

1 **SUPPLEMENTAL MATERIAL**

2 **Four alkaloids from *Portulaca oleracea* L. and their anti-inflammatory**

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27

## 28 **Abstract**

29 Four alkaloids were isolated from *Portulaca oleracea* L., including two new  
30 compounds, 2-(4-amino-6-hydroxy-1,6-dihydropyrimidin-5-yl)-5-  
31 (hydroxymethyl)tetrahydrofuran-3,4-diol, named Olerapyrimidine (**1**) and  
32 (2R,3S,4S,5R,6S)-2-(hydroxymethyl)-6-((6-hydroxypyridin-3-yl)oxy)tetrahydro-2H-  
33 pyran-3,4,5-triol, named Olerapyridine (**2**), and two known compounds including 1H-  
34 imidazole (**3**) and (5S, 6R, 7S, 8R)-5-amino-(2Z, 4Z)-1, 2, 3-trihydroxybuta-2, 4-  
35 dienyloxy-pentane-6, 7, 8, 9-tetraol (**4**) from *P. oleracea* for the first time. Their  
36 structures were determined by spectroscopic methods, including UHPLC-ESI-Q-  
37 TOF/MS, 1D and 2D NMR spectra. Both Olerapyrimidine and Olerapyridine at 20 $\mu$ M  
38 could inhibit the inflammatory factor, IL-1 $\beta$  and TNF- $\alpha$  in the RAW 264.7 cells induced  
39 by LPS.

40 **Keywords:** *Portulaca oleracea* L.; alkaloid; anti-inflammatory

## 41 **Supporting information**

42 Supplementary material relating to this article is available online, alongside, Tables S1-  
43 S2 and Figures S1-S30.

44 Table S1. Full NMR data of Olerapyrimidine (**1**) in CD<sub>3</sub>OD-*d*<sub>4</sub>.

45 Table S2. Full NMR data of Olerapyridine (**2**) in CD<sub>3</sub>OD-*d*<sub>4</sub>.

46 Figure S1. Structures of compounds **1-4**.

47 Figure S2. Structure of Olerapyrimidine (**1**).

48 Figure S3. Key HMBC correlations of Olerapyrimidine (**1**).

- 49 Figure S4. Key  $^1\text{H}$ - $^1\text{H}$  COSY and ROESY correlations of Olerapyrimidine (**1**).
- 50 Figure S5.  $^1\text{H}$  NMR (600 MHz) spectrum of Olerapyrimidine (**1**). in  $\text{CD}_3\text{OD}-d_4$ .
- 51 Figure S6.  $^{13}\text{C}$  NMR (150 MHz) spectrum of Olerapyrimidine (**1**). in  $\text{CD}_3\text{OD}-d_4$ .
- 52 Figure S7. DEPT 135 spectrum of Olerapyrimidine (**1**) in  $\text{CD}_3\text{OD}-d_4$ .
- 53 Figure S8. HSQC spectrum of Olerapyrimidine (**1**) in  $\text{CD}_3\text{OD}-d_4$ .
- 54 Figure S9. HMBC spectrum of Olerapyrimidine (**1**) in  $\text{CD}_3\text{OD}-d_4$ .
- 55 Figure S10.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of Olerapyrimidine (**1**) in  $\text{CD}_3\text{OD}-d_4$ .
- 56 Figure S11. ROESY spectrum of Olerapyrimidine (**1**) in  $\text{CD}_3\text{OD}-d_4$ .
- 57 Figure S12. UHPLC-ESI-Q-TOF/MS of Olerapyrimidine (**1**).
- 58 Figure S13. Structure of Olerapyridine (**2**).
- 59 Figure S14. Key HMBC correlations of Olerapyridine (**2**).
- 60 Figure S15. Key  $^1\text{H}$ - $^1\text{H}$  COSY and ROESY correlations of Olerapyridine (**2**).
- 61 Figure S16.  $^1\text{H}$  NMR (600 MHz) spectrum of Olerapyridine (**2**) in  $\text{CD}_3\text{OD}-d_4$ .
- 62 Figure S17.  $^{13}\text{C}$  NMR (150 MHz) spectrum of Olerapyridine (**2**) in  $\text{CD}_3\text{OD}-d_4$ .
- 63 Figure S18. DEPT 135 spectrum of Olerapyridine (**2**) in  $\text{CD}_3\text{OD}-d_4$ .
- 64 Figure S19. HSQC spectrum of Olerapyridine (**2**) in  $\text{CD}_3\text{OD}-d_4$ .
- 65 Figure S20. HMBC spectrum of Olerapyridine (**2**) in  $\text{CD}_3\text{OD}-d_4$ .
- 66 Figure S21.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of Olerapyridine (**2**) in  $\text{CD}_3\text{OD}-d_4$ .
- 67 Figure S22. ROESY spectrum of Olerapyridine (**2**) in  $\text{CD}_3\text{OD}-d_4$ .

68 Figure S23. UHPLC-ESI-Q-TOF/MS of Olerapyridine (**2**).

69 Figure S24. Cell viability of the LPS-induced macrophage RAW 264.7 cells pretreated  
70 with compounds **1–2**.

71 Figure S25. Inhibitory effect on the secretion of inflammatory cytokine IL-1 $\beta$  in LPS-  
72 activated RAW 264.7 macrophages cells pretreated with compounds **1–2**.

73 Figure S26. Inhibitory effect on the secretion of inflammatory cytokine TNF- $\alpha$  in LPS-  
74 activated RAW 264.7 macrophages cells pretreated with compounds **1–2**.

75 Figure S27.  $^1\text{H}$  NMR (600 MHz) spectrum of compound **3** in  $\text{CD}_3\text{OD}-d_4$ .

76 Figure S28.  $^{13}\text{C}$  NMR (150 MHz) spectrum of compound **3** in  $\text{CD}_3\text{OD}-d_4$ .

77 Figure S29.  $^1\text{H}$  NMR (600 MHz) spectrum of compound **4** in  $\text{CD}_3\text{OD}-d_4$ .

78 Figure S30.  $^{13}\text{C}$  NMR (150 MHz) spectrum of compound **4** in  $\text{CD}_3\text{OD}-d_4$ .

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80 **Compound characterization**

81 <sup>1</sup>H-imidazole (**3**).

82 The compound is colorless oil.

83 <sup>1</sup>H-NMR: 6.51, 7.12, 7.64

84 <sup>13</sup>C-NMR: 148.07, 118.80, 113.19

85 (5S, 6R, 7S, 8R)-5-amino-(2Z, 4Z)-1, 2, 3-trihydroxybuta-2, 4-dienyloxy-pentane-6, 7,

86 8, 9-tetraol (**4**).

87 The compound is yellow oil.

88 <sup>1</sup>H-NMR: 8.25, 8.11, 5.90, 4.67, 4.26, 4.10, 3.81, 3.69

89 <sup>13</sup>C-NMR: 157.59, 153.47, 149.96, 142.03, 91.25, 88.20, 75.44, 72.69, 63.49

90

91 Table S1. Full NMR data of Olerapyrimidine (**1**) in CD<sub>3</sub>OD-*d*<sub>4</sub>.

NO.	δ <sub>C</sub>	type	δ <sub>H</sub> , mult, ( <i>J</i> in Hz)	HMBC	H-H COSY	ROESY
1		NH				
2	141.54	CH	8.17, d, (8.22)	4,6		
3		N				
4	148.02	C				
5	123.28	C				
6	91.29	CH	5.87, d, (6.54)	2,4		2'
1'		O				
2'	75.45	CH	4.66, d (1.32)	4,6	3'	6
3'	72.74	CH	4.23, m	5, 5'	2'	
4'	65.02	CH	3.90, m	2'	5'	

5'	88.24	CH	4.08, m	2', 3'	4', 6'
6'	63.53	CH <sub>2</sub>	3.65, dd, (2.25, 12.54)	4'	4',

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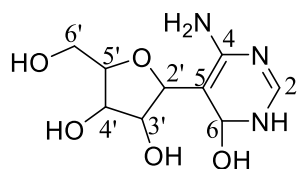
Table S2. Full NMR data of Olerapyridine (**2**) in CD<sub>3</sub>OD-*d*<sub>4</sub>.

NO.	$\delta_C$	type	$\delta_H$ , mult, ( <i>J</i> in Hz)	HMBC	H-H COSY	ROESY
1		N				
2	119.41	CH	7.52, s	4, 6		
3	151.13	C		5		
4	126.21	CH	7.16, d, (8.7)	2, 6	5	2'
5	118.50	CH	6.74, d, (8.7)	3	4	
6	158.15	C				
1'		O				
2'	103.63	CH	4.69, d, 7.08)	5, 4', 6'	3'	4, 4', 6'
3'	74.94	CH	3.34, s	1, 4, 1''	2', 4'	
4'	77.92	CH	3.31, m	2', 6'	3', 5'	2'
5'	71.26	CH	3.32, m	3'	4', 6'	
6'	78.08	CH	3.35, m	2', 4'	5'	2'
7'	62.50	CH <sub>2</sub>	3.63, dd, (1.80, 10.98)	5'	6'	

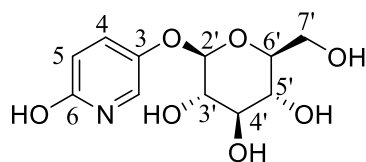
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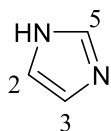
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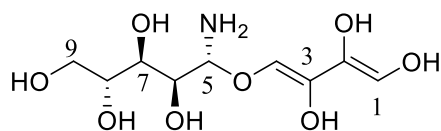
Olerapyrimidine (1)



Olerapyridine (2)



1H-imidazole (3)

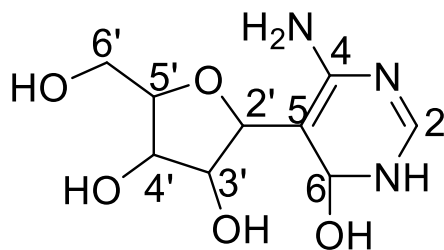


(5S, 6R, 7S, 8R)-5-amino-(2Z, 4Z)-1, 2, 3-trihydroxybuta-2, 4-dienyloxy-pentane-6, 7, 8, 9-tetraol (4)

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Figure S1. Structures of compounds **1-4**.

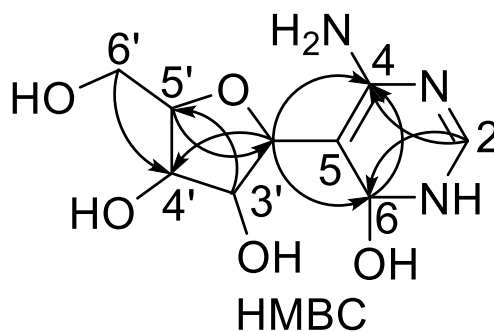


Olerapyrimidine (1)

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Figure S2. Structure of Olerapyrimidine (**1**).

