

# Australindolones, new aminopyrimidine substituted indolone alkaloids from an Antarctic tunicate *Synoicum* sp.

Sofia Kokkaliari,<sup>1</sup> Kim Pham,<sup>1</sup> Nargess Shahbazi,<sup>2,5</sup> Laurent Calcul,<sup>1</sup> Lukasz Wojtas,<sup>1</sup> Nerida G. Wilson,<sup>3</sup> Alexander D. Crawford<sup>2,4</sup> and Bill J. Baker<sup>1,4,\*</sup>

<sup>1</sup> Department of Chemistry, University of South Florida, 4202 E. Fowler Ave., CHE205, Tampa, Florida 33620, USA

<sup>2</sup> Department of Preclinical Sciences and Pathology, Norwegian University of Life Sciences (NMBU), Ås, Norway

<sup>3</sup> Research & Collections, Western Australia Museum, 49 Kew Street, Welshpool 6106 and School of Biological Sciences, University of Western Australia, 35 Stirling Highway, Crawley 6009, Western Australia, Australia

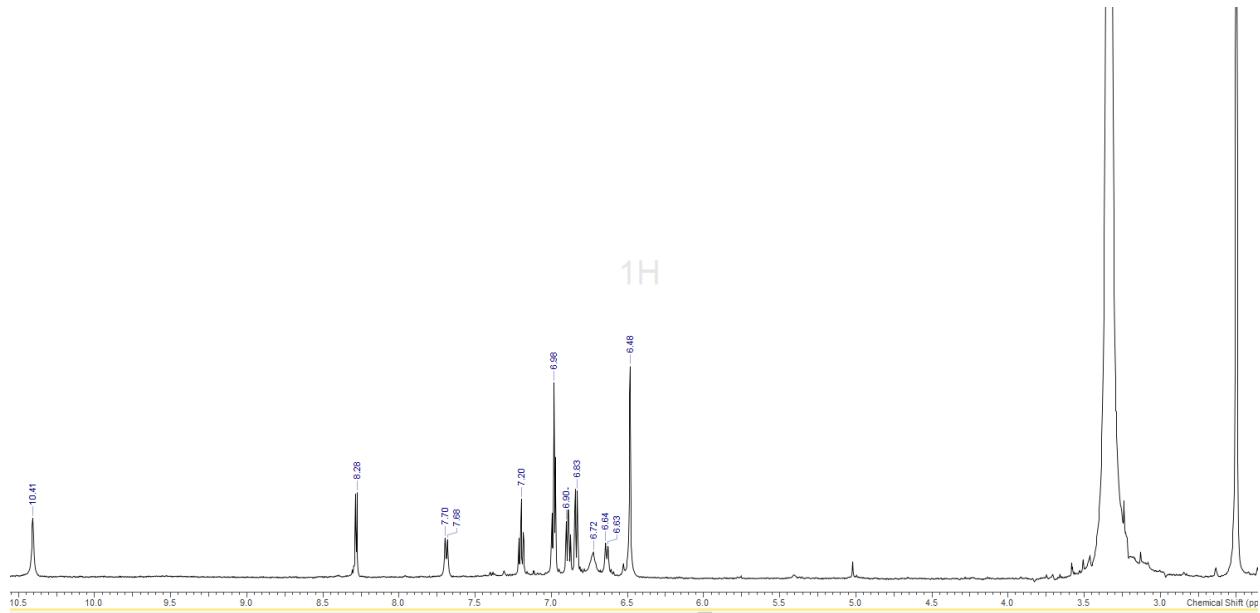
<sup>4</sup> Institute for Arctic and Antarctic Biodiscovery, Medford, Oregon, USA

<sup>5</sup> Current address: Institute of Molecular Biology, University of Innsbruck, Innsbruck, Austria

