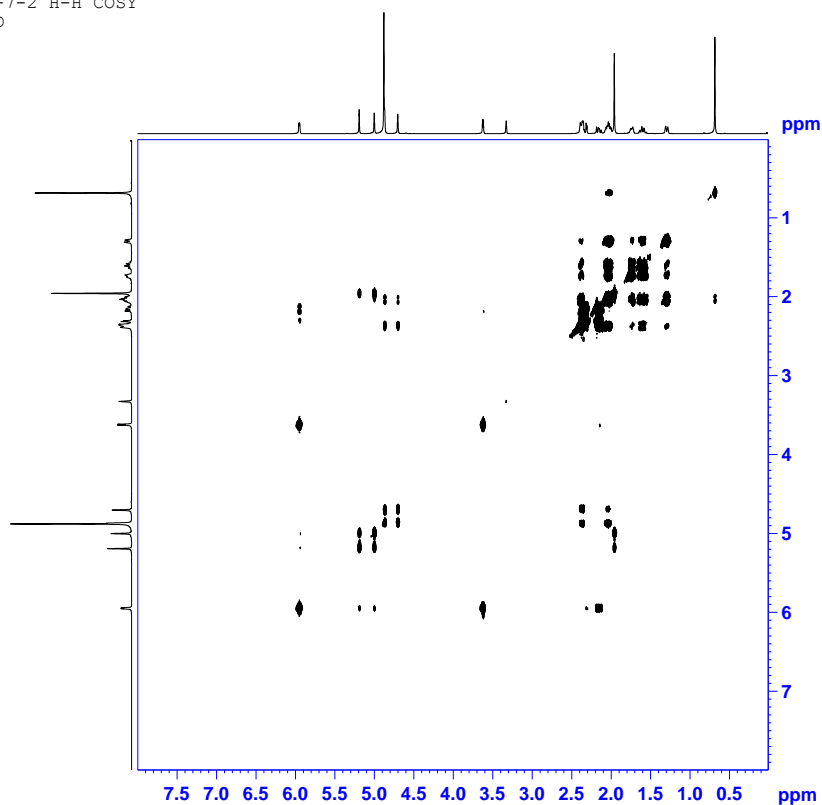


Figure S10 DEPT135 (125 MHz) spectrum of compound 1 in methanol- $d_4$

WBZ-7-2 H-H COSY  
MeOD



```
NAME WBZ-7-2
EXPNO 4
PROCNO 1
Date_ 20190920
Time 11.07 h
INSTRUM spect
PROBHD Z119470 0014 (
PULPROG cosygpmfgf
TD 2048
SOLVENT MeOD
NS 4
DS 16
SWH 4000.000 Hz
FIDRES 3.906250 Hz
AQ 0.2560500 sec
RG 202.63
DW 125.000 usec
DE 6.50 usec
TE 298.1 K
D0 0.00000300 sec
D1 2.000000000 sec
D13 0.00000400 sec
D16 0.00020000 sec
IN0 0.00025000 sec
ND0 1
TD 128
SFO1 500.132 MHz
FIDRES 31.2500000 Hz
SW 7.997 ppm
FrMODE QF
SI 1024
SF 500.1900000 MHz
WDW SINE
SSB 0
LB 0.00 Hz
PC 1.40
SI 1024
MC2 QF
SF 500.1900000 MHz
WDW SINE
SSB 0
LB 0.00 Hz
GB 0
```

Figure S11  $^1\text{H}$ - $^1\text{H}$  COSY (500 MHz) spectrum of compound 1 in methanol- $d_4$



