

## Supporting Information

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### (8S, 9R)-Dihydroisoflavipucine, a New Isoflavipucine Derivative from the Endophytic Fungi *Botryosphaeria dothidea*

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Table of Contents	Page
<b>Figure S1</b> : The ITS sequences of <i>Botryosphaeria dothidea</i> (D4-2).	3
<b>Figure S2</b> : Extraction, fractionation and purification procedures for compounds <b>1</b> and <b>2</b> .	4
<b>Figure S3</b> : The semi-preparative HPLC separation of <b>1</b> and <b>2</b> .	5
<b>Figure S4</b> : Chiral HPLC chromatogram of compound <b>1</b> .	6
<b>Figure S5</b> : Chiral HPLC chromatogram of compound <b>2</b> .	7
<b>Figure S6</b> : <sup>1</sup> H (400 MHz) NMR Spectroscopic Data of (8S, 9R)-dihydroisoflavipucine ( <b>1</b> ) in MeOD.	8
<b>Figure S7</b> : <sup>13</sup> C (100 MHz) NMR Spectroscopic Data of (8S, 9R)-dihydroisoflavipucine ( <b>1</b> ) in MeOD.	9
<b>Figure S8</b> : DEPT135 Spectrum of (8S, 9R)-dihydroisoflavipucine ( <b>1</b> ) in MeOD.	10
<b>Figure S9</b> : <sup>1</sup> H- <sup>1</sup> H COSY Spectrum of (8S, 9R)-dihydroisoflavipucine ( <b>1</b> ) in MeOD.	11

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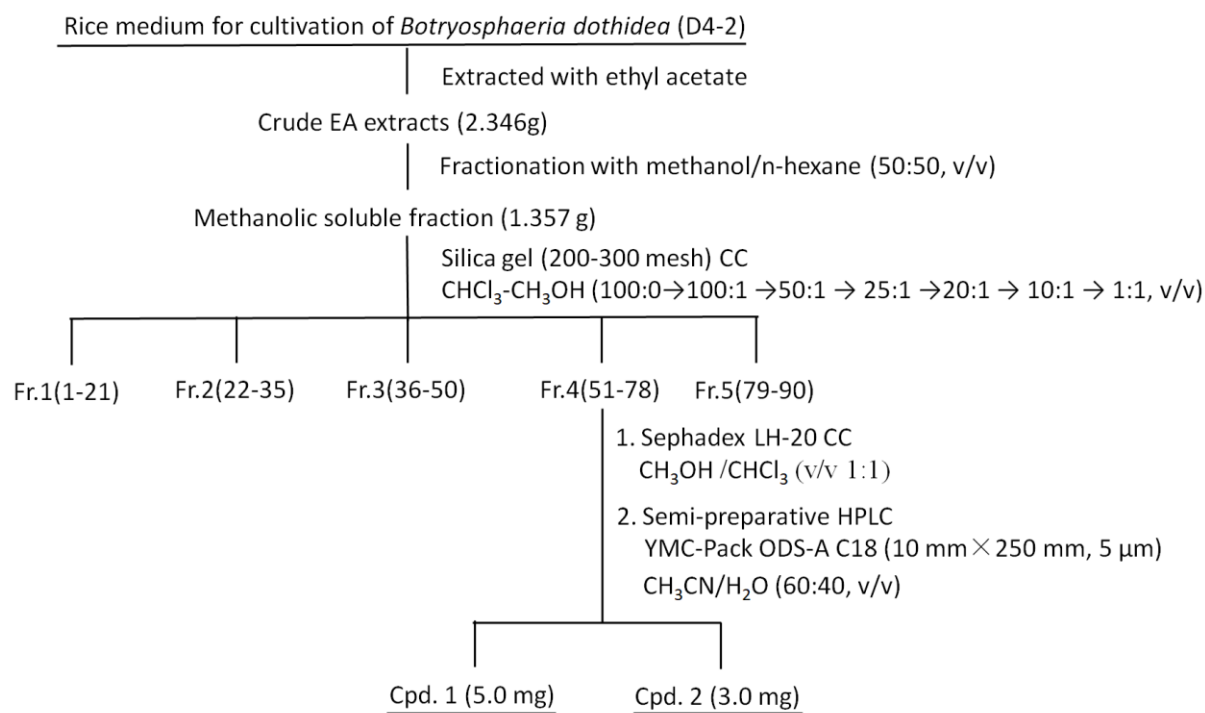
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<b>Figure S10</b> : HSQC Spectrum of (8 <i>S</i> , 9 <i>R</i> )-dihydroisoflavipucine ( <b>1</b> ) in MeOD.	12
<b>Figure S11</b> : HMBC Spectrum of (8 <i>S</i> , 9 <i>R</i> )-dihydroisoflavipucine ( <b>1</b> ) in MeOD.	13
<b>Figure S12</b> : <sup>1</sup> H (400 MHz) NMR Spectroscopic Data of (8 <i>S</i> , 9 <i>S</i> )-dihydroisoflavipucine ( <b>2</b> ) in MeOD.	14
<b>Figure S13</b> : <sup>13</sup> C (100 MHz) NMR Spectroscopic Data of (8 <i>S</i> , 9 <i>S</i> )-dihydroisoflavipucine ( <b>2</b> ) in MeOD.	15
<b>Figure S14</b> : HRESIMS Spectrum of (8 <i>S</i> , 9 <i>R</i> )-dihydroisoflavipucine ( <b>1</b> ).	16
<b>Figure S15</b> : HRESIMS Spectrum of (8 <i>S</i> , 9 <i>S</i> )-dihydroisoflavipucine ( <b>2</b> ).	17
<b>Figure S16</b> : CD Spectrum of (8 <i>S</i> , 9 <i>R</i> )-dihydroisoflavipucine ( <b>1</b> ) and (8 <i>S</i> , 9 <i>S</i> )-dihydroisoflavipucine ( <b>2</b> ) in MeOH.	18
<b>Figure S17</b> : Antiproliferative effects of (8 <i>S</i> , 9 <i>R</i> )-dihydroisoflavipucine ( <b>1</b> ) and (8 <i>S</i> , 9 <i>S</i> )-dihydroisoflavipucine ( <b>2</b> ) on breast cancer cells MDA-MB-231, MCF7 and 4T1.	19
<b>Figure S18</b> : The Scifinder search for the new compound (8 <i>S</i> , 9 <i>R</i> )- <b>1</b> .	20