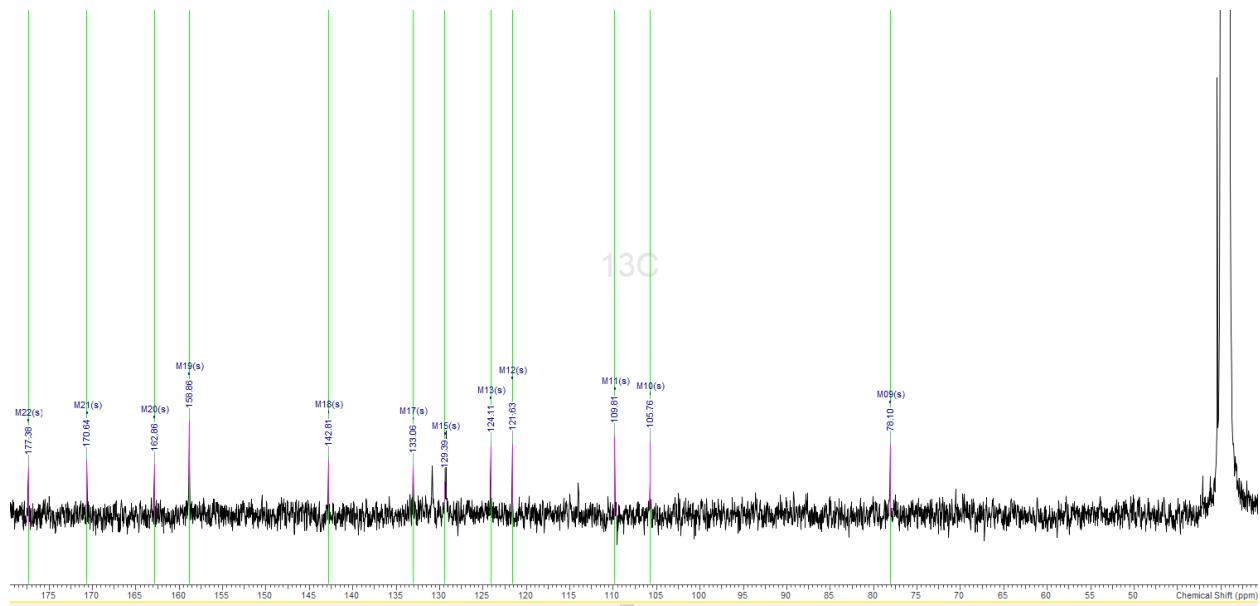
\* Correspondence: bjbaker@usf.edu

Data	Page
Figure S1. Australindolone A (1) <sup>1</sup> H NMR spectrum (500 MHz, DMSO-d <sub>6</sub> )	2
Figure S2. Australindolone A (1) <sup>13</sup> C NMR spectrum (125 MHz, DMSO-d <sub>6</sub> )	3
Figure S3. Australindolone A (1) COSY NMR spectrum (500 MHz, DMSO-d <sub>6</sub> )	3
Figure S4. Australindolone A (1) HMBC NMR spectrum (500 MHz, DMSO-d <sub>6</sub> )	4
Figure S5. Australindolone A (1) HSQC NMR spectrum (500 MHz, DMSO-d <sub>6</sub> )	4
Figure S6. Australindolone A (1) HRESIMS(+)	5
Figure S7. Australindolone B (2) <sup>1</sup> H NMR spectrum (500 MHz, DMSO-d <sub>6</sub> )	5
Figure S8. Australindolone B (2) <sup>13</sup> C NMR spectrum (125 MHz, DMSO-d <sub>6</sub> )	6
Figure S9. Australindolone B (2) COSY NMR spectrum (500 MHz, DMSO-d <sub>6</sub> )	6
Figure S10. Australindolone B (2) HMBC NMR spectrum (500 MHz, DMSO-d <sub>6</sub> )	7
Figure S11. Australindolone B (2) HSQC NMR spectrum (500 MHz, DMSO-d <sub>6</sub> )	7
Figure S12. Australindolone B (2) HRESIMS(+)	8
Figure S13. Australindolone C (3) <sup>1</sup> H NMR spectrum (500 MHz, DMSO-d <sub>6</sub> )	8
Figure S14. Australindolone C (3) <sup>13</sup> C NMR spectrum (125 MHz, DMSO-d <sub>6</sub> )	9
Figure S15. Australindolone C (3) COSY NMR spectrum (500 MHz, DMSO-d <sub>6</sub> )	9