WBZ-7-2 HSQC  
MeOD

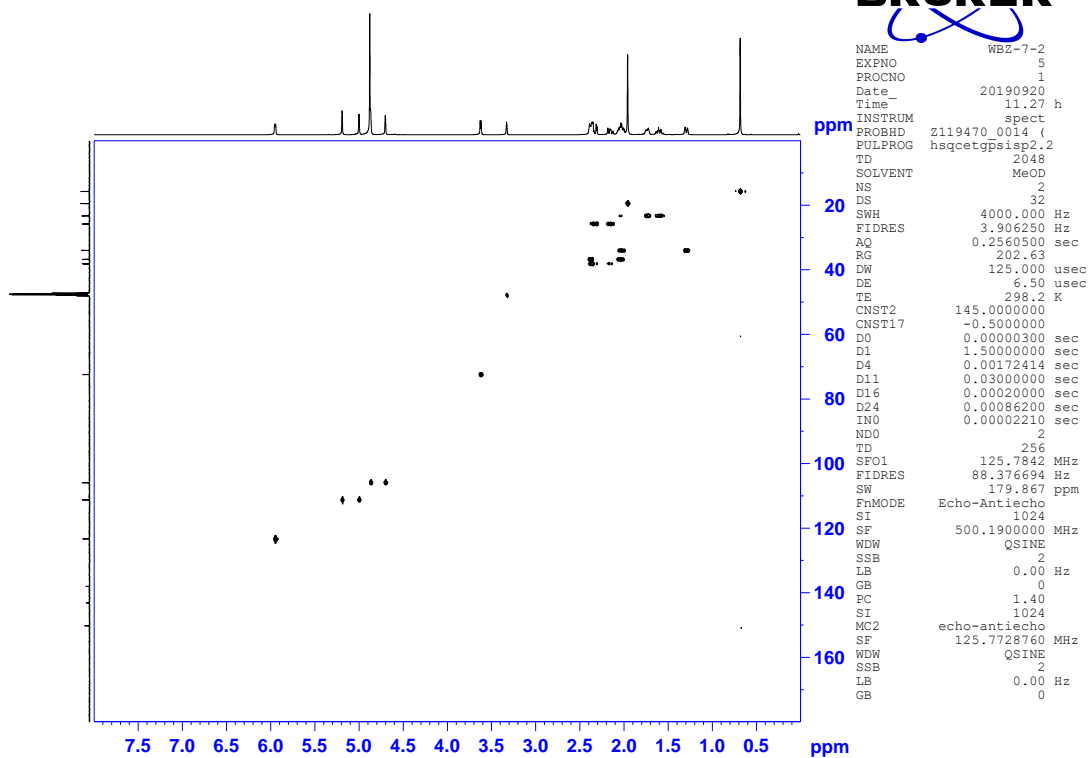


Figure S12 HSQC (500 MHz) spectrum of compound **1** in methanol-*d*<sub>4</sub>