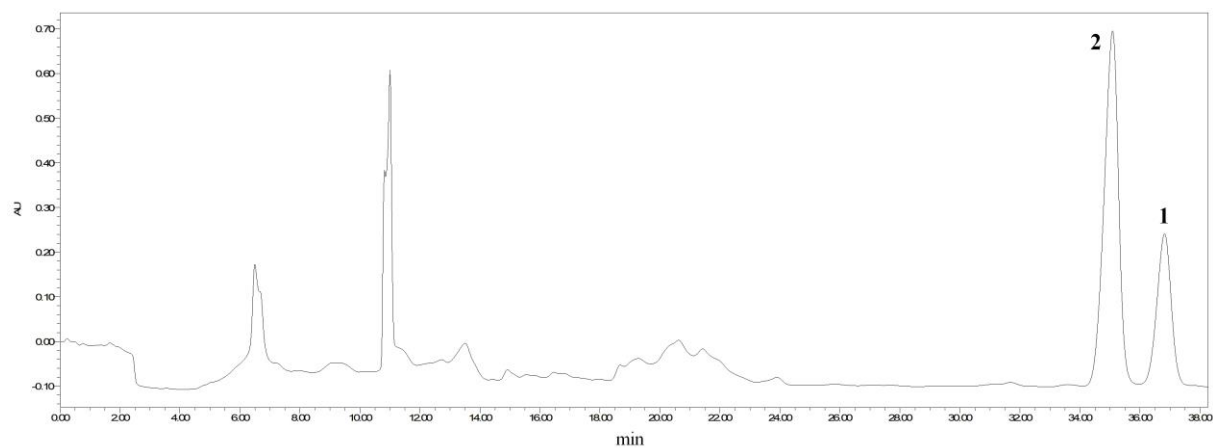
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TGTGTTGGGCCCGTCCCCCGCCGGGGGACGCGCCCTAAAAGCAGCGGCAGCA  
CCGCGTCTTGAGTTCTCGAGCGCATGG

**Figure S1:** The ITS sequences of *Botryosphaeria dothidea* (D4-2).



**Figure S2 :** Extraction, fractionation and purification procedures for compounds **1** and **2**



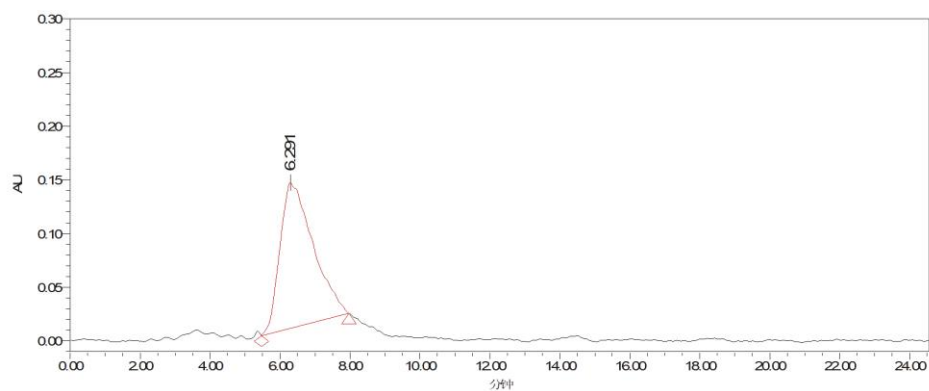
**Figure S3** : The semi-preparative HPLC separation of **1** ( $t_R=35.5$  min) and **2** ( $t_R=34.5$  min).

Column: YMC-Pack ODS-A C18 (10 mm  $\times$  250 mm, 5  $\mu$ m)

Mobile phase: Acetonitrile/H<sub>2</sub>O (60:40)

Flow rate: 1.5 mL/min

Wavelength: 254 nm



#### Analysis results

Compound	Retention Time (min)	Height ( $\mu\text{V}$ )	Area ( $\mu\text{V}\cdot\text{S}$ )	Area(%)
<b>1</b>	6.291	136144	8902462	100%

Column: Daicel chiralpak IA-H (4.6 mm  $\times$  250mmL, 5  $\mu\text{m}$ )

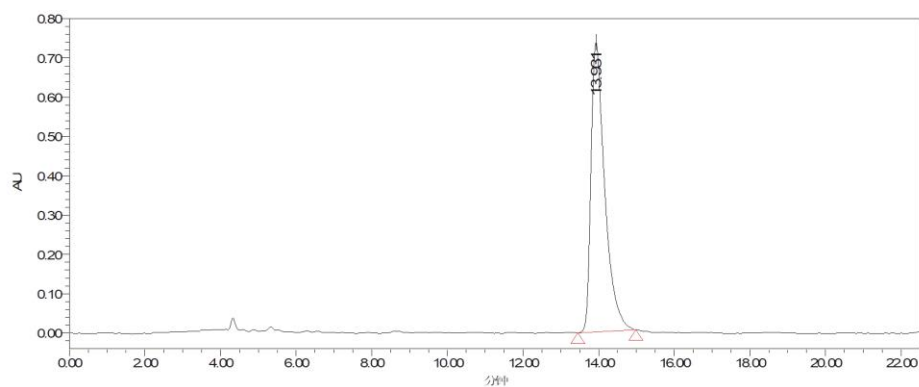
Mobile phase: n-hexane/ Isopropanol (90:10)

Flow rate: 1.0 mL/min

Wavelength: 210 nm

Injection volume : 5.0 $\mu\text{L}$

**Figure S4** : Chiral HPLC chromatogram of compound **1** ( $t_{\text{R}}$ =6.291 min).



#### Analysis results

Compound	Retention Time (min)	Height ( $\mu\text{V}$ )	Area ( $\mu\text{V}\cdot\text{S}$ )	Area(%)
<b>2</b>	13.931	737468	18623660	100%

Column: Daicel chiralpak IA-H (4.6 mm  $\times$  250mmL, 5  $\mu\text{m}$ )