Figure S16. Australindolone C (3) HMBC NMR spectrum (500 MHz, DMSO-d <sub>6</sub> )	10
Figure S17. Australindolone C (3) HSQC NMR spectrum (500 MHz, DMSO-d <sub>6</sub> )	10
Figure S18. Australindolone D (4) HRESIMS(+)	11
Figure S19. Australindolone D (4) <sup>1</sup> H NMR spectrum (500 MHz, DMSO-d <sub>6</sub> )	11
Figure S20. Australindolone D (4) <sup>13</sup> C NMR spectrum (125 MHz, DMSO-d <sub>6</sub> )	12
Figure S21. Australindolone D (4) COSY NMR spectrum (500 MHz, DMSO-d <sub>6</sub> )	12
Figure S22. Australindolone D (4) HMBC NMR spectrum (500 MHz, DMSO-d <sub>6</sub> )	13
Figure S23. Australindolone D (4) HSQC NMR spectrum (500 MHz, DMSO-d <sub>6</sub> )	13
Figure S24. Australindolone D (4) HRESIMS(+)	14
Figure S25. Meridianin A (5) <sup>1</sup> H NMR spectrum (400 MHz, DMSO-d <sub>6</sub> )	14
Figure S26. Meridianin A (5) <sup>13</sup> C NMR spectrum (100 MHz, DMSO d <sub>6</sub> )	15
Figure S27. Meridianin A (5) HRSEIMS(+)	15
Figure S28. Meridianin B (6) <sup>1</sup> H NMR spectrum (400 MHz, DMSO-d <sub>6</sub> )	16
Figure S29. Meridianin B (6) HRESIMS	16
Figure S30. Meridianin C (7) <sup>1</sup> H NMR spectrum (400 MHz, DMSO-d <sub>6</sub> )	17
Figure S31. Meridianin C (7) <sup>13</sup> C NMR spectrum (100 MHz, DMSO-d <sub>6</sub> )	17