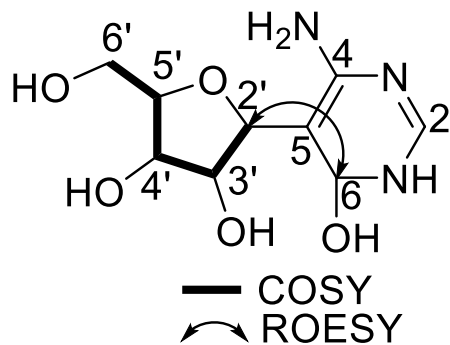


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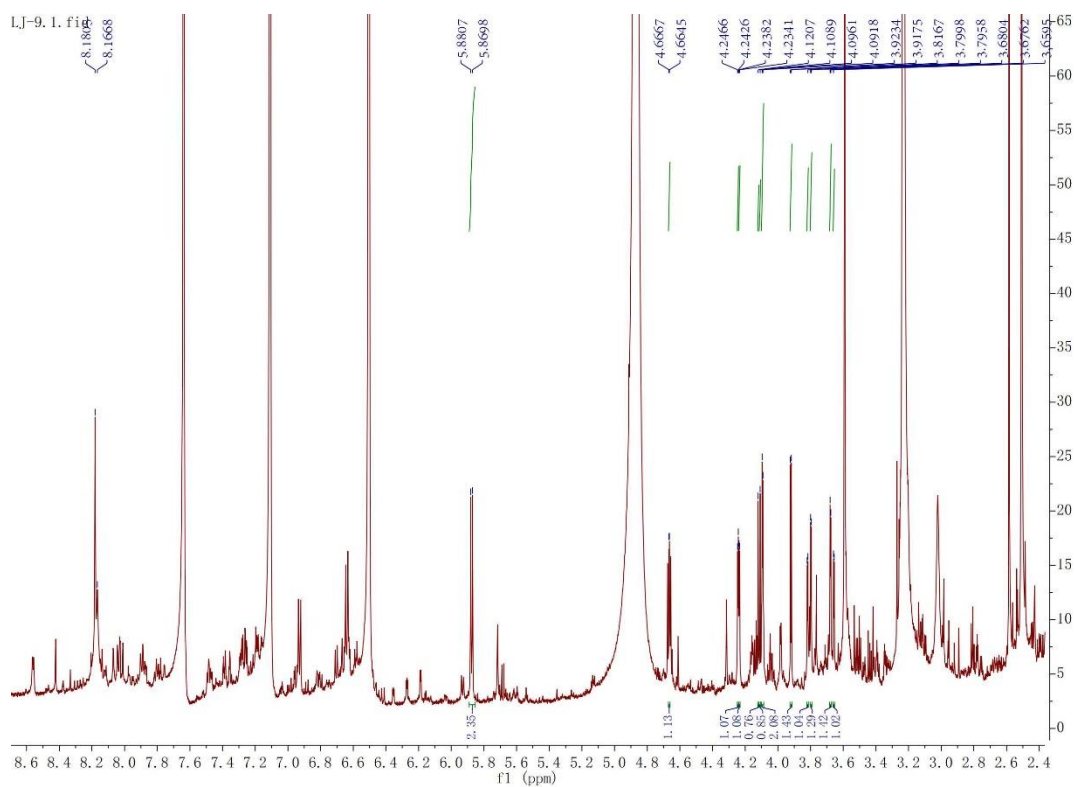
Figure S3. Key HMBC correlations of Olerapyrimidine (**1**).





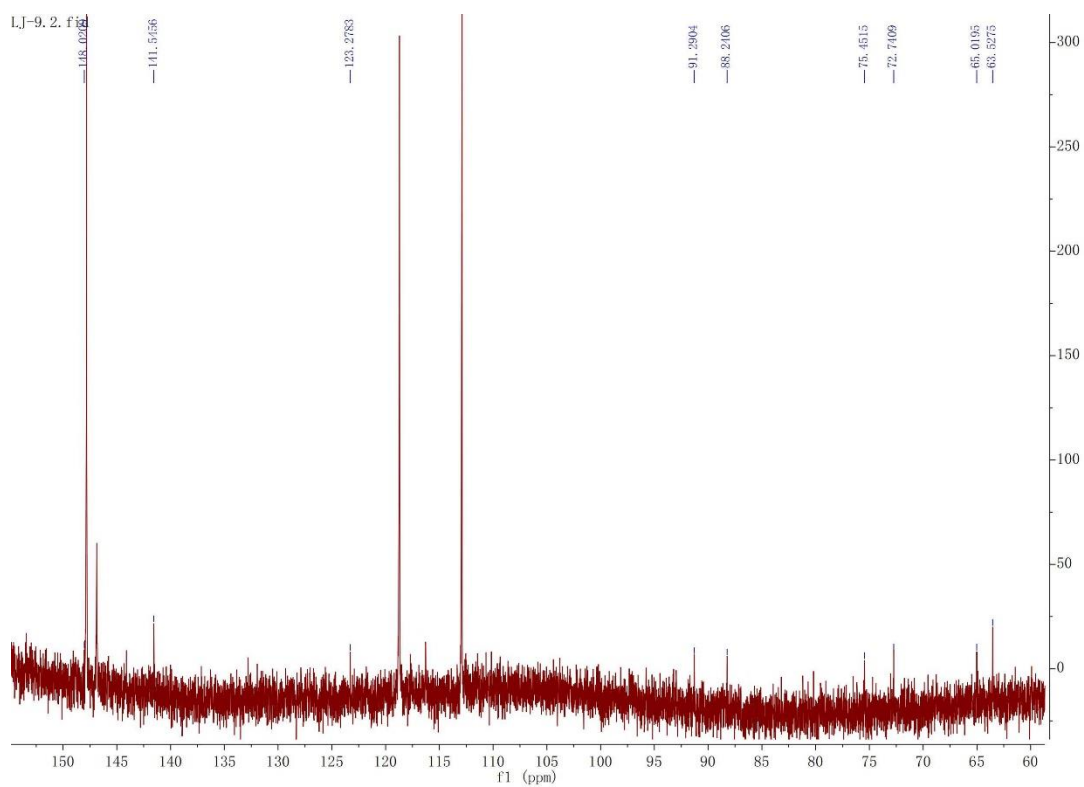
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104 Figure S4. Key  $^1\text{H}$ - $^1\text{H}$  COSY and ROESY correlations of Olerapyrimidine (**1**).

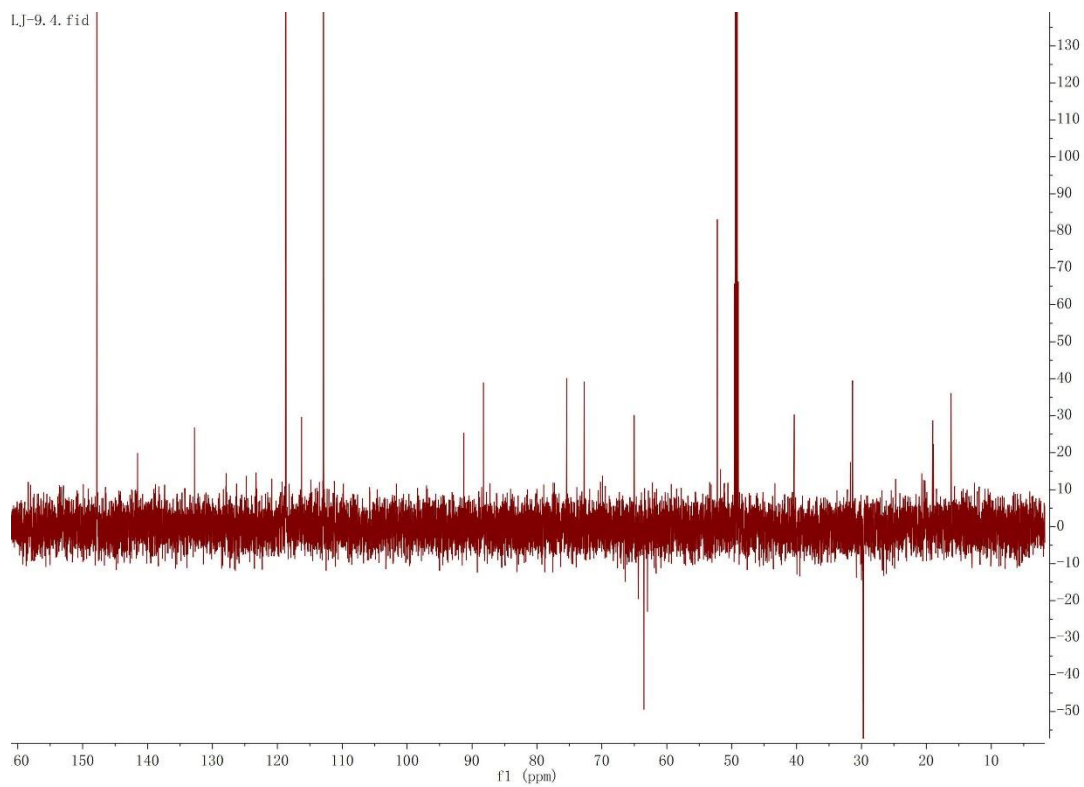


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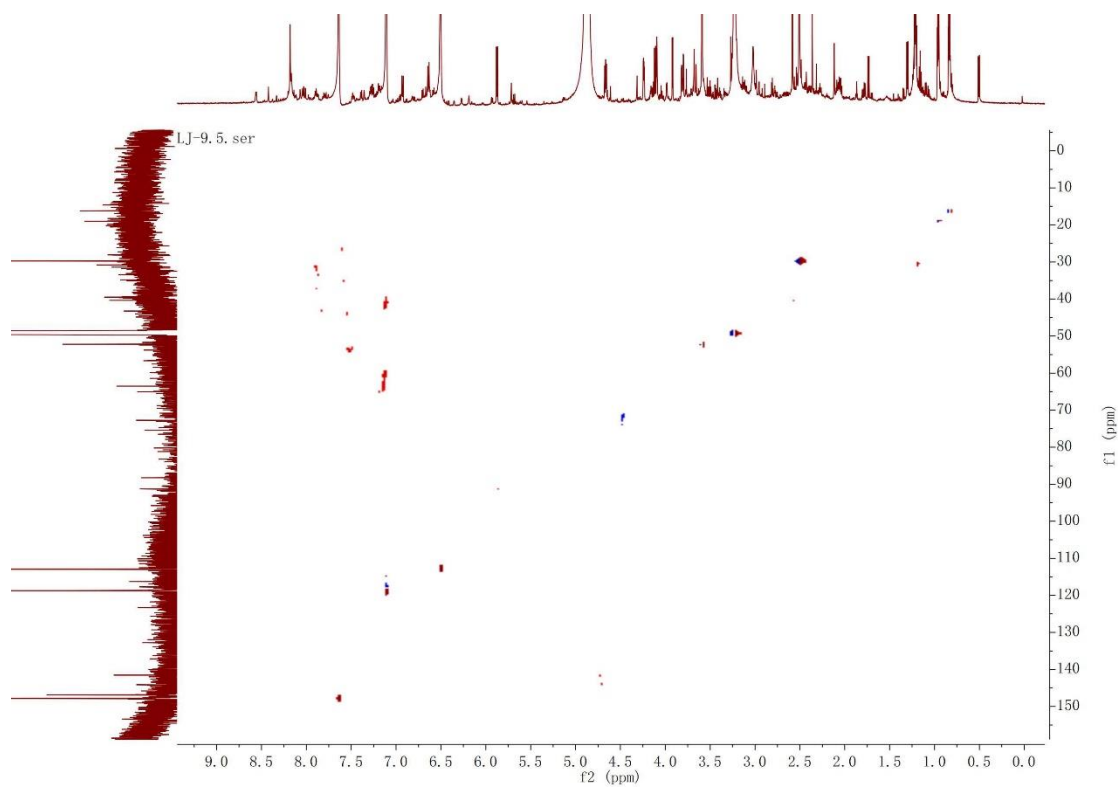
106 Figure S5.  $^1\text{H}$  NMR (600 MHz) spectrum of Olerapyrimidine (**1**), in  $\text{CD}_3\text{OD}-d_4$ .