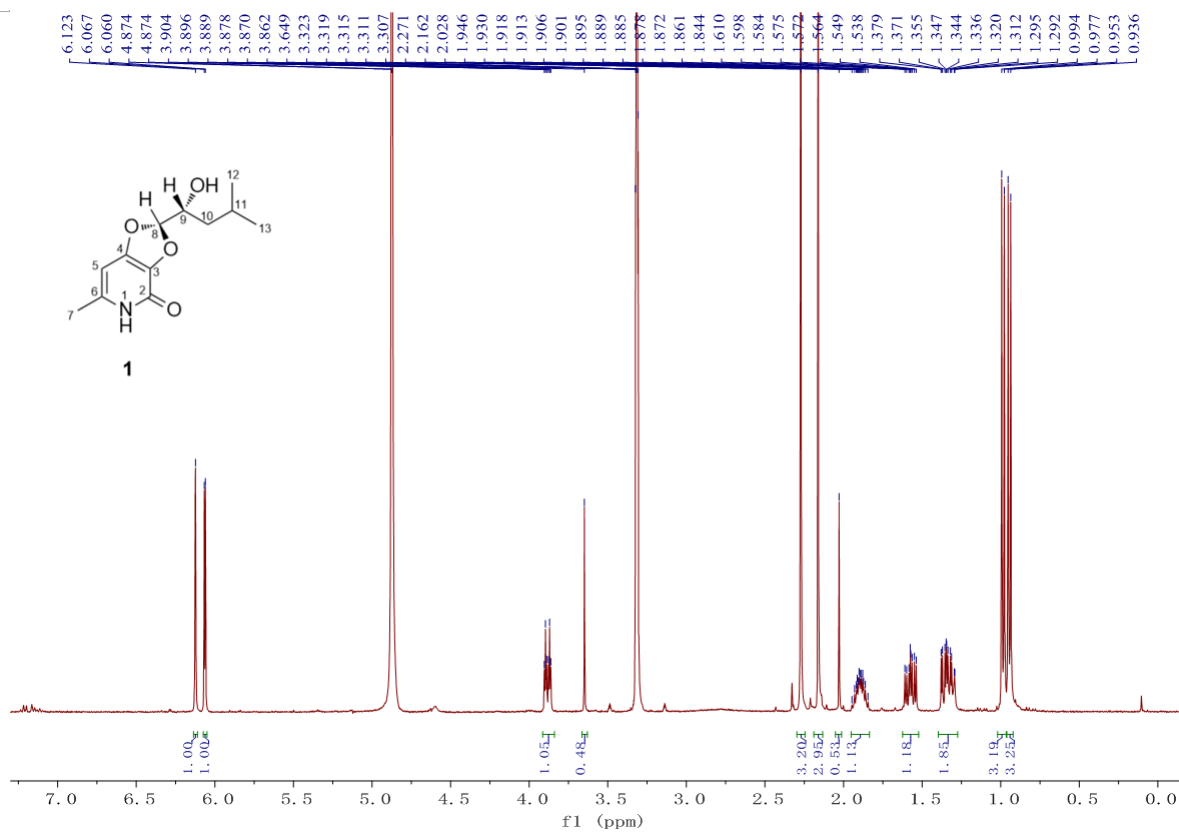
Mobile phase: n-hexane/ Isopropanol (90:10)