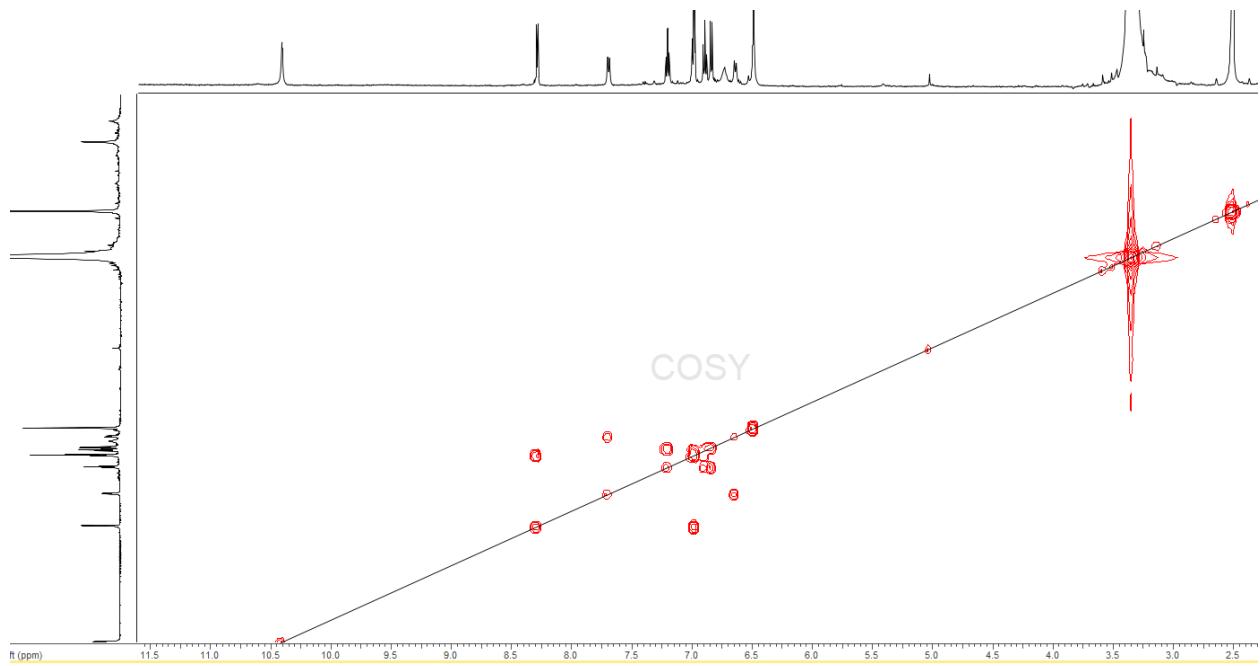
<b>Figure S32.</b> Meridianin C (7) HRESIMS(+)	18
<b>Figure S33.</b> Meridianin D (8) $^1\text{H}$ NMR spectrum (400 MHz, $\text{DMSO}-d_6$ )	18
<b>Figure S34.</b> Meridianin D (8) HRESIMS(+)	19
<b>Figure S35.</b> Meridianin E (9) $^1\text{H}$ NMR spectrum (400 MHz, $\text{DMSO}-d_6$ )	19
<b>Figure S36.</b> Meridianin E (9) HRESIMS(+)	20
<b>Figure S37.</b> Meridianin F (10) $^1\text{H}$ NMR spectrum (400 MHz, $\text{CD}_3\text{OD}$ )	20
<b>Figure S38.</b> Meridianin F (10) HRESIMS(+)	21
<b>Figure S39.</b> Meridianin G (11) $^1\text{H}$ NMR spectrum (400 MHz, $\text{CD}_3\text{OD}$ )	21
<b>Figure S40.</b> Meridianin G (11) HRESIMS(+)	22
<b>Figure S41.</b> Meridianin H (12) $^1\text{H}$ NMR spectrum (500 MHz, $\text{DMSO}-d_6$ )	22
<b>Figure S42.</b> Meridianin H (12) $^{13}\text{C}$ NMR spectrum (125 MHz, $\text{DMSO}-d_6$ )	23
<b>Figure S43.</b> Meridianin H (12) COSY NMR spectrum (500 MHz, $\text{DMSO}-d_6$ )	23
<b>Figure S44.</b> Meridianin H (12) HMBC NMR spectrum (500 MHz, $\text{DMSO}-d_6$ )	24
<b>Figure S45.</b> Meridianin H (12) HSQC NMR spectrum (500 MHz, $\text{DMSO}-d_6$ )	24
<b>Figure S46.</b> Meridianin H (12) HRESIMS(+)	25
Table S1. Crystal data and structure refinement for australindolone B (2)	26