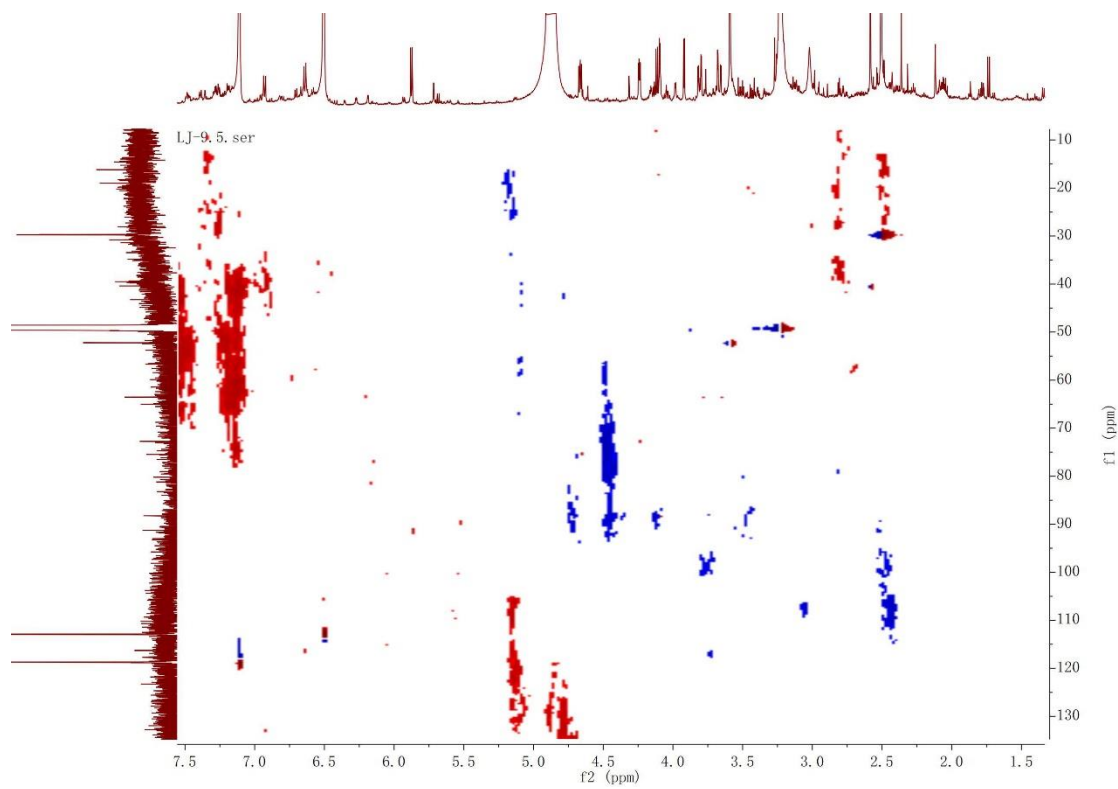
108 Figure S6.  $^{13}\text{C}$  NMR (150 MHz) spectrum of Olerapyrimidine (**1**). in  $\text{CD}_3\text{OD}-d_4$ .



110 Figure S7. DEPT 135 spectrum of Olerapyrimidine (**1**) in  $\text{CD}_3\text{OD}-d_4$ .



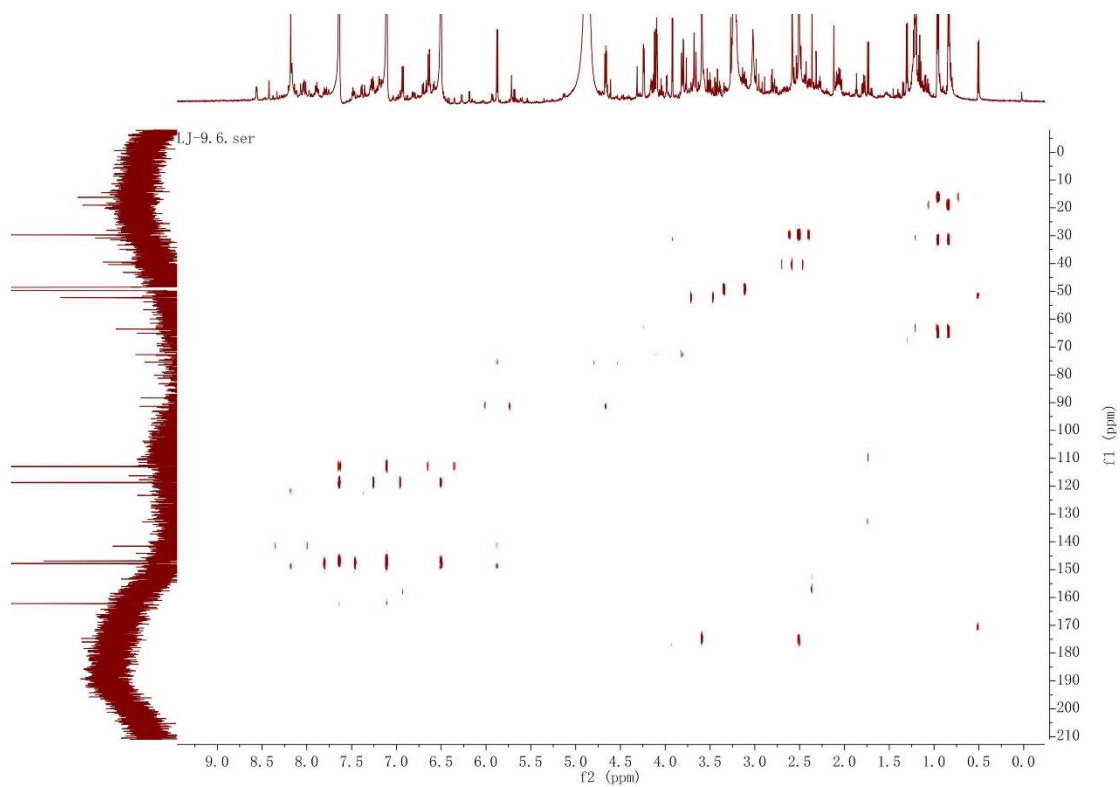
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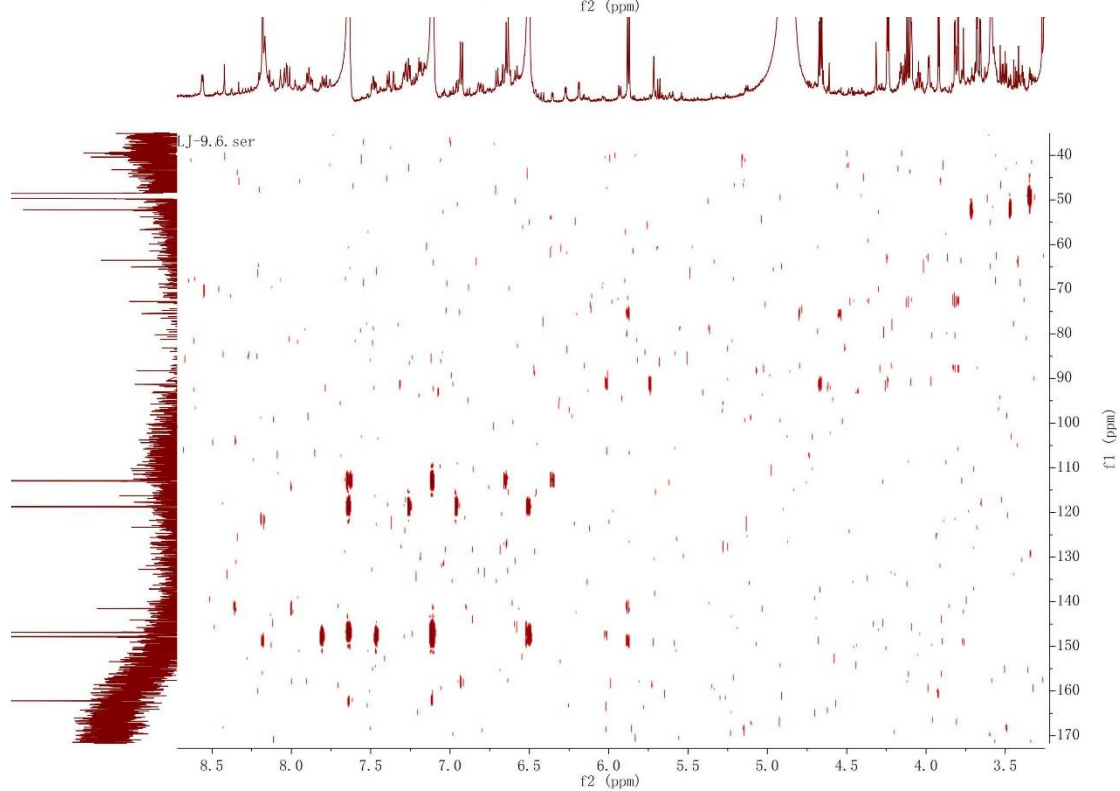
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Figure S8. HSQC spectrum of Olerapyrimidine (**1**) in  $CD_3OD-d_4$ .