WBZ-7-2 HMBC  
MeOD

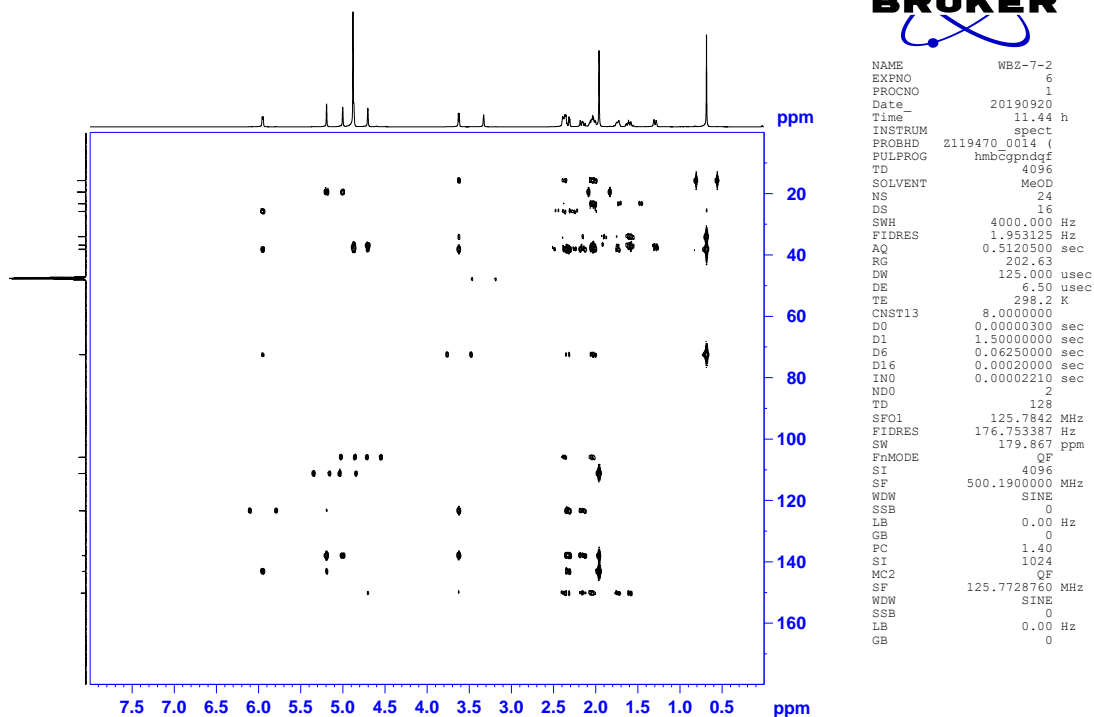


Figure S13 HMBC (500 MHz) spectrum of compound **1** in methanol-*d*<sub>4</sub>