Flow rate: 1.0 mL/min

Wavelength: 210 nm

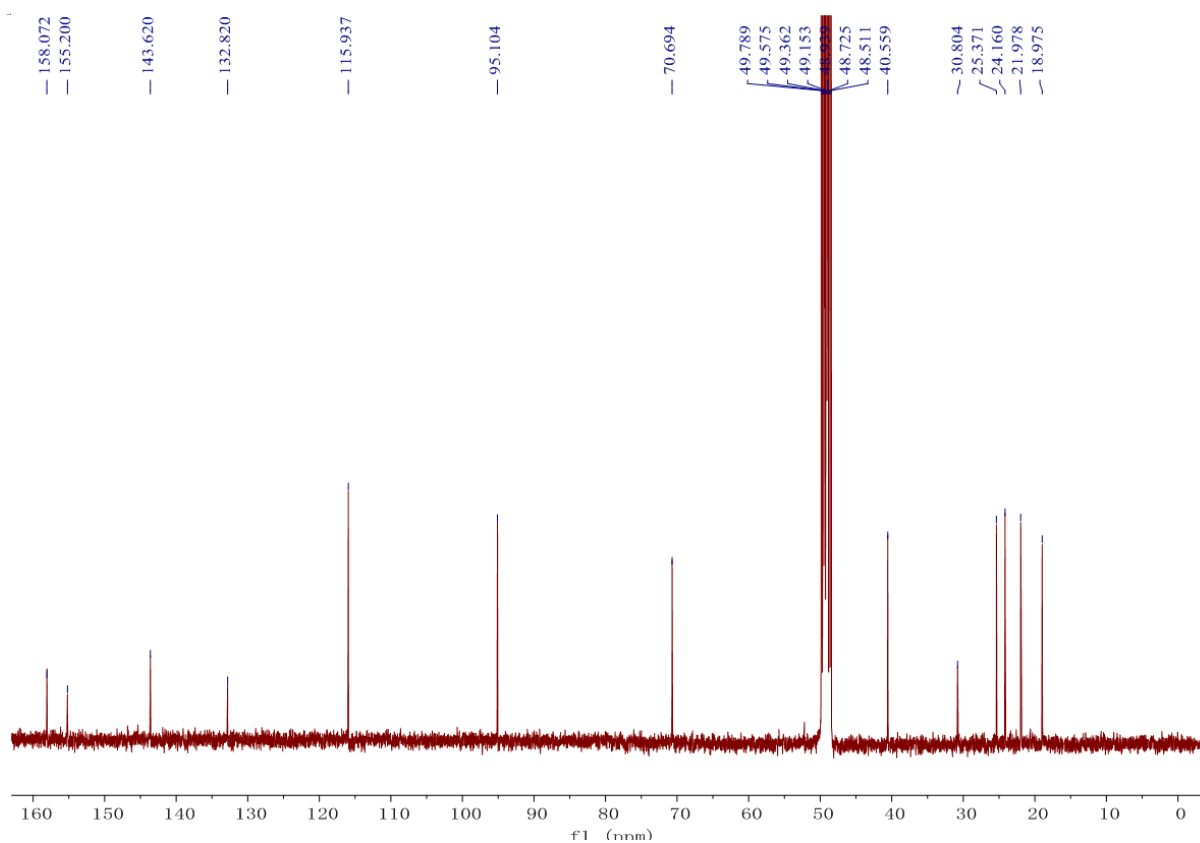
Injection volume : 5.0 $\mu\text{L}$

**Figure S5** : Chiral HPLC chromatogram of compound **2** ( $t_{\text{R}}$ =13.931 min).

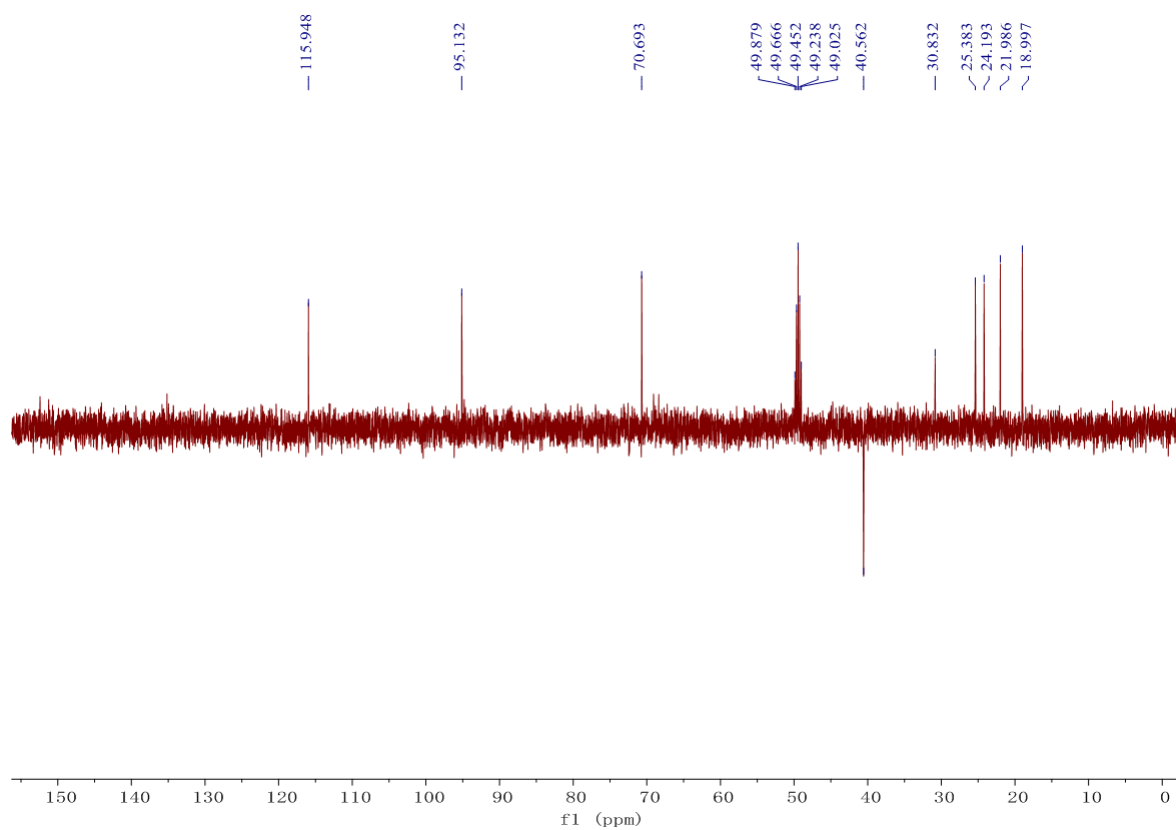


**Figure S6 :** <sup>1</sup>H (400 MHz) NMR Spectroscopic Data of (8S, 9R)-Dihydroisoflavipucine (**1**) in MeOD.