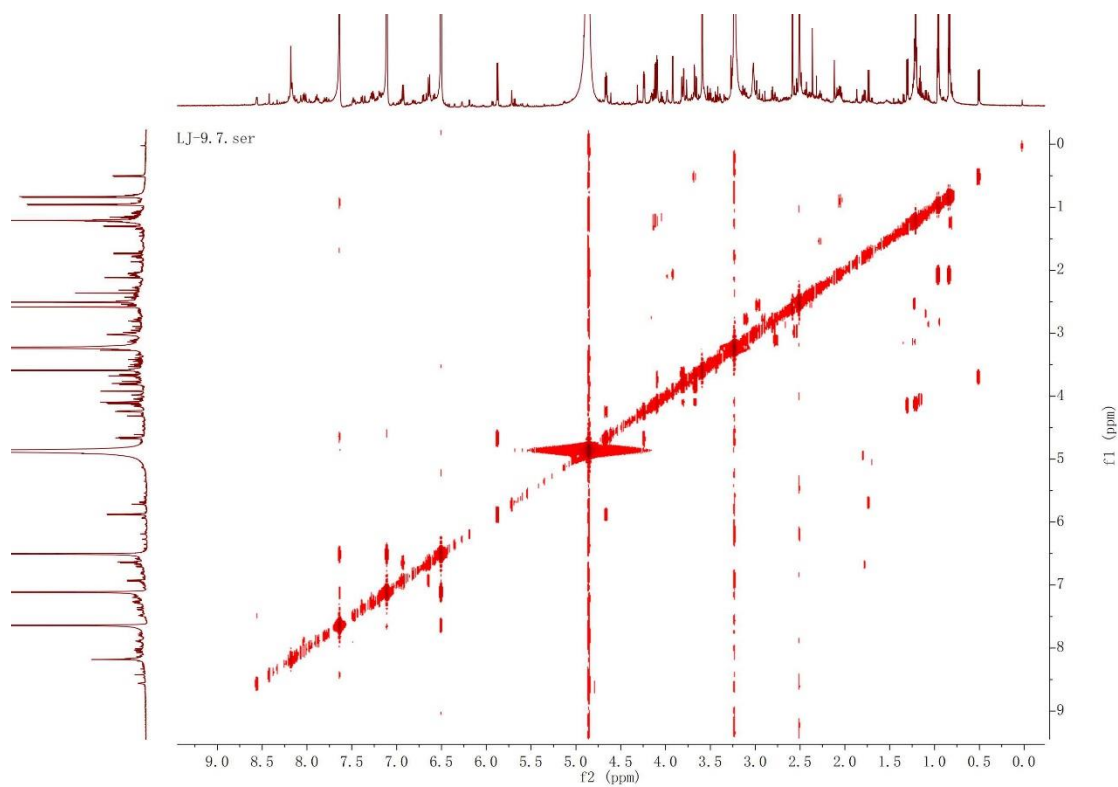
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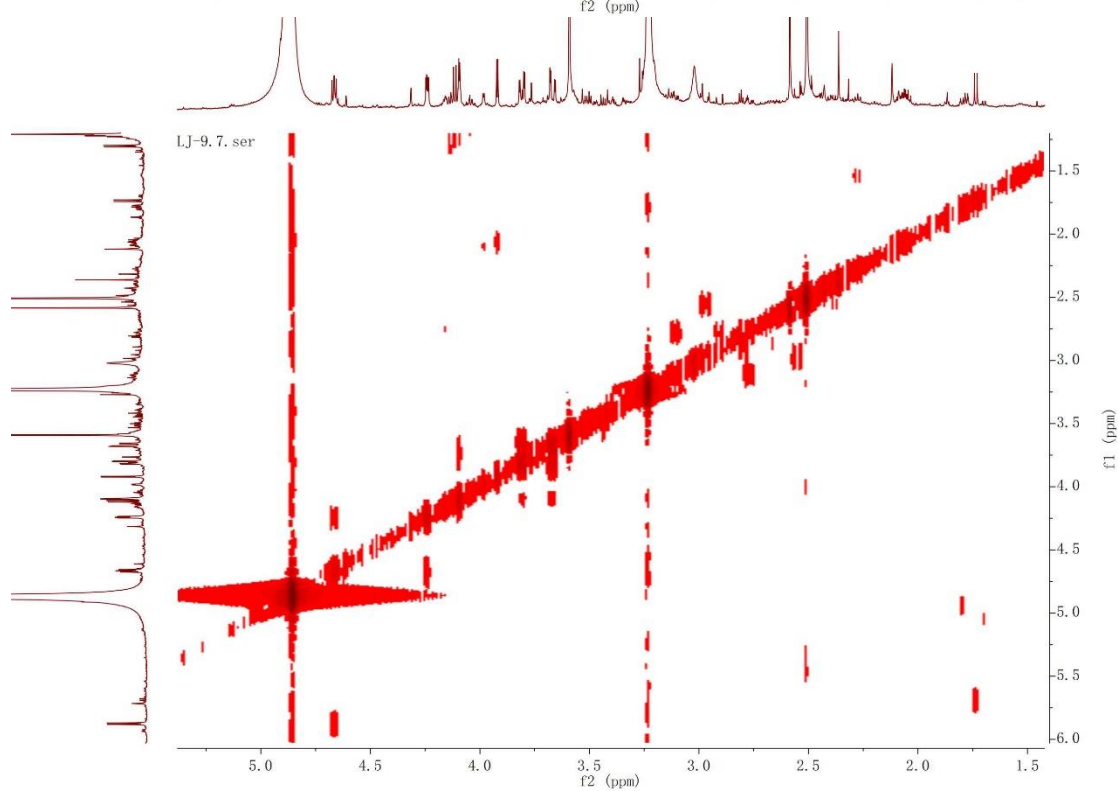
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Figure S9. HMBC spectrum of Olerapyrimidine (**1**) in CD<sub>3</sub>OD-*d*<sub>4</sub>.



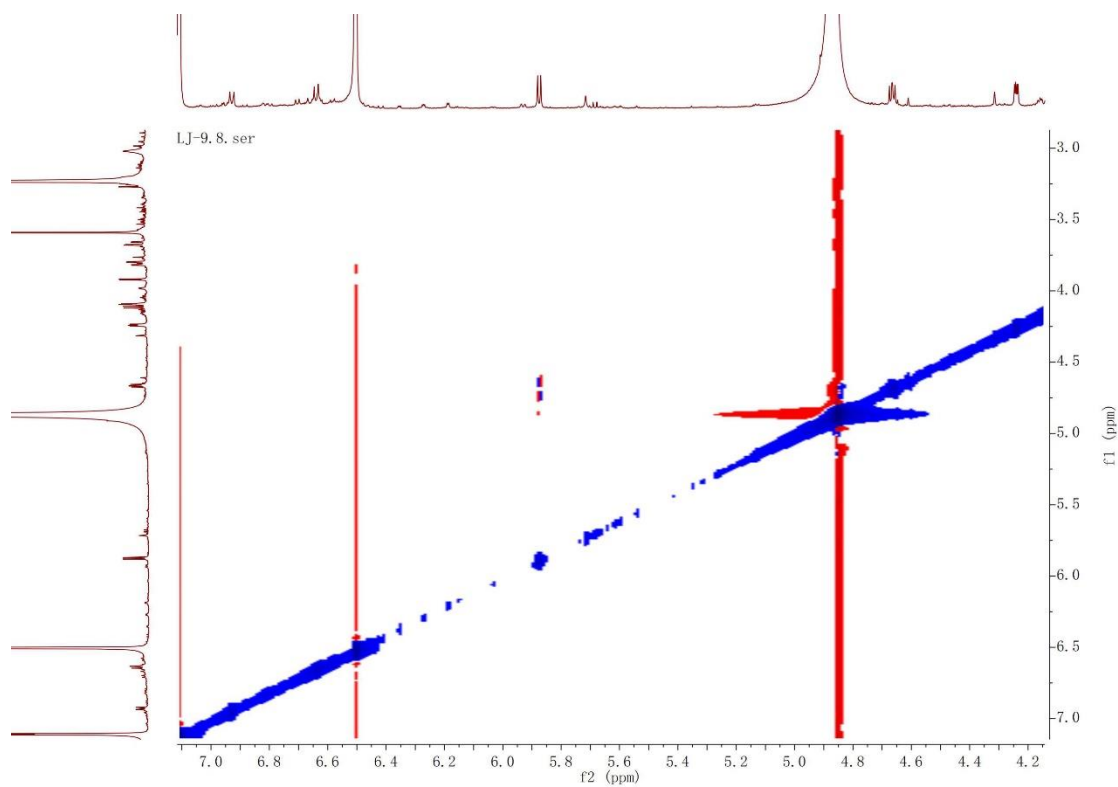
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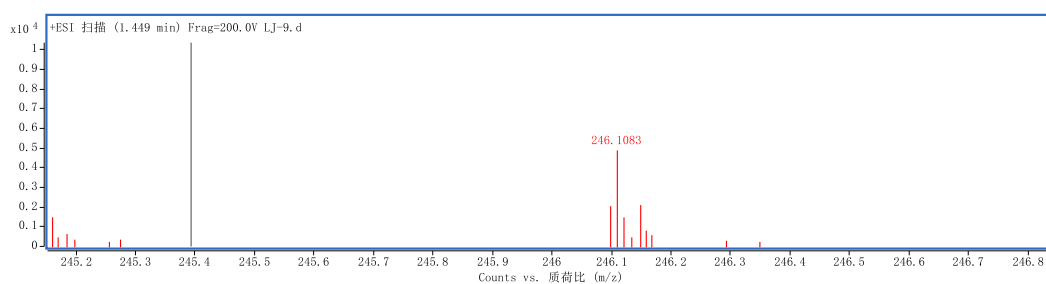
Figure S10.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of Olerapyrimidine (**1**) in  $\text{CD}_3\text{OD}-d_4$ .



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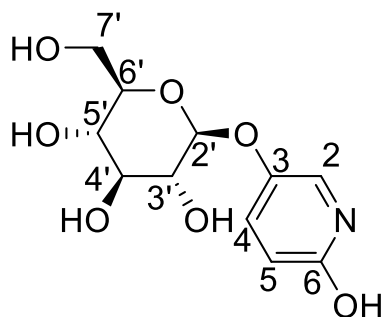
Figure S11. ROESY spectrum of Olerapyrimidine (**1**) in  $\text{CD}_3\text{OD}-d_4$ .



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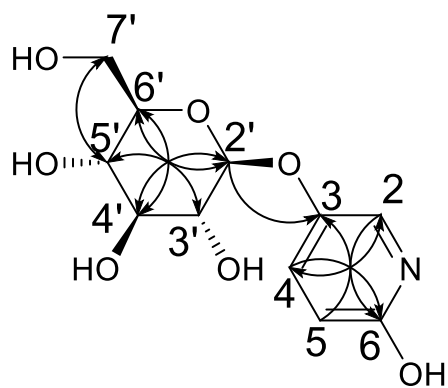
Figure S12. UHPLC-ESI-Q-TOF/MS of Olerapyrimidine (**1**).



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Figure S13. Structure of Olerapyrimidine (**2**).