WBZ-7-2 NOE  
MeOD

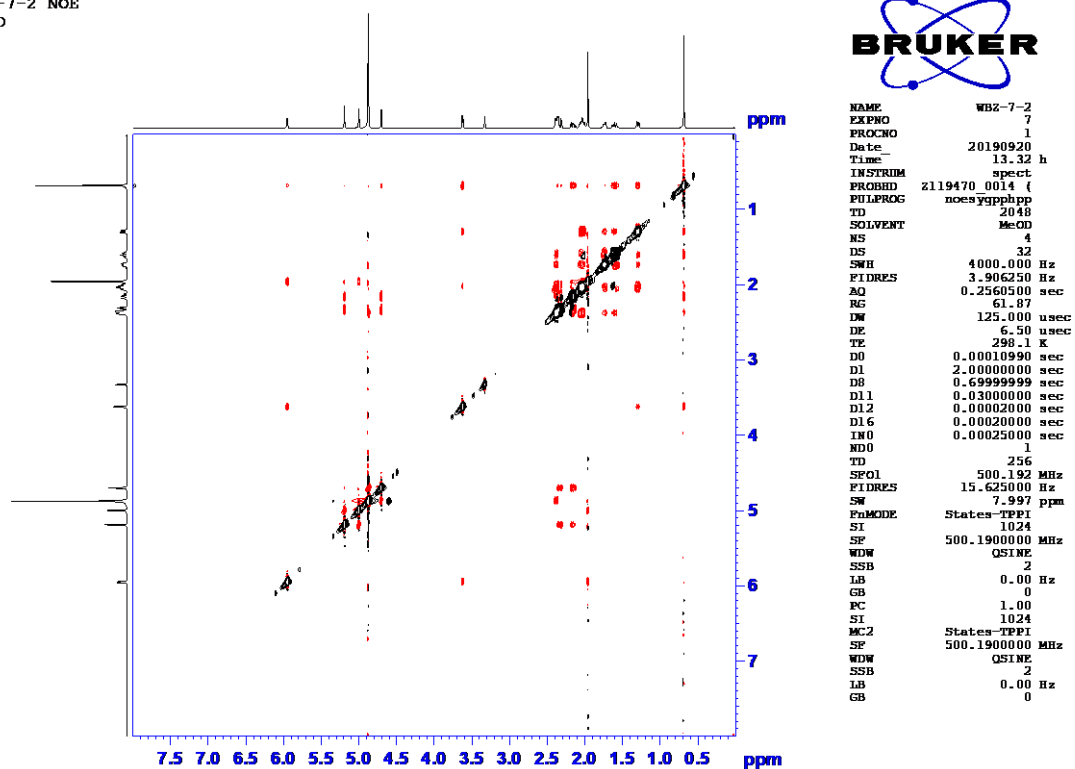
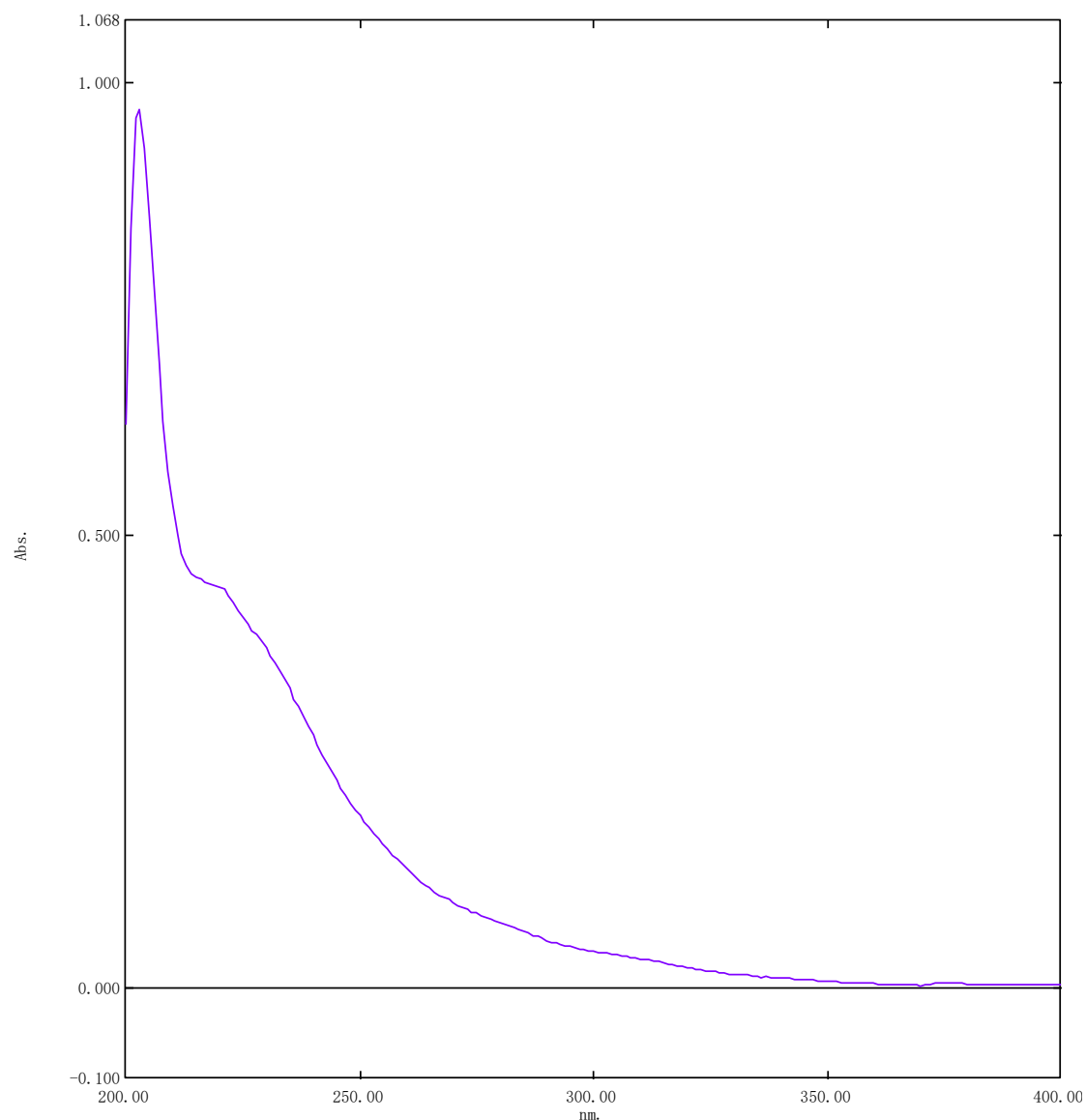
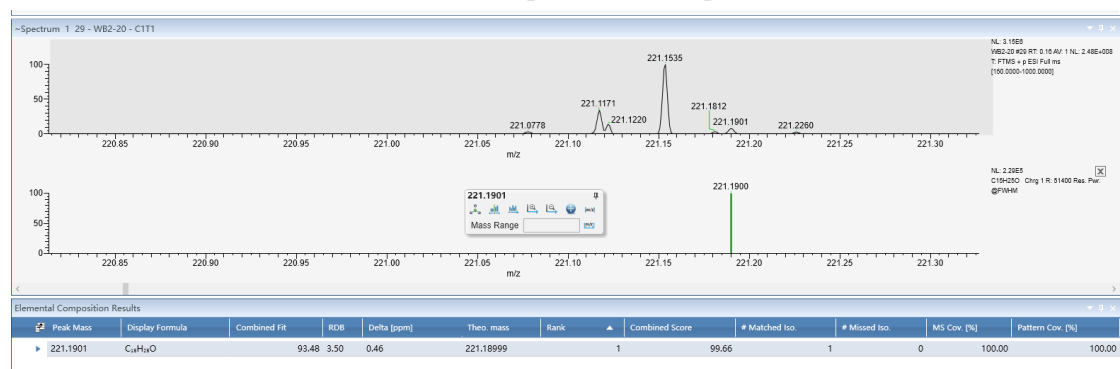