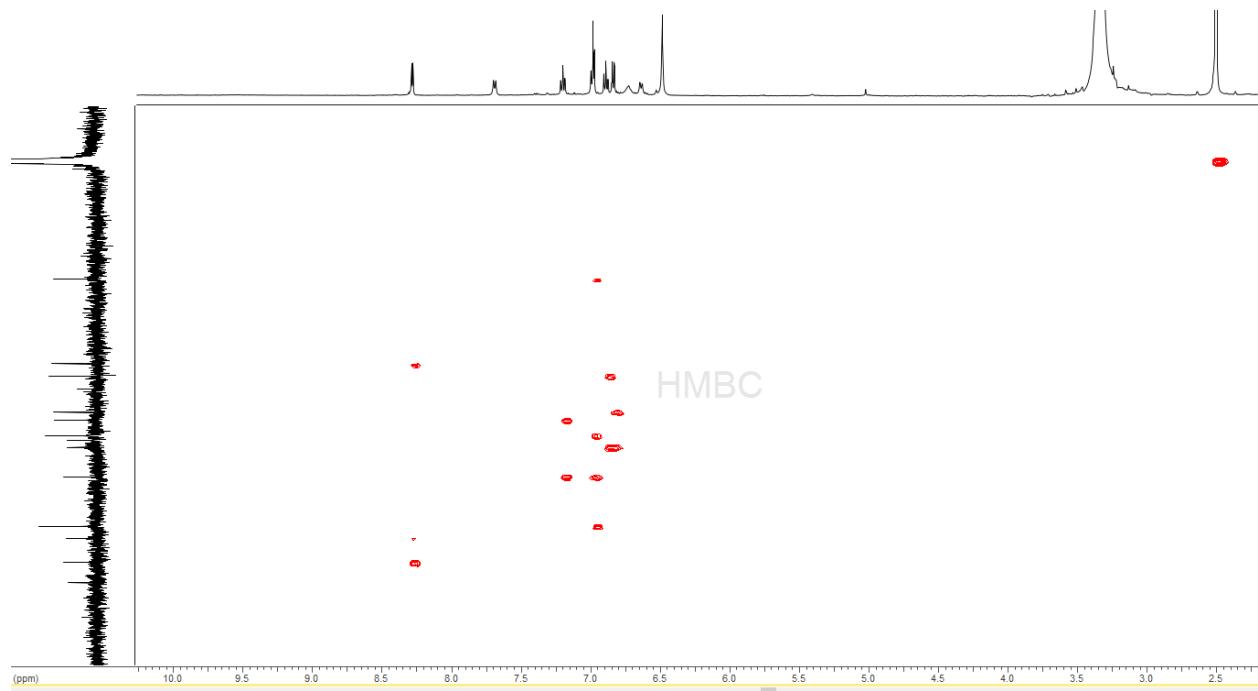
**Figure S1.** Australindolone A (1)  $^1\text{H}$  NMR spectrum (500 MHz,  $\text{DMSO}-d_6$ )



**Figure S2.** Australindolone A (**1**)  $^{13}\text{C}$  NMR spectrum (125 MHz,  $\text{DMSO}-d_6$ )



**Figure S3.** Australindolone A (**1**) COSY NMR spectrum (500 MHz,  $\text{DMSO}-d_6$ )



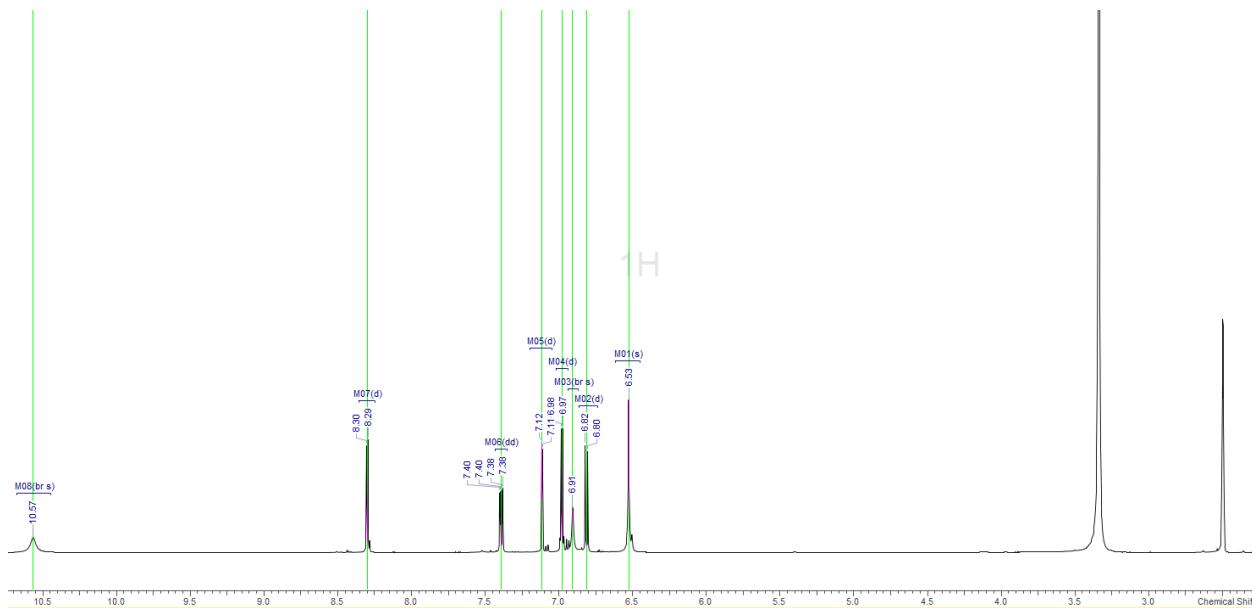
**Figure S4.** Australindolone A (**1**) HMBC NMR spectrum (500 MHz,  $\text{DMSO}-d_6$ )