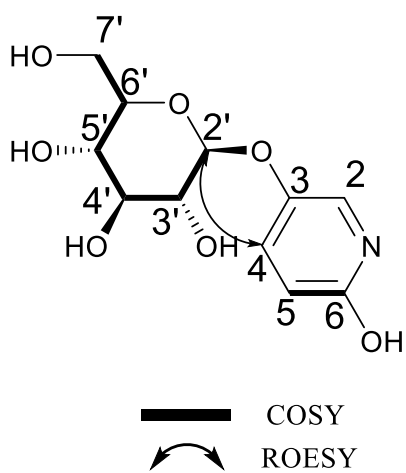


HMBC

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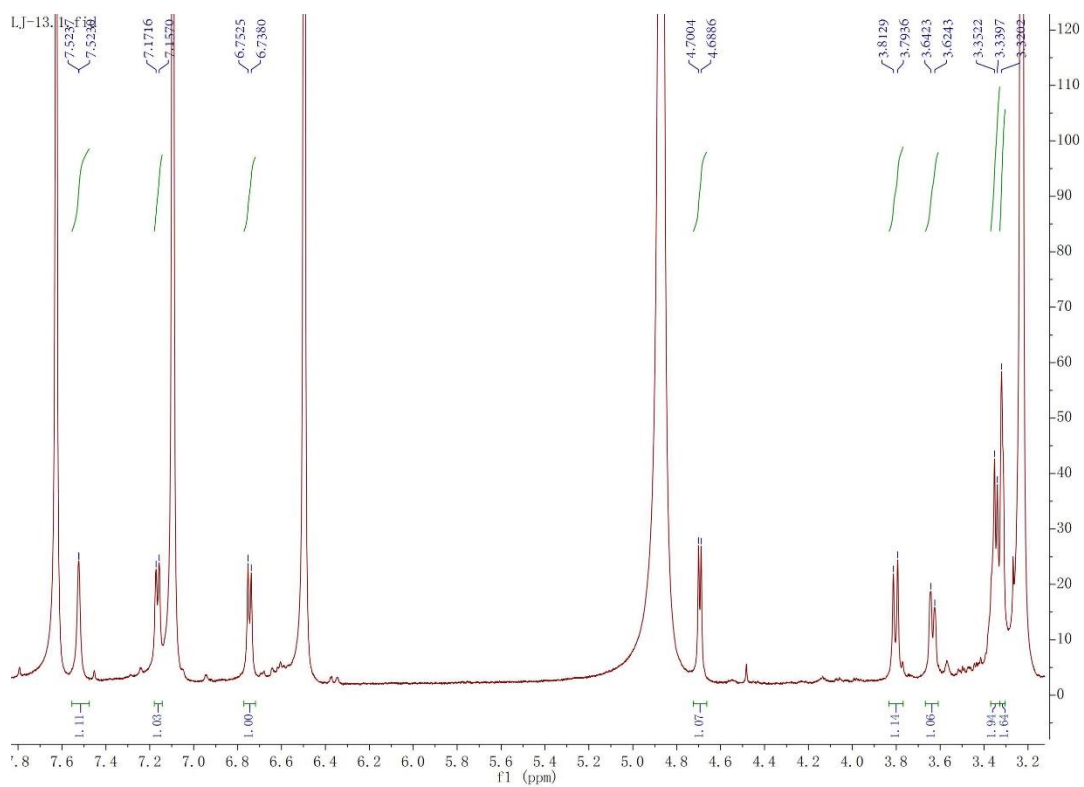
Figure S14. Key HMBC correlations of Olerapyridine (**2**).



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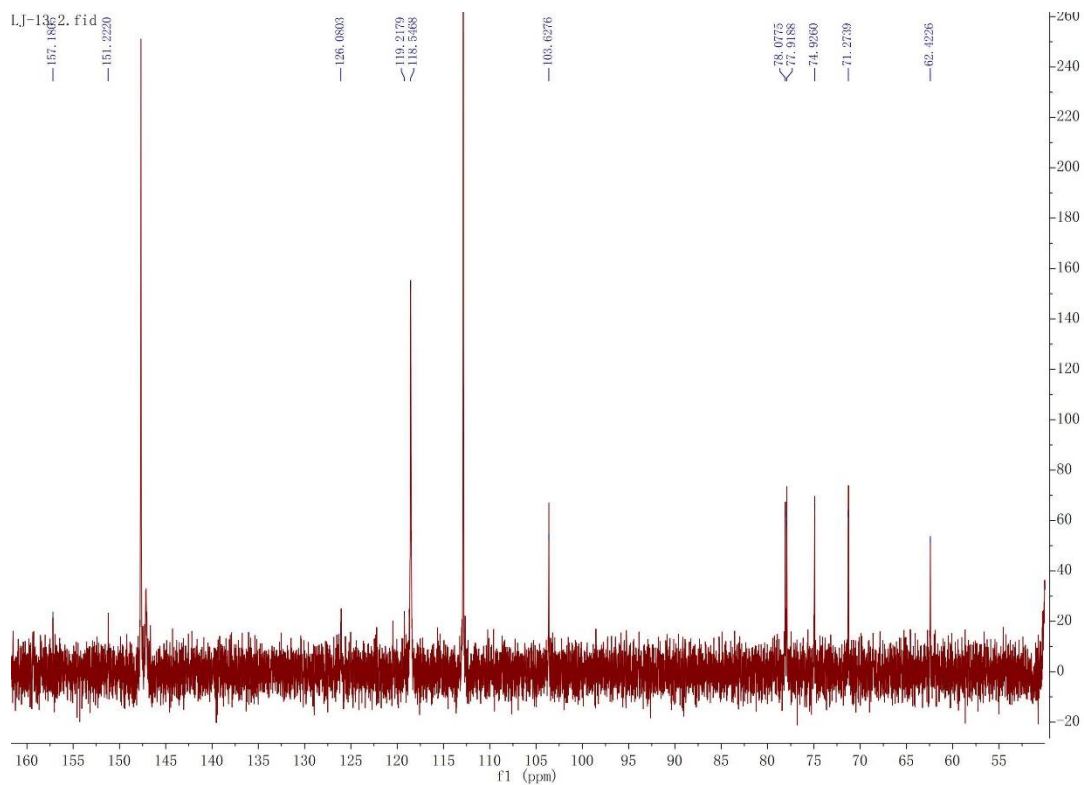
Figure S15. Key  $^1\text{H}$ - $^1\text{H}$  COSY and ROESY correlations of Olerapyridine (**2**).



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Figure S16. <sup>1</sup>H NMR (600 MHz) spectrum of Olerapyridine (2) in CD<sub>3</sub>OD-*d*<sub>4</sub>.

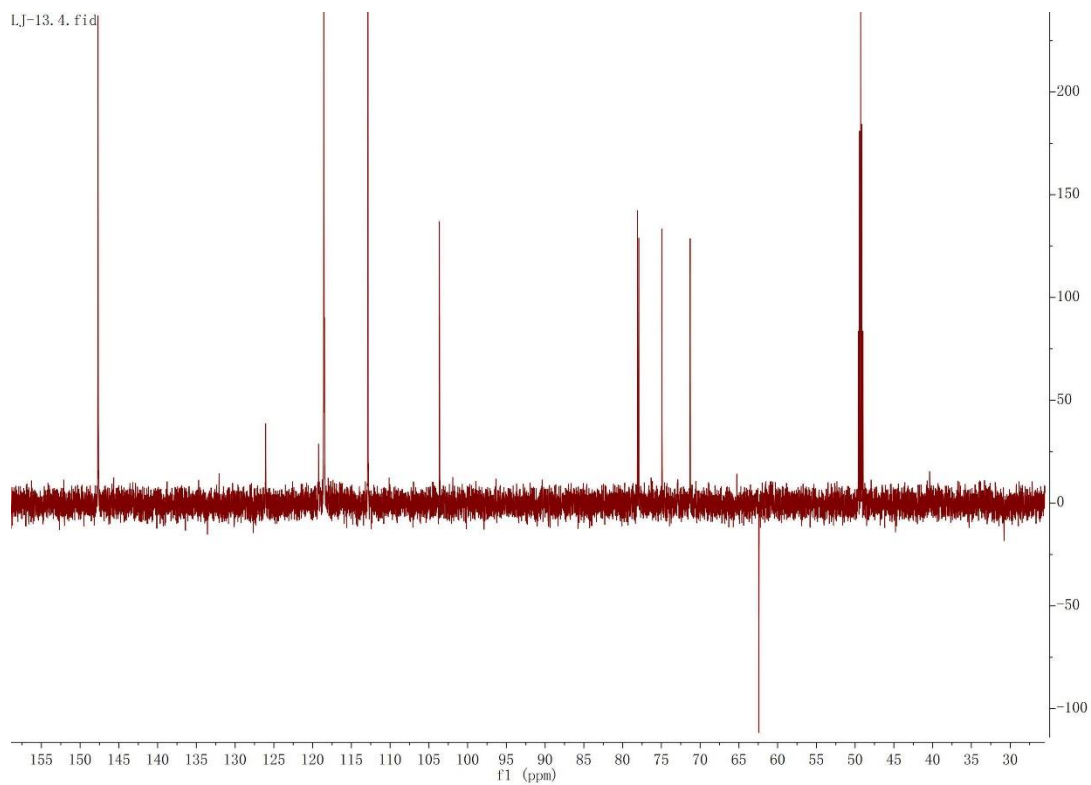


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Figure S17. <sup>13</sup>C NMR (150 MHz) spectrum of Olerapyridine (2) in CD<sub>3</sub>OD-*d*<sub>4</sub>.