Figure S14 NOESY (500 MHz) spectrum of compound **1** in methanol- $d_4$



**Figure S15** UV spectrum of compound **2**



**Figure S16** HRESIMS spectrum of compound **2**

WBZ-20 H  
CDC13

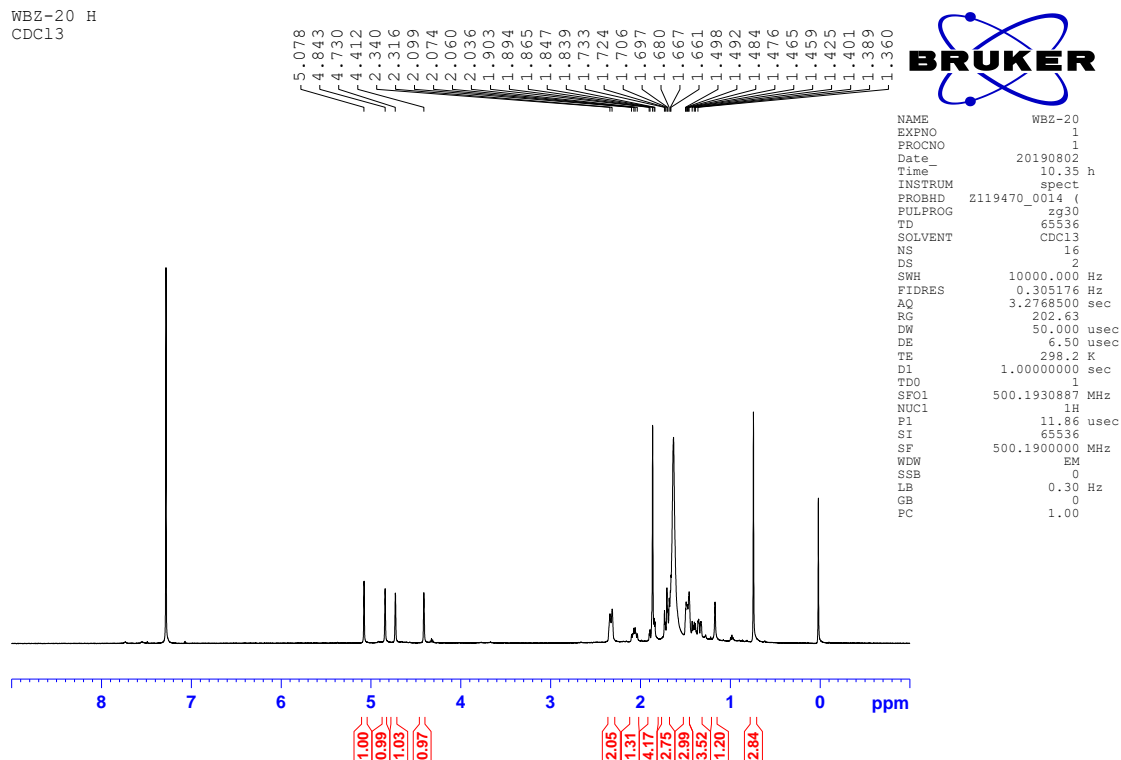


Figure S17  $^1\text{H}$  NMR (500 MHz) spectrum of compound **2** in  $\text{CDCl}_3$

WBZ-20 C  
CDC13

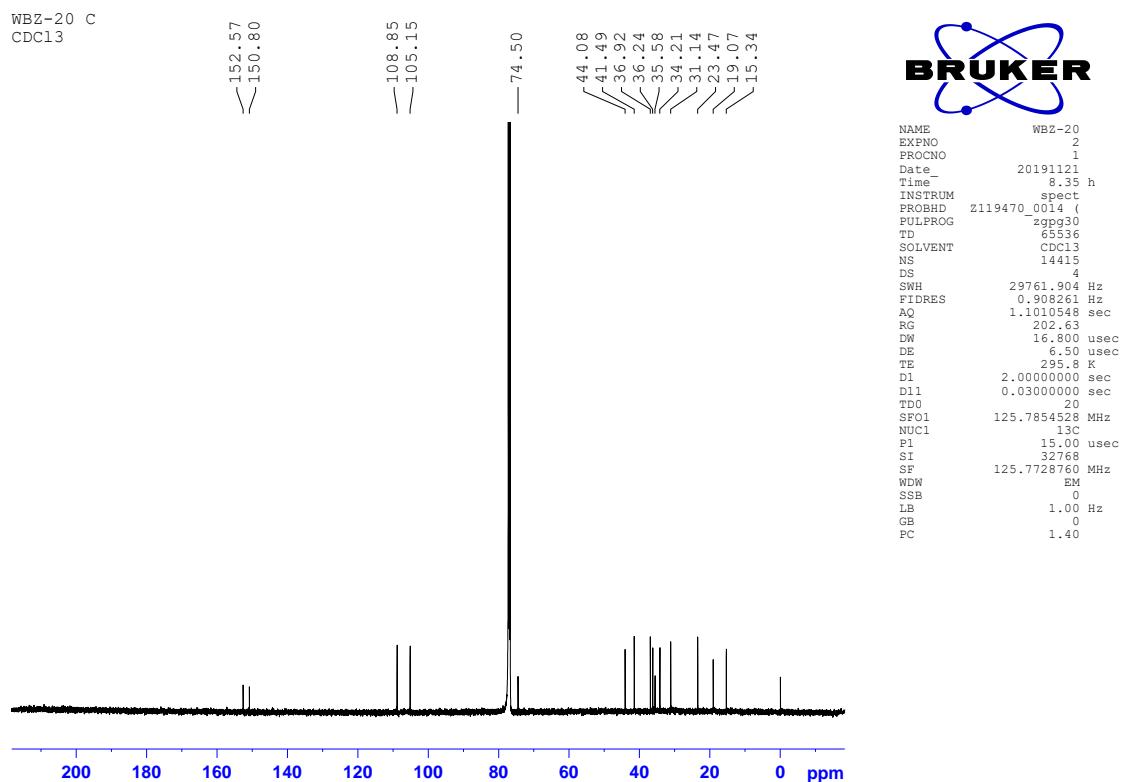
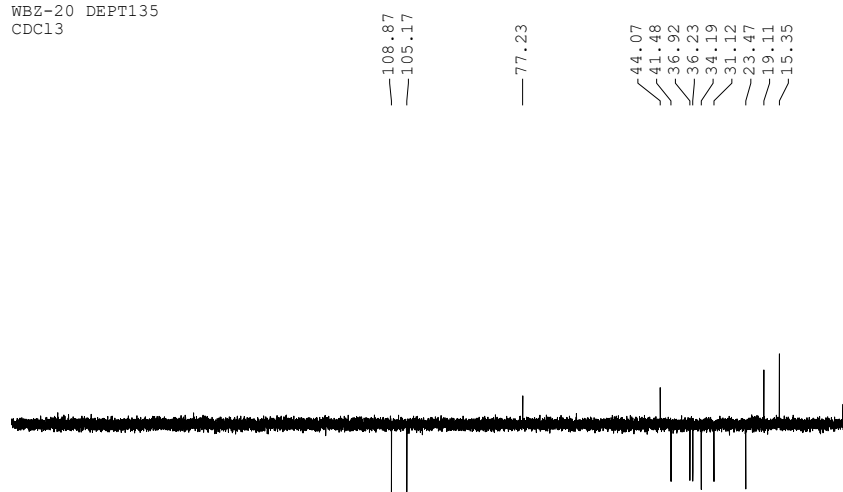