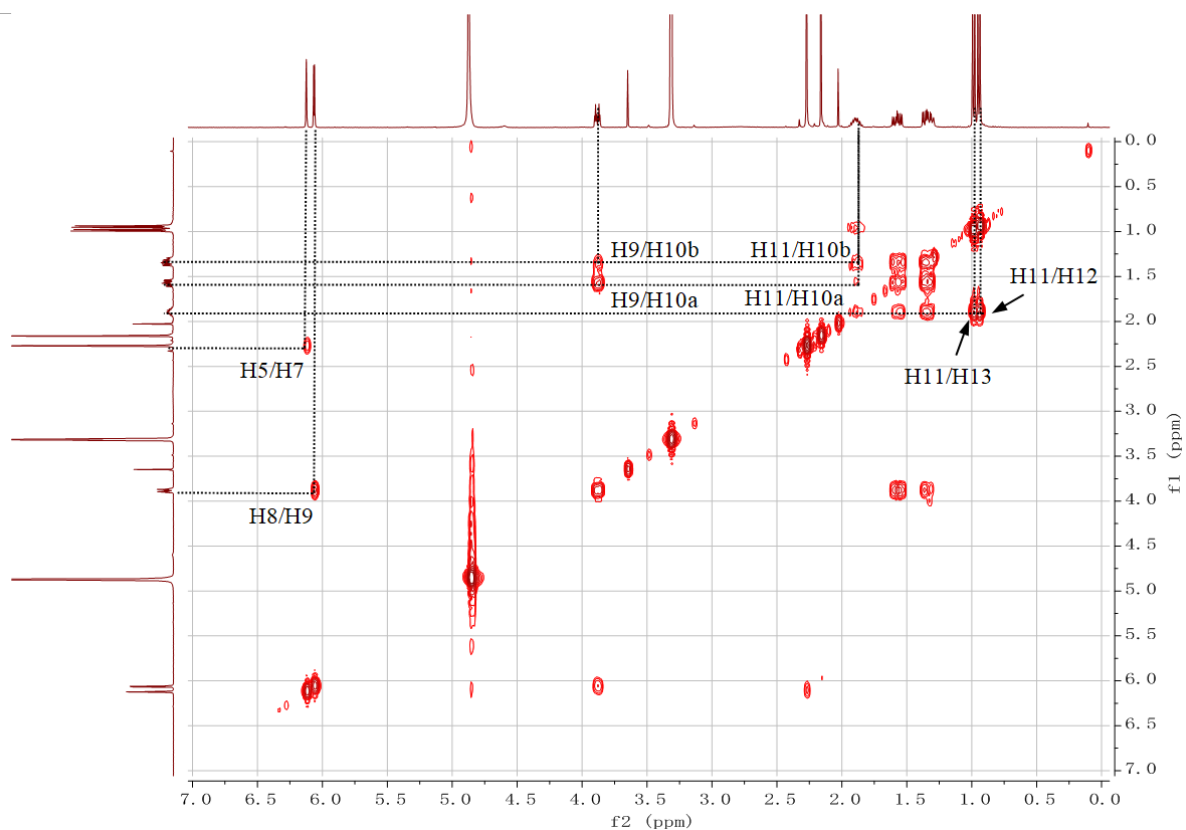




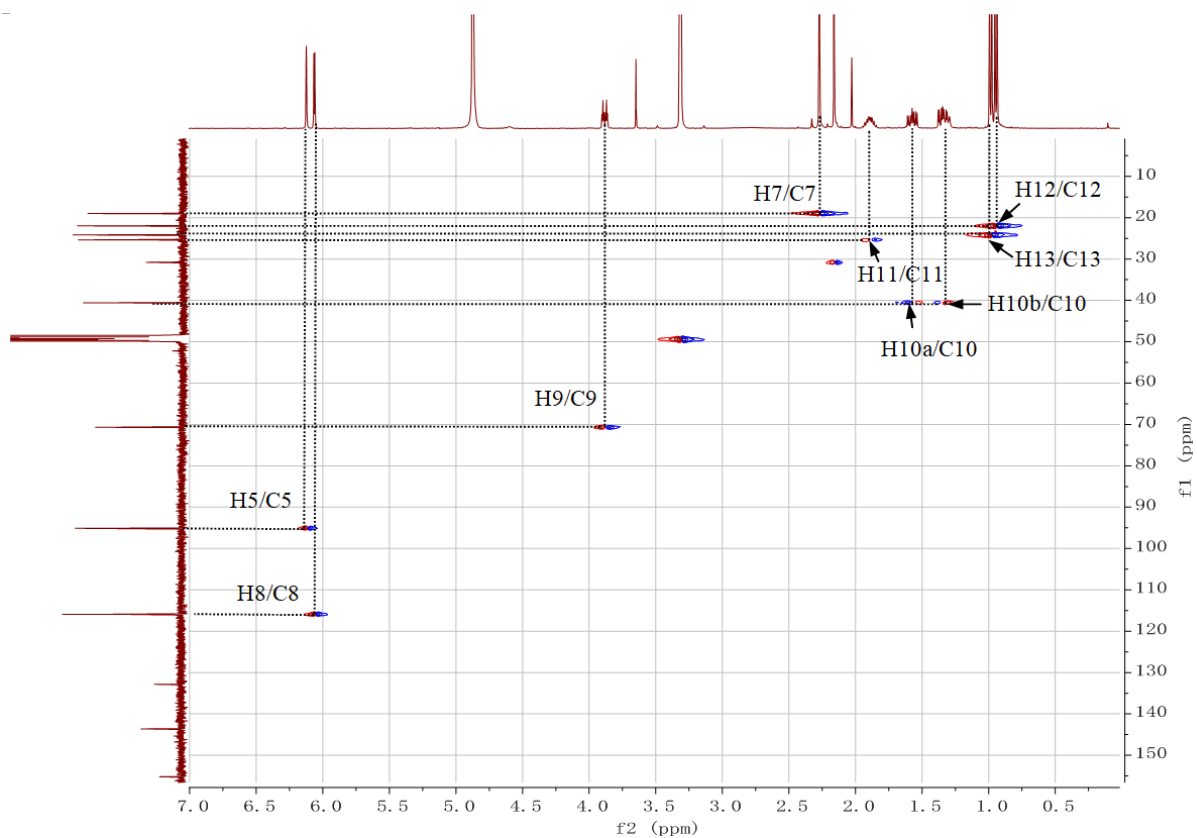
**Figure S7 :**  $^{13}\text{C}$  (100 MHz) NMR Spectroscopic Data of (8S, 9R)-Dihydroisoflavipucine (**1**) in MeOD.



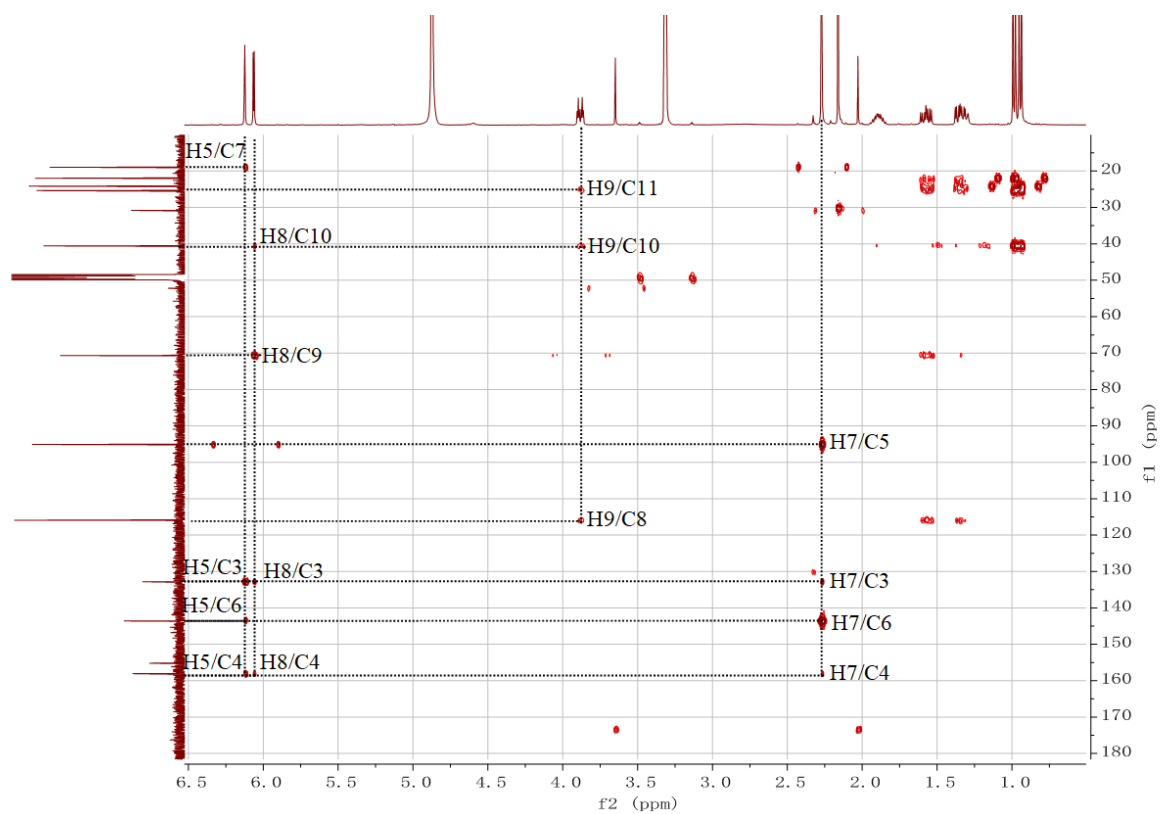
**Figure S8** : DEPT135 Spectrum of (8S, 9R)-Dihydroisoflavipucine (**1**) in MeOD.