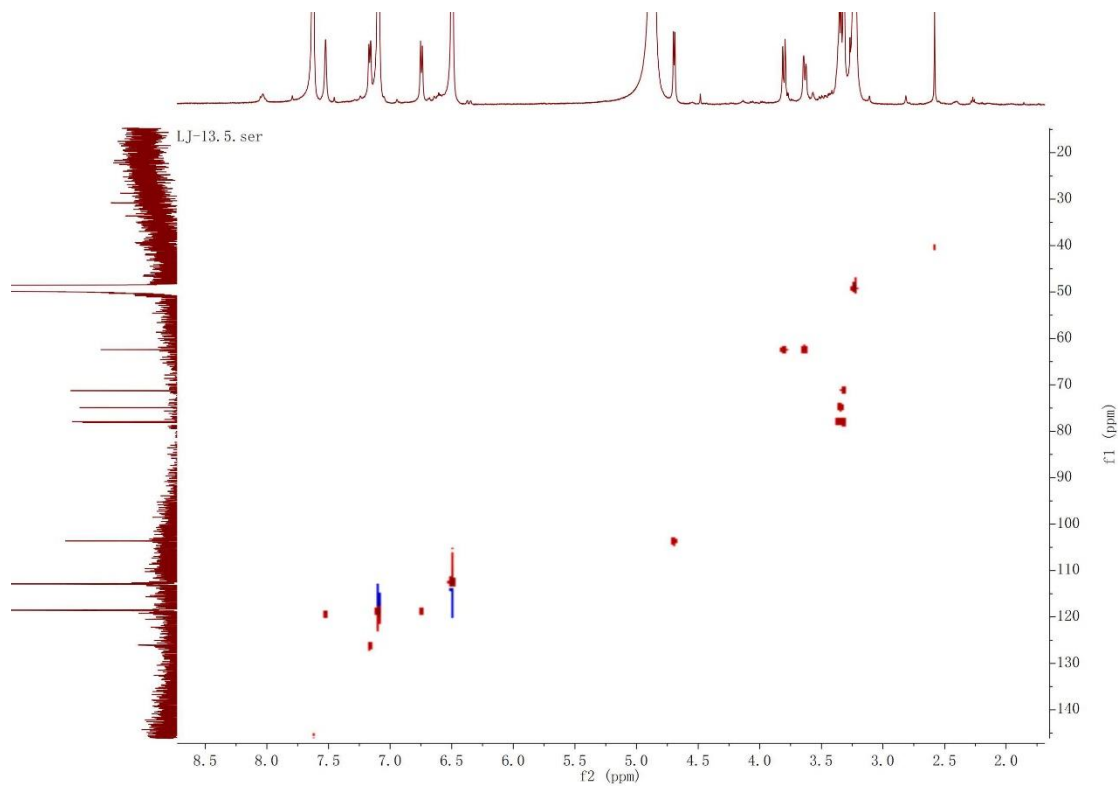




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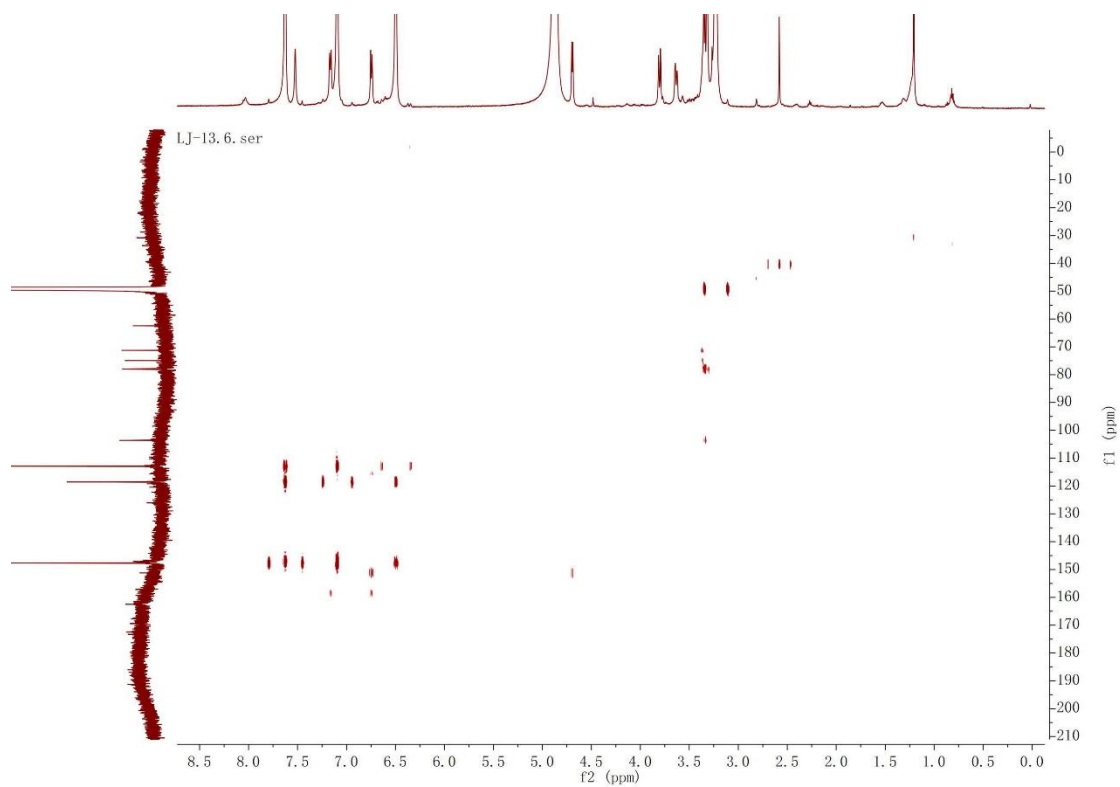
Figure S18. DEPT 135 spectrum of Olerapyridine (**2**) in  $\text{CD}_3\text{OD}-d_4$ .



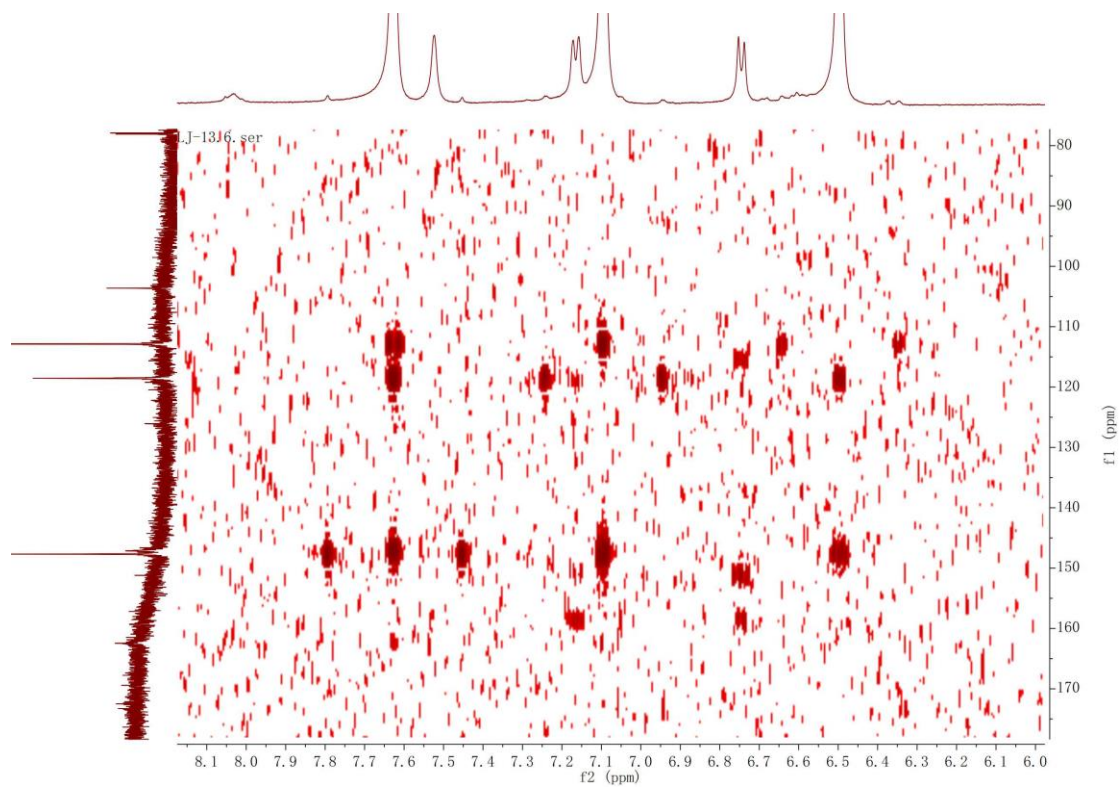
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Figure S19. HSQC spectrum of Olerapyridine (**2**) in  $\text{CD}_3\text{OD}-d_4$ .



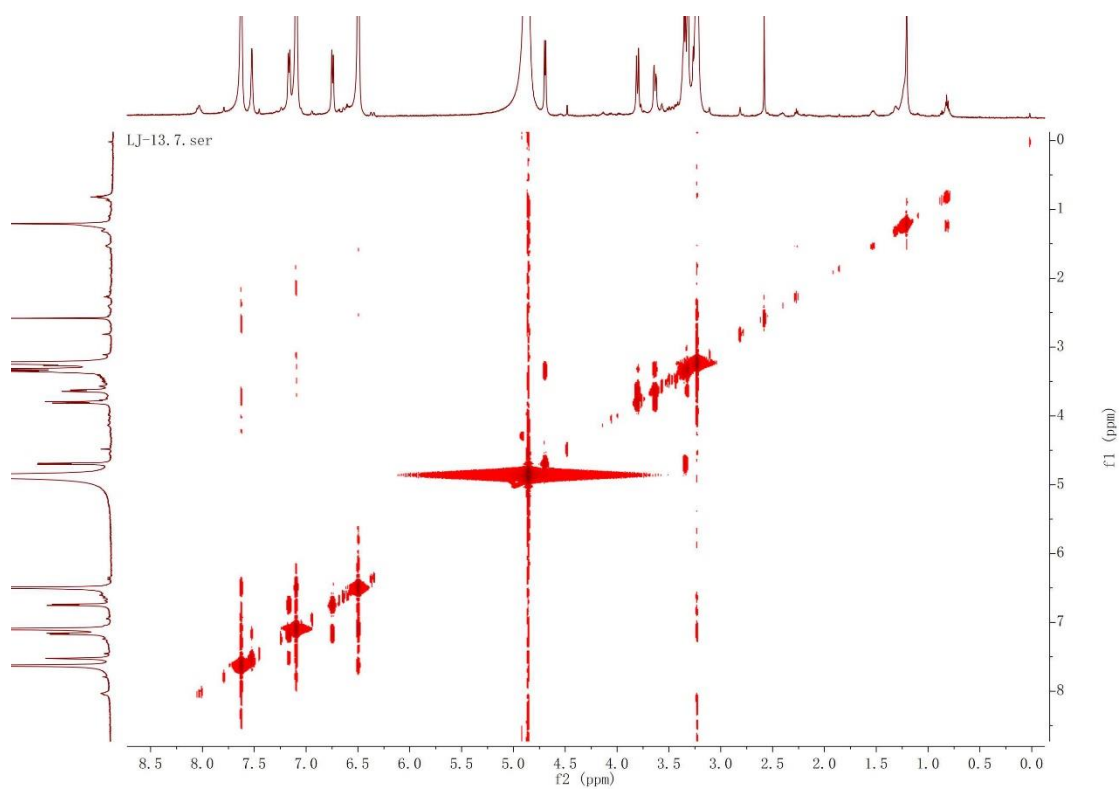
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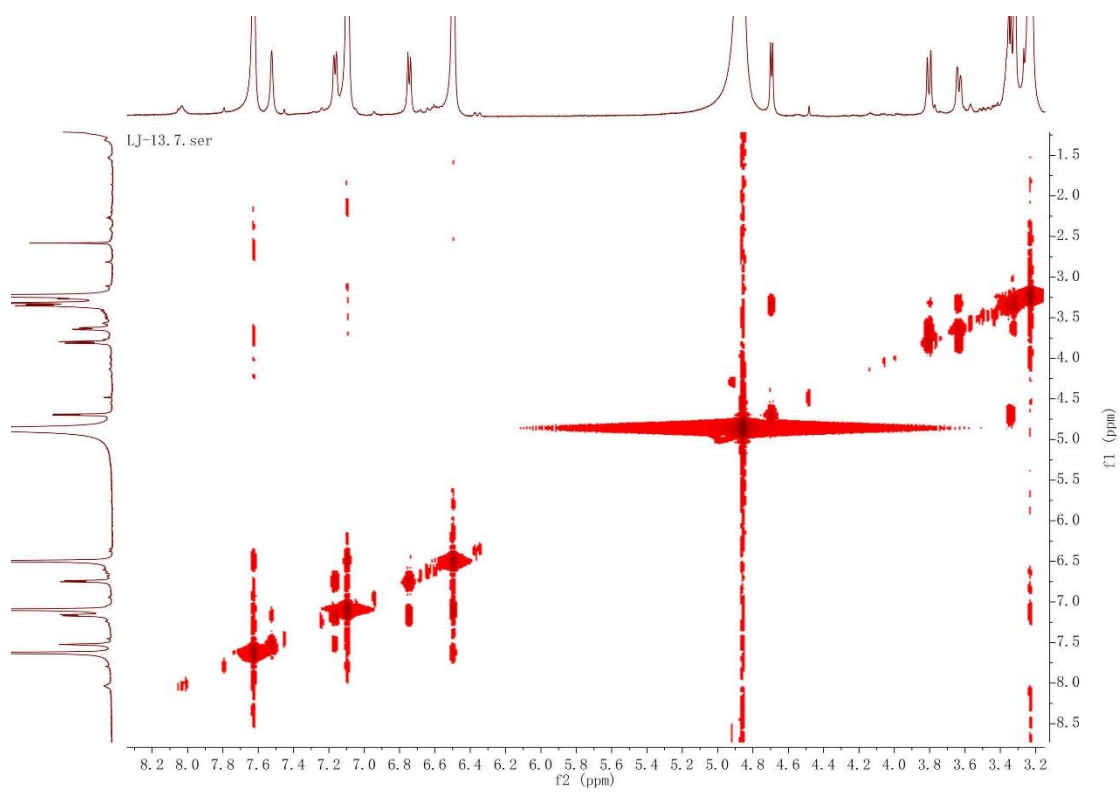
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Figure S20. HMBC spectrum of Olerapyridine (**2**) in CD<sub>3</sub>OD-*d*<sub>4</sub>.