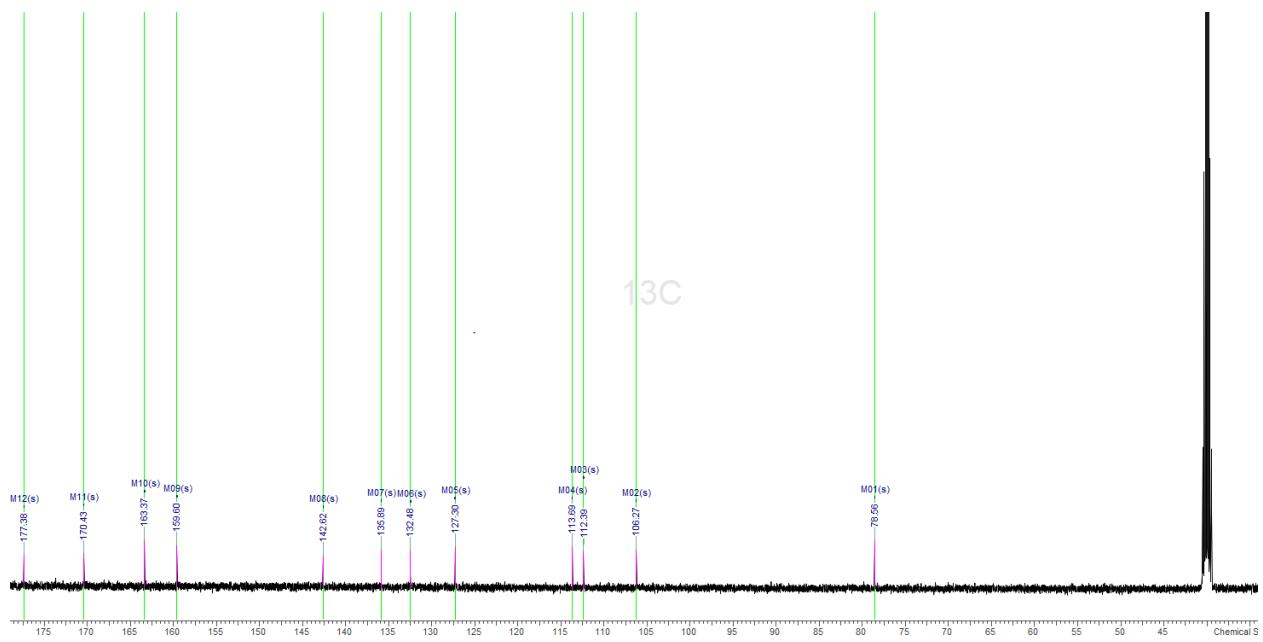
**Figure S5.** Australindolone A (**1**) HSQC NMR spectrum (500 MHz,  $\text{DMSO}-d_6$ )