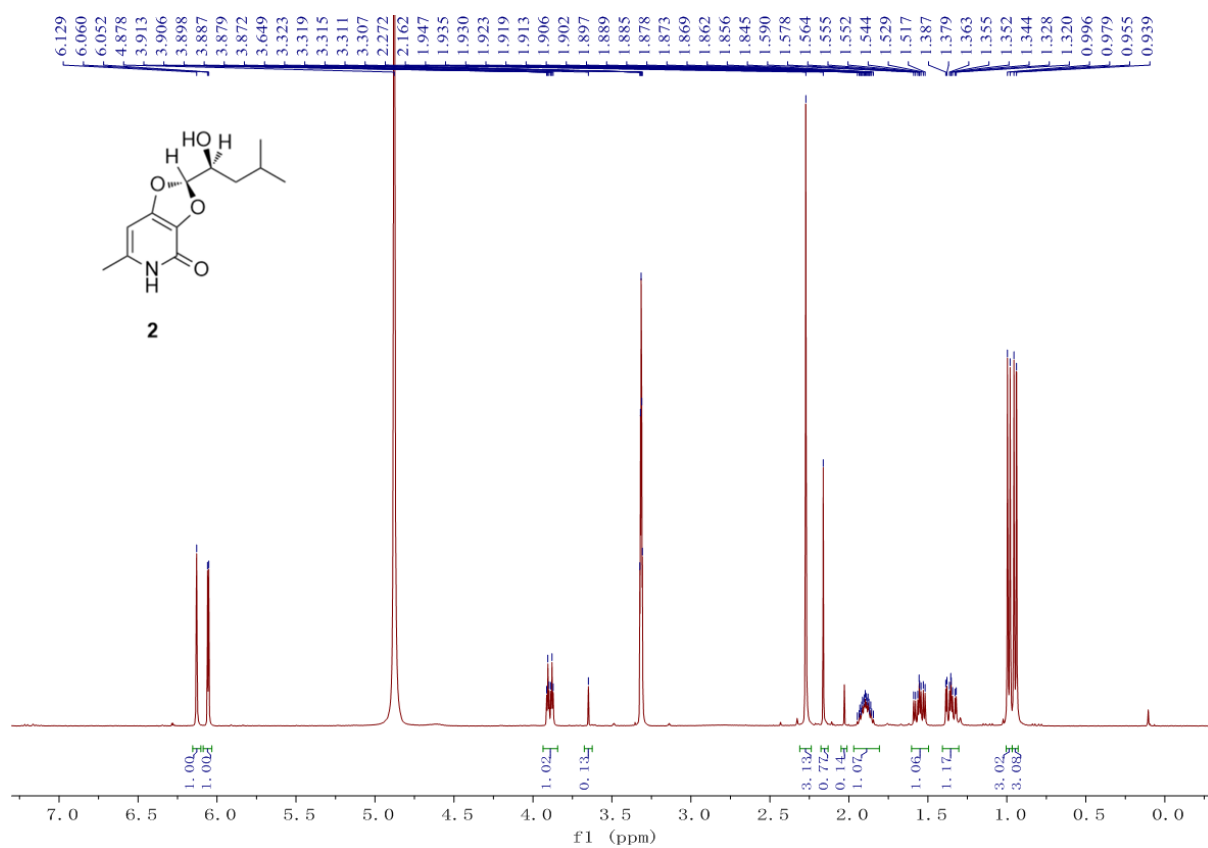
**Figure S9** :  $^1\text{H}$ - $^1\text{H}$  COSY Spectrum of (8*S*, 9*R*)-Dihydroisoflavipicine (**1**) in MeOD.



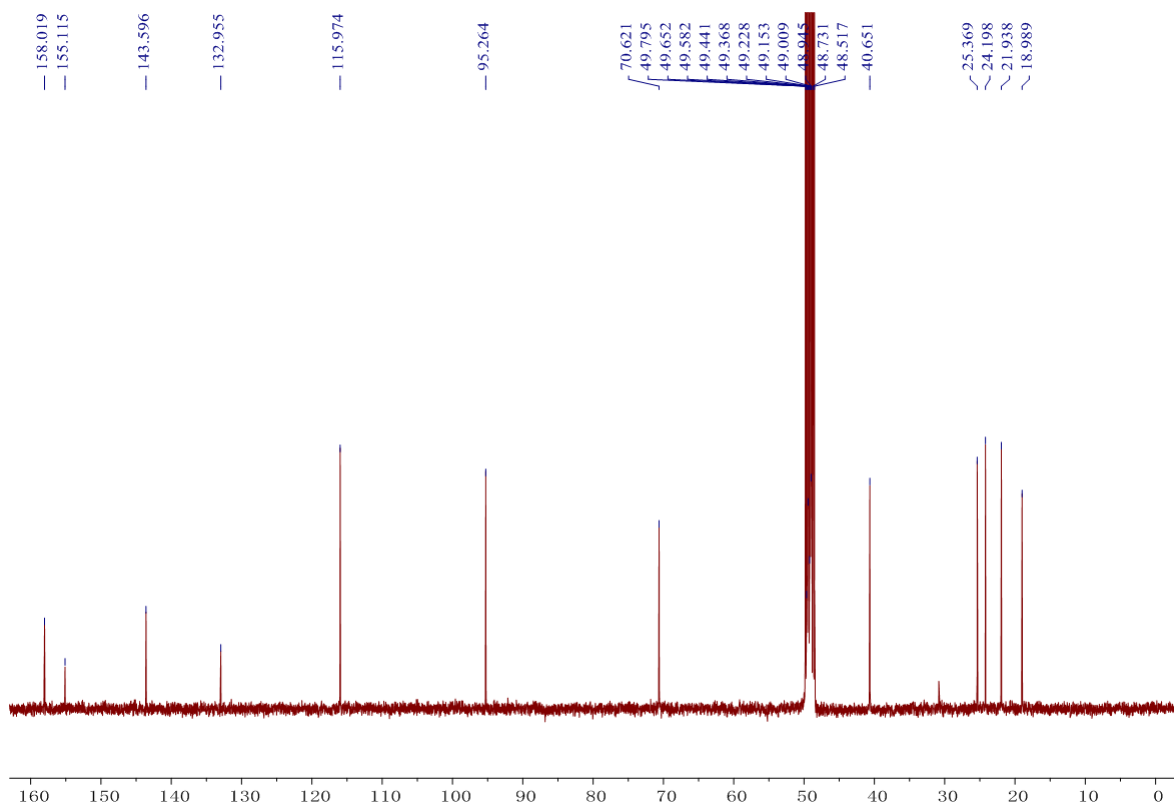
**Figure S10** : HSQC Spectrum of (8S, 9R)-Dihydroisoflavipucine (1) in MeOD.