Figure S18  $^{13}\text{C}$  NMR (125 MHz) spectrum of compound **2** in  $\text{CDCl}_3$

WBZ-20 DEPT135  
CDCl<sub>3</sub>



```
NAME WBZ-20
EXPNO 3
PROCNO 1
Date_ 20191114
Time 21.58 h
INSTRUM spect
PROBHD Z119470 0014 (
PULPROG deptsp135
TD 65536
SOLVENT CDCl3
NS 1280
DS 8
SWH 25252.525 Hz
FIDRES 0.770646 Hz
AQ 1.2976629 sec
RG 202.63
DW 19.800 usec
DE 6.50 usec
TE 294.5 K
CNST2 145.0000000
D1 2.0000000 sec
D2 0.00344828 sec
D12 0.0002000 sec
TD0 5
SF01 125.7854533 MHz
NUC1 13
PI 15.00 usec
P13 2000.00 usec
SI 32768
SF 125.7728760 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
```

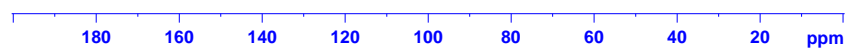
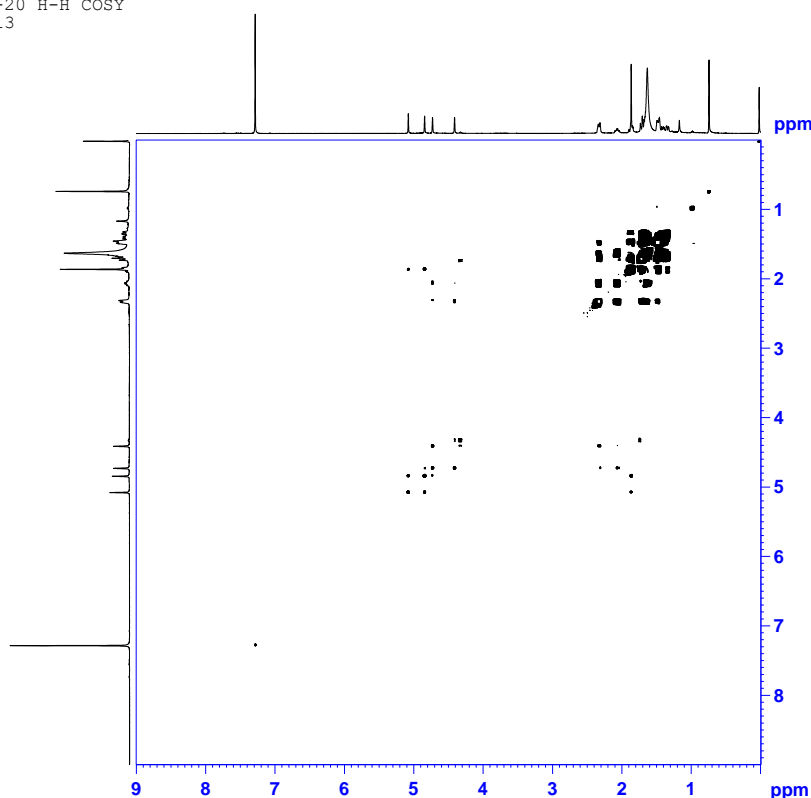