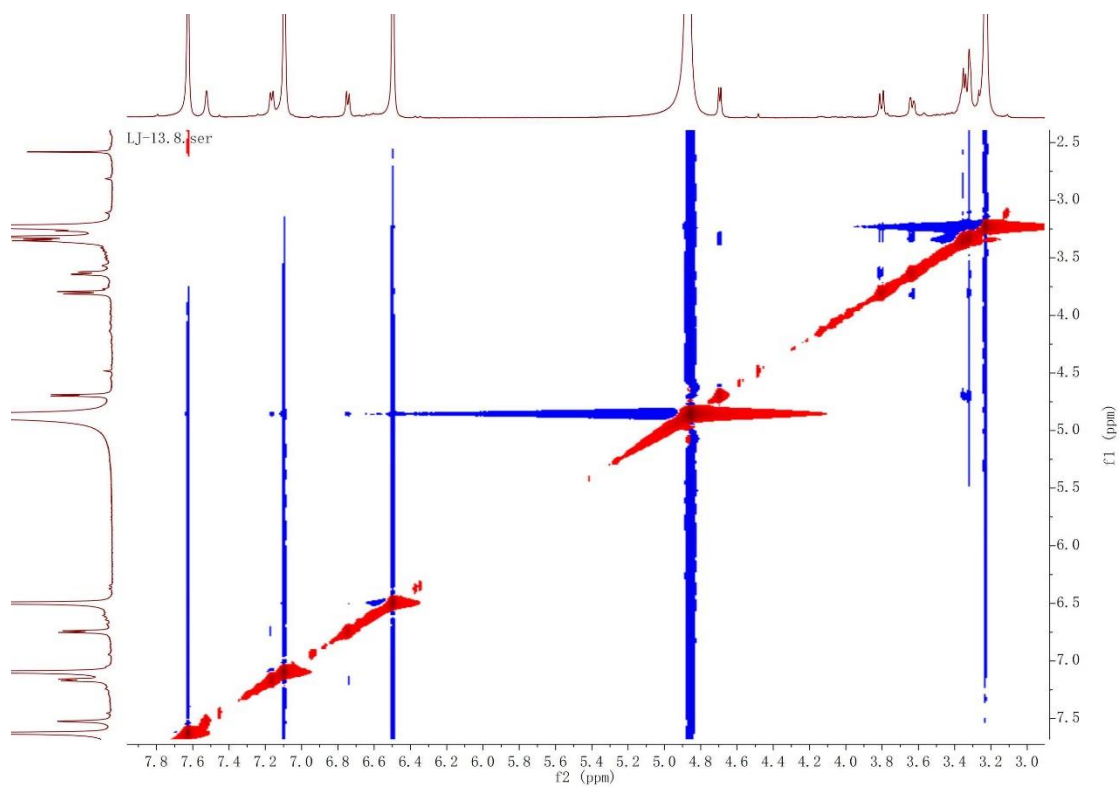
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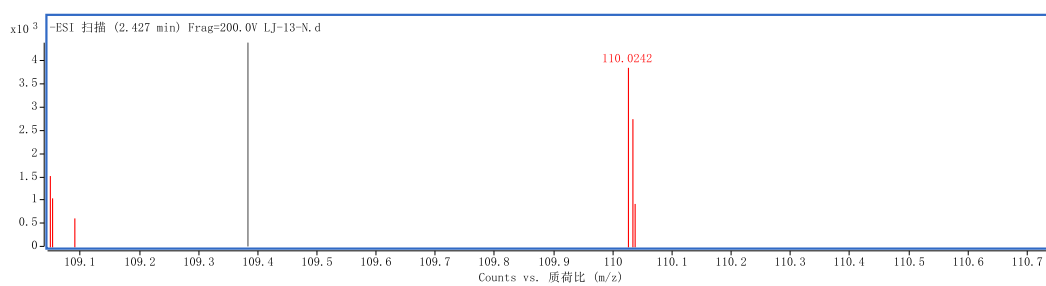
Figure S21.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of Olerapyridine (**2**) in  $\text{CD}_3\text{OD}-d_4$ .



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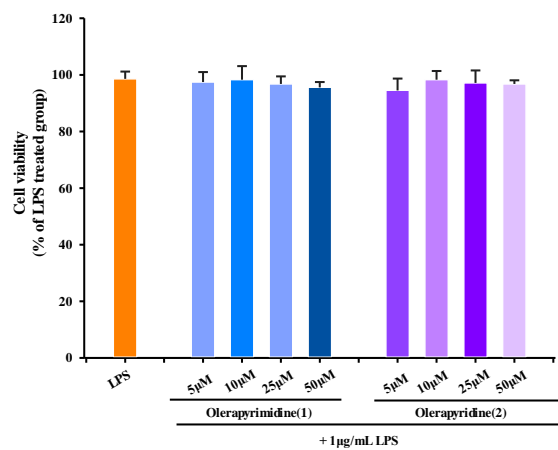
Figure S22. ROESY spectrum of Olerapyridine (2) in CD<sub>3</sub>OD-*d*<sub>4</sub>.



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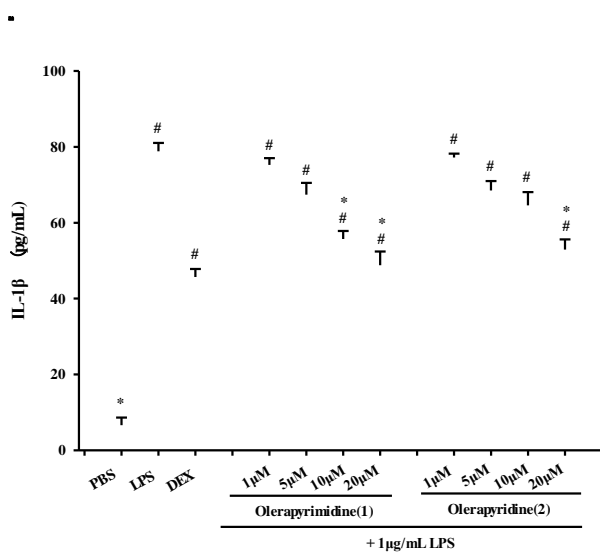
Figure S23. UHPLC-ESI-Q-TOF/MS of Olerapyridine (2).



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149 Figure S24. Cell viability of the LPS-induced macrophage RAW 264.7 cells

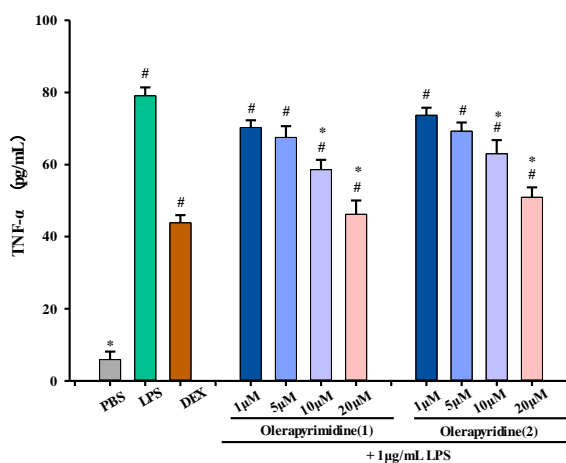
150 pretreated with compounds 1–2.



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152 Figure S25. Inhibitory effect on the secretion of inflammatory cytokine IL-1 $\beta$  in LPS-

153 activated RAW 264.7 macrophages cells pretreated with compounds 1–2.