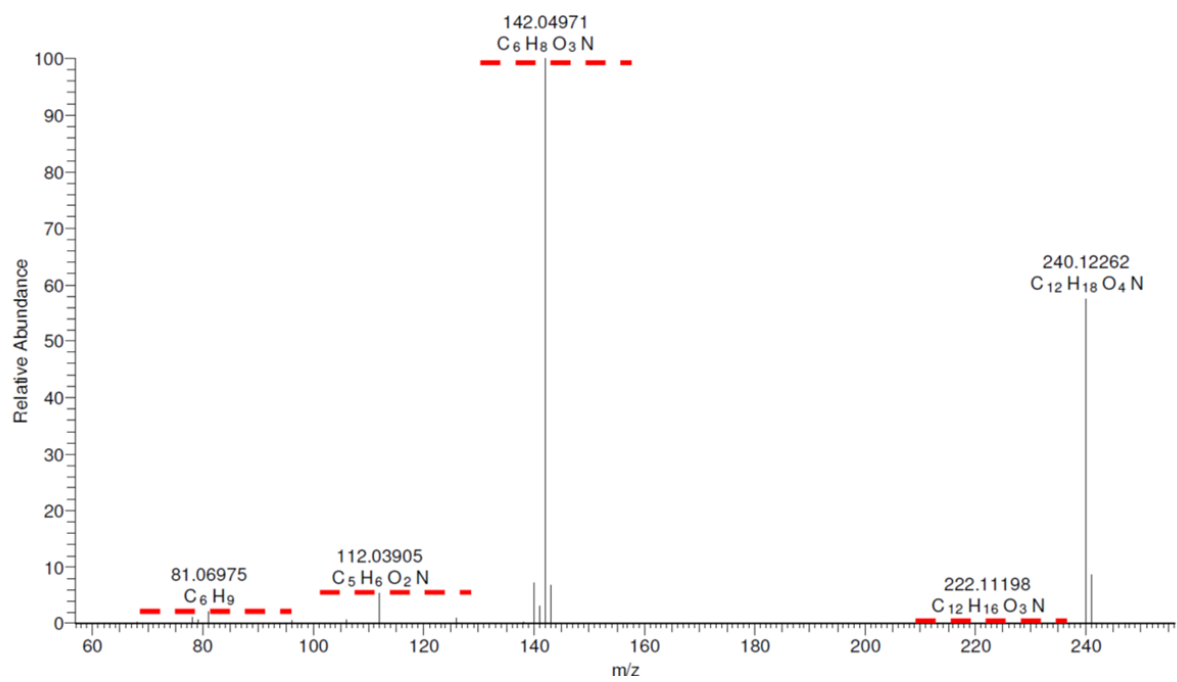
**Figure S11** : HMBC Spectrum of (8S, 9R)-Dihydroisoflavipucine (1) in MeOD.



**Figure S12** :  $^1\text{H}$  (400 MHz) NMR Spectroscopic Data of (8S, 9S)-Dihydroisoflavipucine (2) in MeOD.

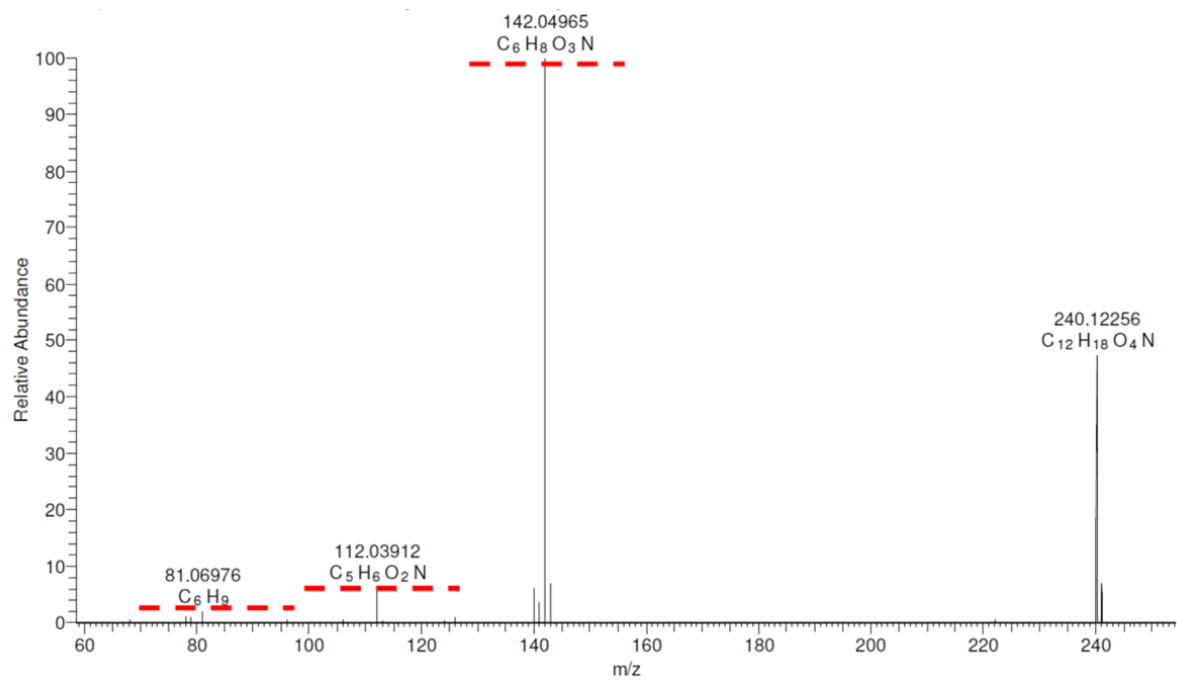


**Figure S13 :**  $^{13}\text{C}$  (100 MHz) NMR Spectroscopic Data of (8*S*, 9*S*)-Dihydroisoflavipucine (**2**) in MeOD.