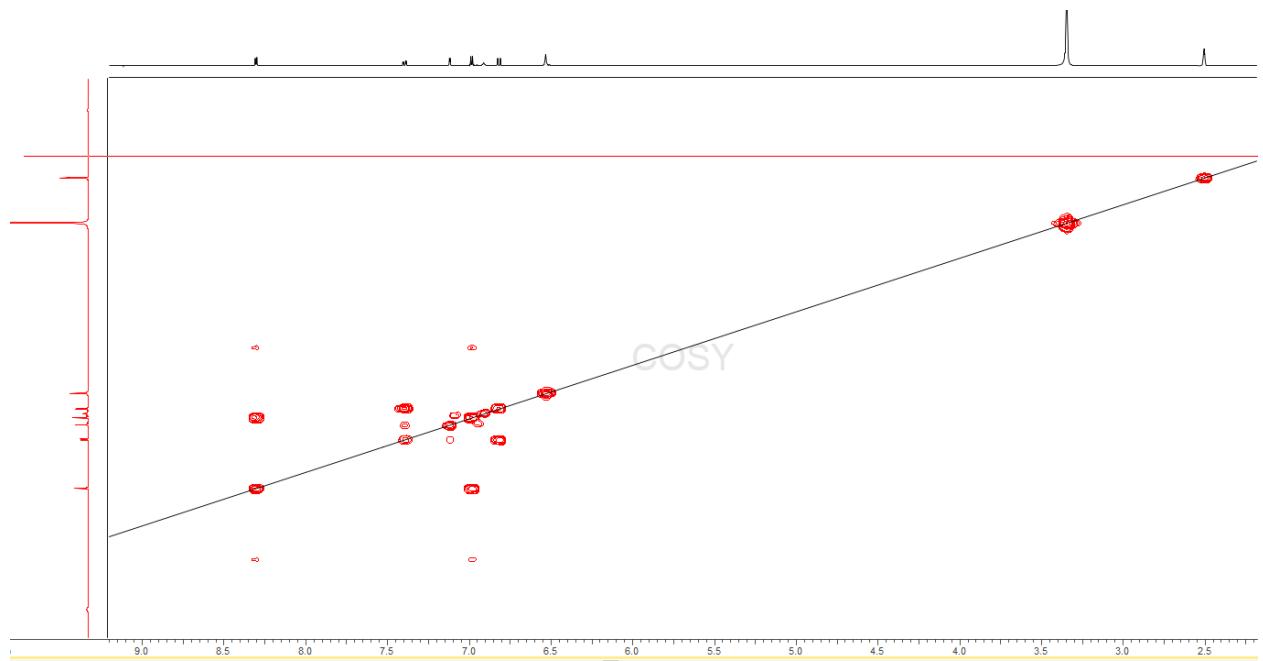
**Figure S6.** Australindolone A (**1**) HRESIMS(+)