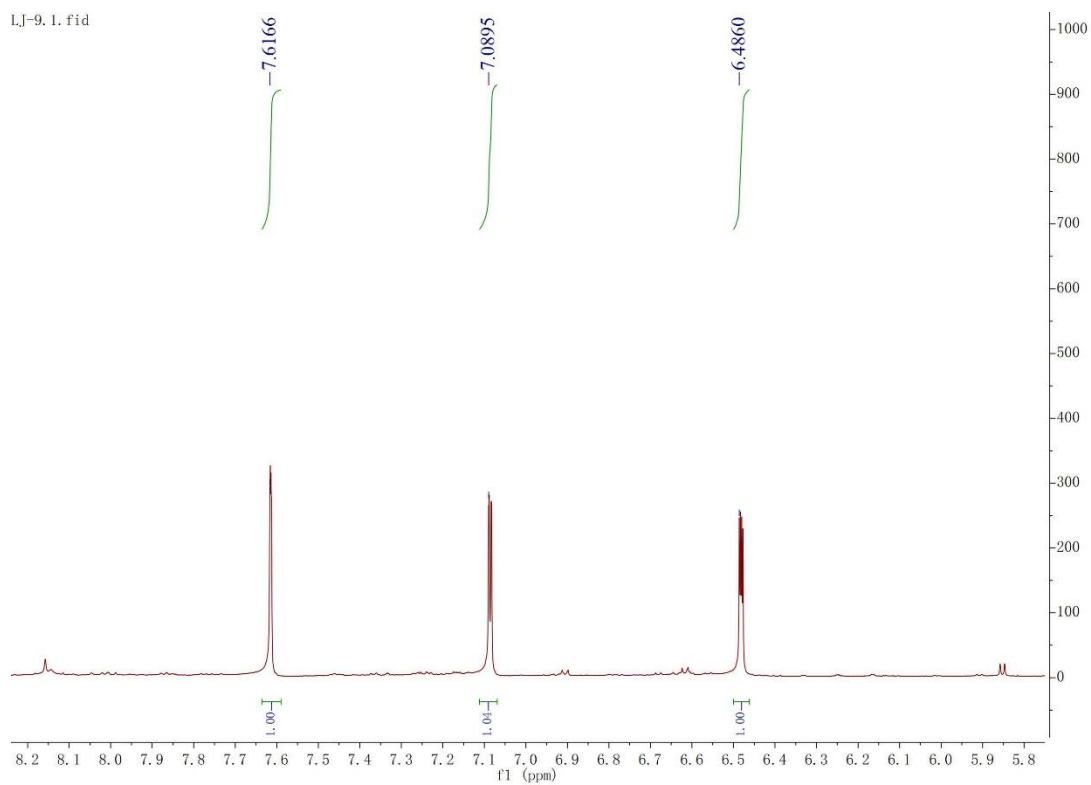


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155 Figure S26. Inhibitory effect on the secretion of inflammatory cytokine TNF- $\alpha$  in

156 LPS-activated RAW 264.7 macrophages cells pretreated with compounds 1–2.

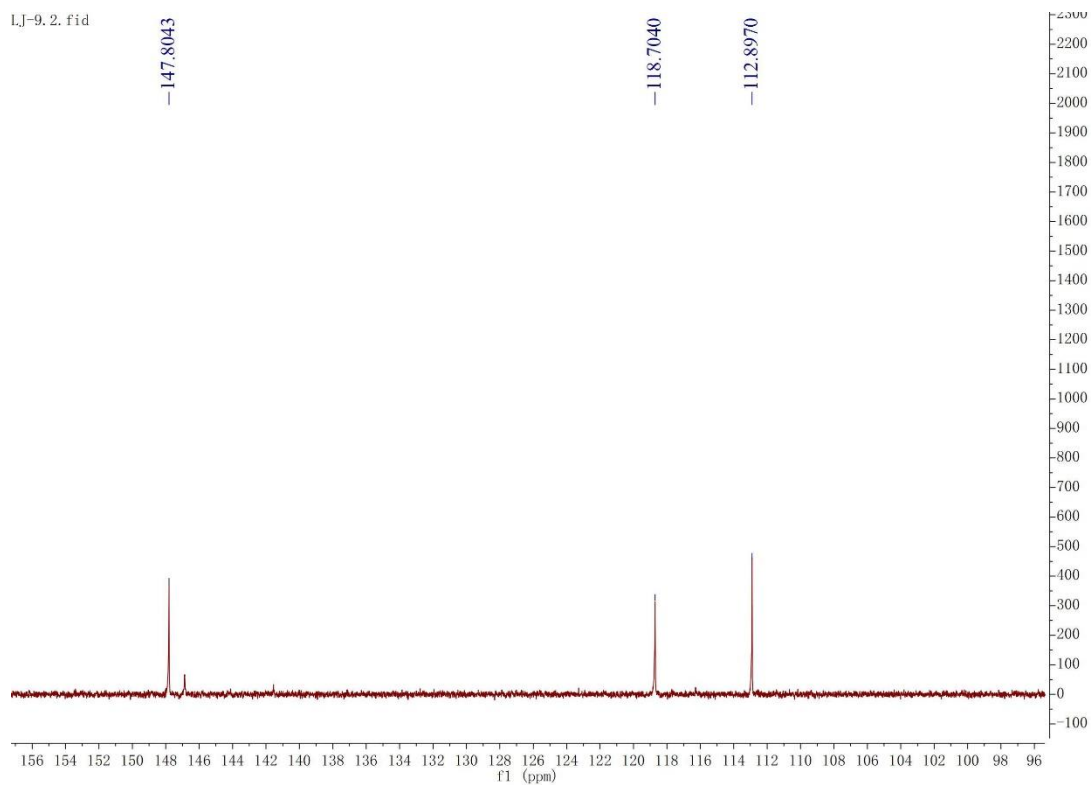
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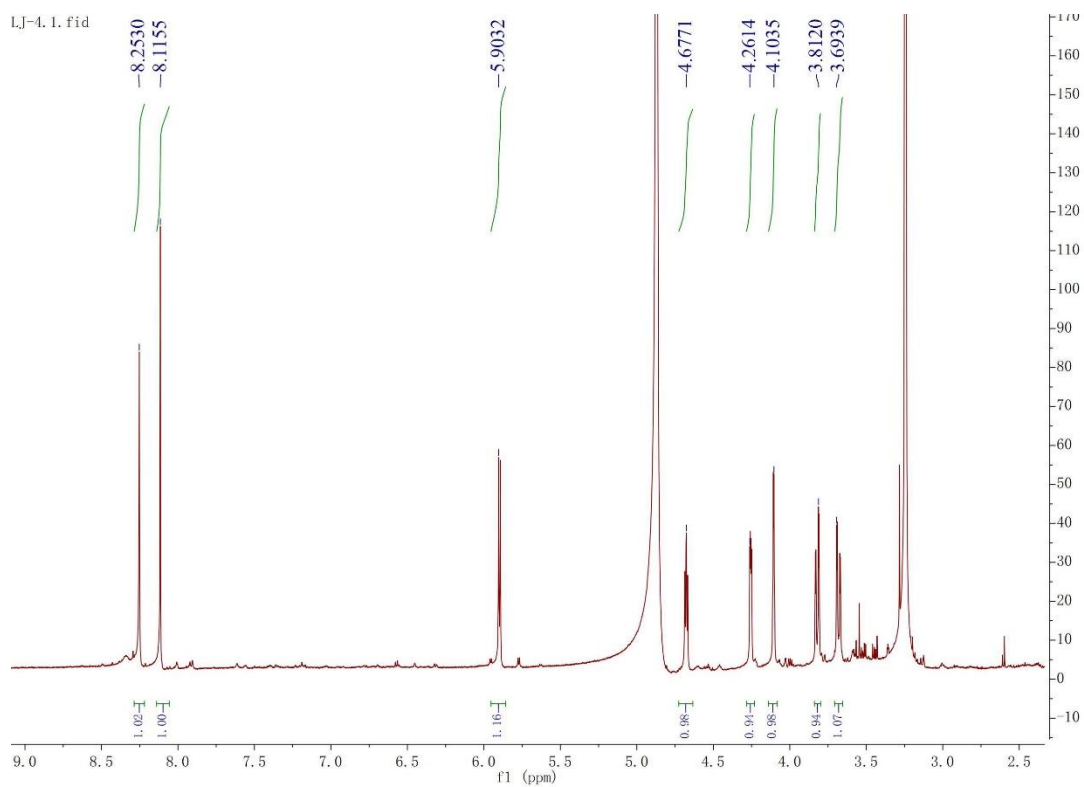
Figure S27.  $^1\text{H}$  NMR (600 MHz) spectrum of compound **3** in  $\text{CD}_3\text{OD}-d_4$ .



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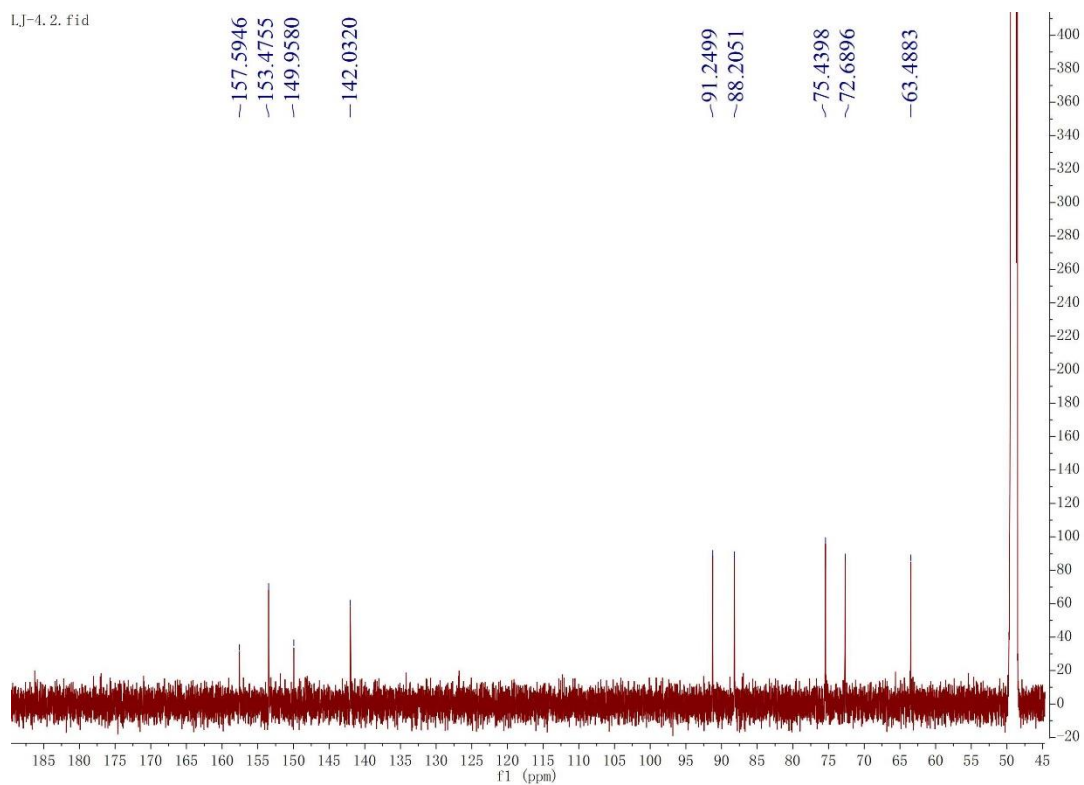
Figure S28.  $^{13}\text{C}$  NMR (150 MHz) spectrum of compound **3** in  $\text{CD}_3\text{OD}-d_4$ .



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Figure S29.  $^1\text{H}$  NMR (600 MHz) spectrum of compound **4** in  $\text{CD}_3\text{OD}-d_4$ .



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Figure S30.  $^{13}\text{C}$  NMR (150 MHz) spectrum of compound **4** in  $\text{CD}_3\text{OD}-d_4$ .

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