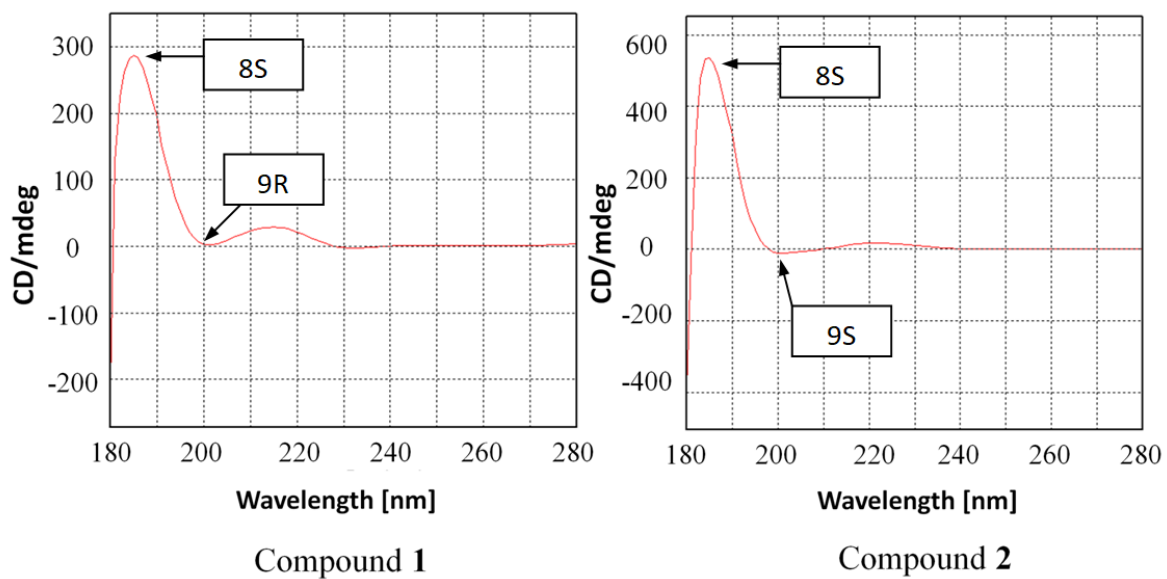


**Figure S14** : HRESIMS Spectrum of (8S, 9R)-Dihydroisoflavipucine (**1**).

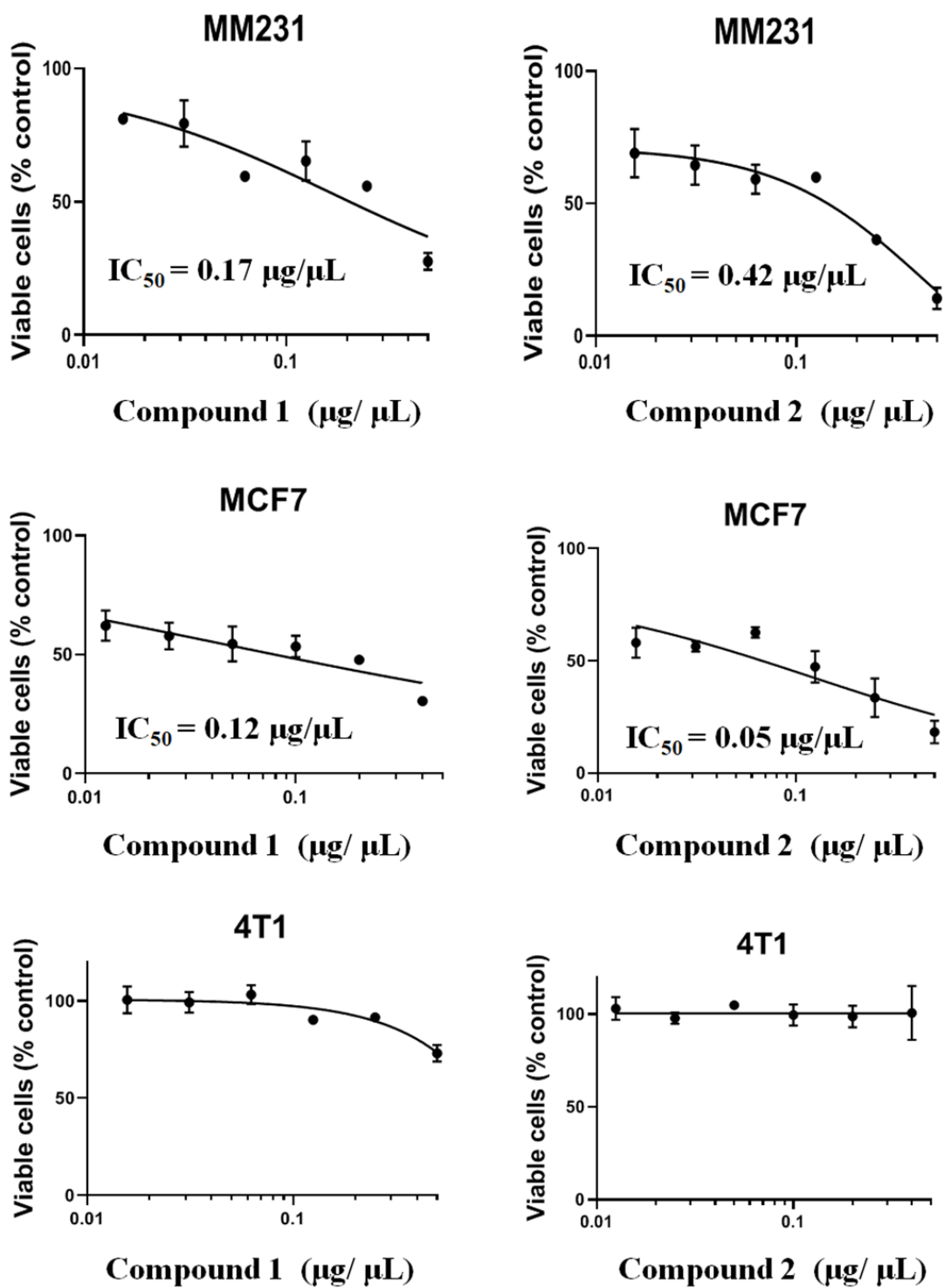




**Figure S15** : HRESIMS Spectrum of (8S, 9S)-Dihydroisoflavipucine (**2**).



**Figure S16** :CD Spectrum of (8S, 9R)-dihydroisoflavipucine (**1**) and (8S, 9S)-dihydroisoflavipucine (**2**) in MeOH.



**Figure S17** : Antiproliferative effects of (8S, 9R)-dihydroisoflavipucine (1) and (8S, 9S)-dihydroisoflavipucine (2) on breast cancer cells MDA-MB-231, MCF7 and 4T1.

Substances search results showing chemical structures and their absolute stereochemistry:

- 1. 65023-24-7: Absolute stereochemistry, Rotation (+).  $(8R,9S)-1$
- 2. 1337957-99-9: Absolute stereochemistry, Rotation (-).  $(8R,9S)-1$
- 4. 1967778-35-3: Absolute stereochemistry, Rotation (+).  $(8S,9S)-1$

The literatures mentioned above and their analysis were followed by a list.

Identified as dihydroisoflavipucine by MS data and stereo-configurations were dimiss.

$(8R,9S)-1$

Identified as dihydroisoflavipucine by MS data and stereo-configurations were dimiss.

$(8R,9S)-1$

Biosynthetic pathways of dihydroisoflavipucine was elucidated and stereo-configurations were dimiss.

Dihydroisoflavipucine was firstly identified through planar structures

$(8R,9S)-1$  and  $(8S,9S)-1$

$(8R,9S)-1$

Figure S18: The Scifinder search for the new compound (8S, 9R)-1.