Figure S19 DEPT135 (125 MHz) spectrum of compound 2 in CDCl<sub>3</sub>

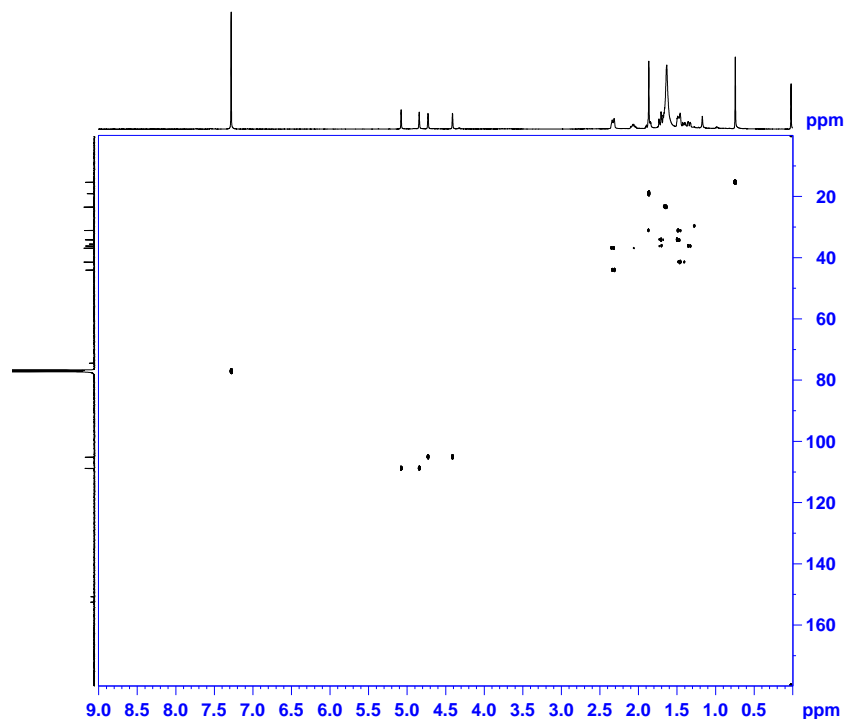
WBZ-20 H-H COSY  
CDCl<sub>3</sub>



```
NAME WBZ-20
EXPNO 4
PROCNO 1
Date_ 20191120
Time 16.07 h
INSTRUM spect
PROBHD Z119470_0014 (
PULPROG cosygpmf
TD 2048
SOLVENT CDCl3
NS 20
DS 16
SWH 4504.504 Hz
FIDRES 4.398930 Hz
AQ 0.2273780 sec
RG 202.63
DW 111.000 usec
DE 6.50 usec
TE 298.1 K
DO 0.00000300 sec
D1 2.00000000 sec
D13 0.00000400 sec
D16 0.00020000 sec
INO 0.00022220 sec
ND0 1
TD 66
SF01 500.1923 MHz
FIDRES 68.188637 Hz
SW 8.997 ppm
FMODE QF
SI 1024
SF 500.1900000 MHz
WDW SINE
SSB 0
LB 0.00 Hz
GB 0
PC 1.40
SI 1024
MC2 QF
SF 500.1900000 MHz
WDW SINE
SSB 0
LB 0.00 Hz
GB 0
```

Figure S20 <sup>1</sup>H-<sup>1</sup>H COSY (500 MHz) spectrum of compound 2 in CDCl<sub>3</sub>

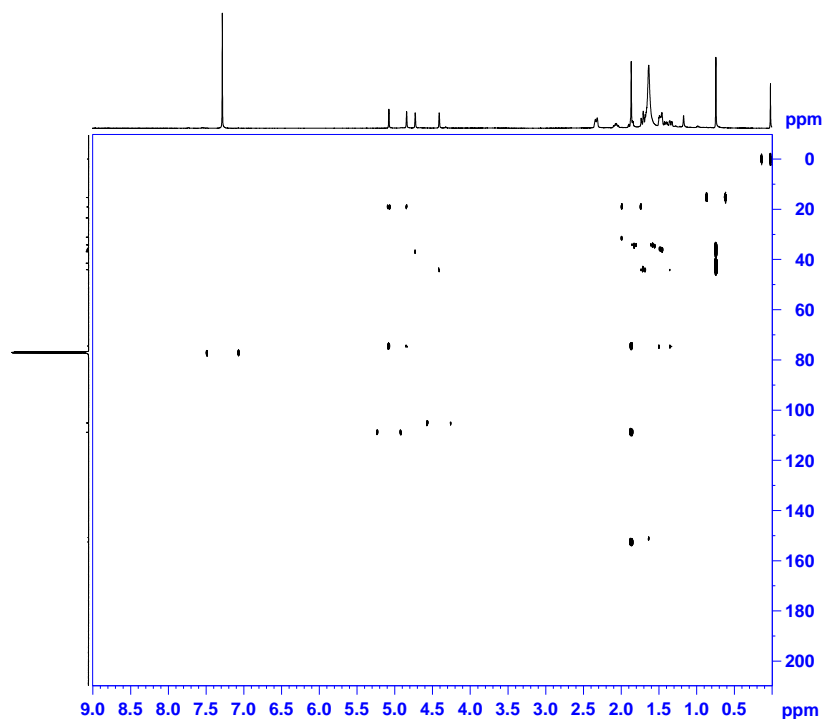
WBZ-20 HSQC  
CDC13



```
NAME WBZ-20
EXPNO 5
PROCNO 1
Date_ 20191129
Time_ 9.07 h
INSTRUM spect
PROBHD Z119470.0014 (
FULPROG hsqcetgpsisp2.2
TD 2048
SOLVENT CDC13
NS 2
DS 32
SWH 4504.504 Hz
FIDRES 4.398930 Hz
AQ 0.2273780 sec
RG 202.63
DW 111.000 usec
DE 6.50 usec
TE 298.2 K
CNST2 145.0000000
CNST17 -0.5000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D24 0.00086200 sec
INO 0.00002210 sec
ND0 2
TD 256
SFO1 125.7842 MHz
FIDRES 88.376694 Hz
SW 179.867 ppm
F1MODE Echo-Antiecho
SI 1024
SF 500.1900000 MHz
WDW QSINE
SSB 2
LB 0.00 Hz
GB 0
PC 1.40
SI 1024
MC2 echo-antiecho
SF 125.7728760 MHz
WDW QSINE
SSB 2
LB 0.00 Hz
GB 0
```

Figure S21 HSQC (500 MHz) spectrum of compound **2** in CDCl<sub>3</sub>

WBZ-20 HMBC  
CDC13



```
NAME WBZ-20
EXPNO 6
PROCNO 1
Date_ 20191120
Time_ 17.32 h
INSTRUM spect
PROBHD Z119470.0014 (
FULPROG hmbcgpndgff
TD 4096
SOLVENT CDC13
NS 40
DS 16
SWH 4504.504 Hz
FIDRES 2.199465 Hz
AQ 0.4547060 sec
RG 202.63
DW 111.000 usec
DE 6.50 usec
TE 298.2 K
CNST13 8.0000000
D0 0.00000300 sec
D1 1.50000000 sec
D6 0.06250000 sec
D16 0.00020000 sec
INO 0.00001810 sec
ND0 2
TD 106
SFO1 125.7855 MHz
FIDRES 260.606689 Hz
SW 219.615 ppm
F1MODE QF
SI 4096
SF 500.1900000 MHz
WDW SINE
SSB 0
LB 0.00 Hz
GB 0
PC 1.40
SI 1024
MC2 QF
SF 125.7728760 MHz
WDW SINE
SSB 0
LB 0.00 Hz
GB 0
```

Figure S22 HMBC (500 MHz) spectrum of compound **2** in CDCl<sub>3</sub>

WBZ-20 NOE  
CDC13

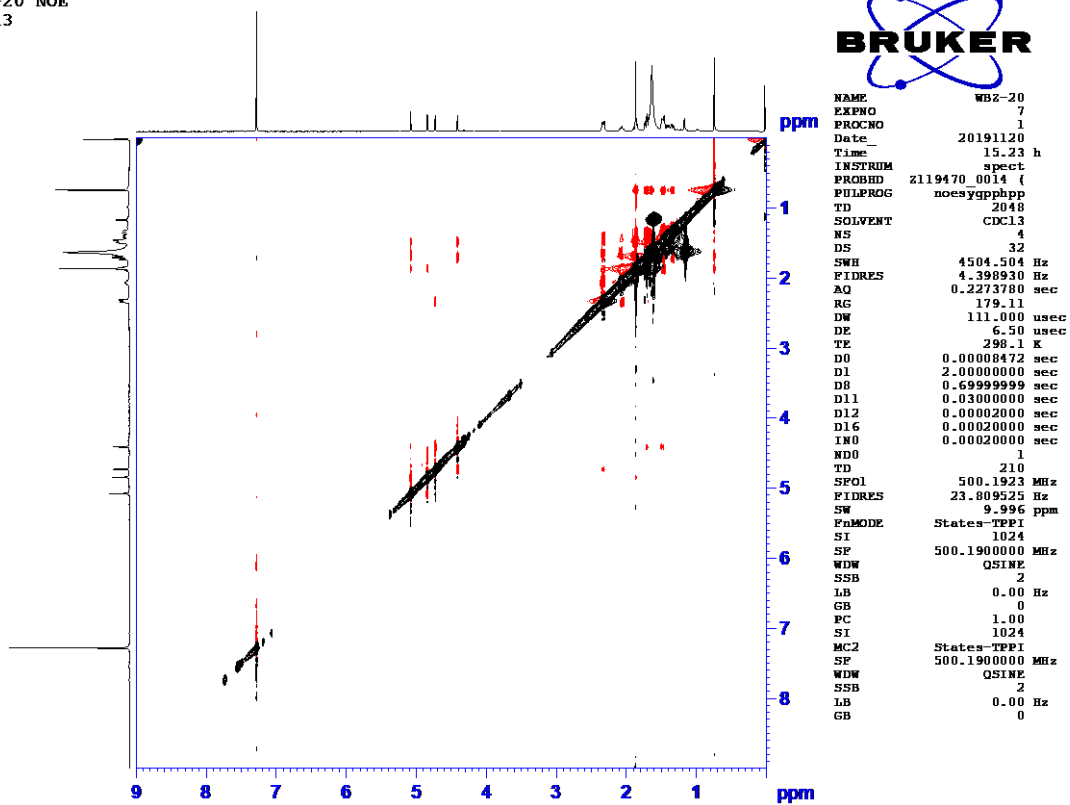


Figure S23 NOESY (500 MHz) spectrum of compound **2** in CDCl<sub>3</sub>