**Figure S7.** Australindolone B (**2**) <sup>1</sup>H NMR spectrum (500 MHz, DMSO-*d*<sub>6</sub>)



**Figure S8.** Australindolone B (2)  $^{13}\text{C}$  NMR spectrum (125 MHz,  $\text{DMSO}-d_6$ )



**Figure S9.** Australindolone B (2) COSY NMR spectrum (500 MHz,  $\text{DMSO}-d_6$ )

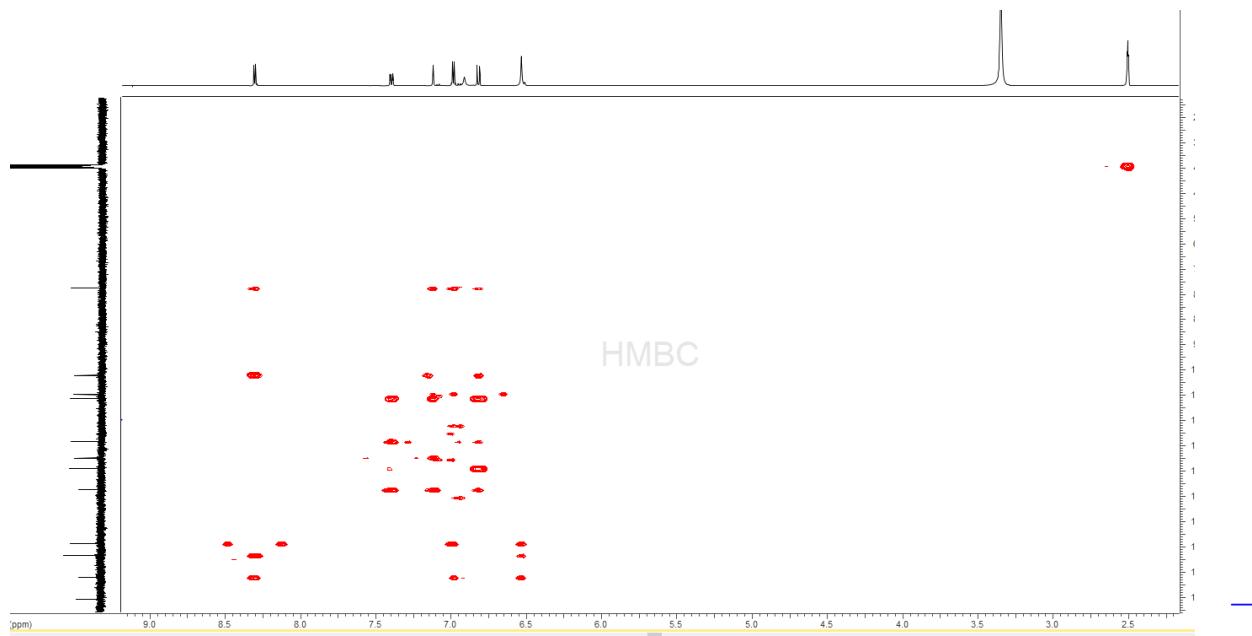


Figure S10. Australindolone B (2) HMBC NMR spectrum (500 MHz,  $\text{DMSO}-d_6$ )

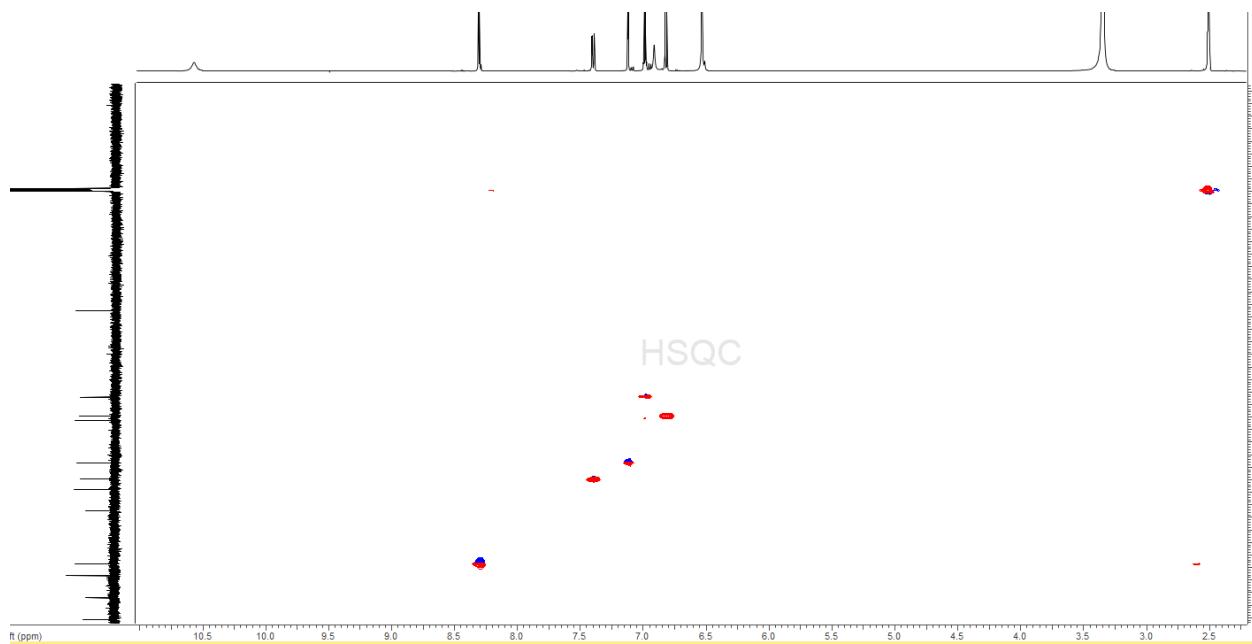
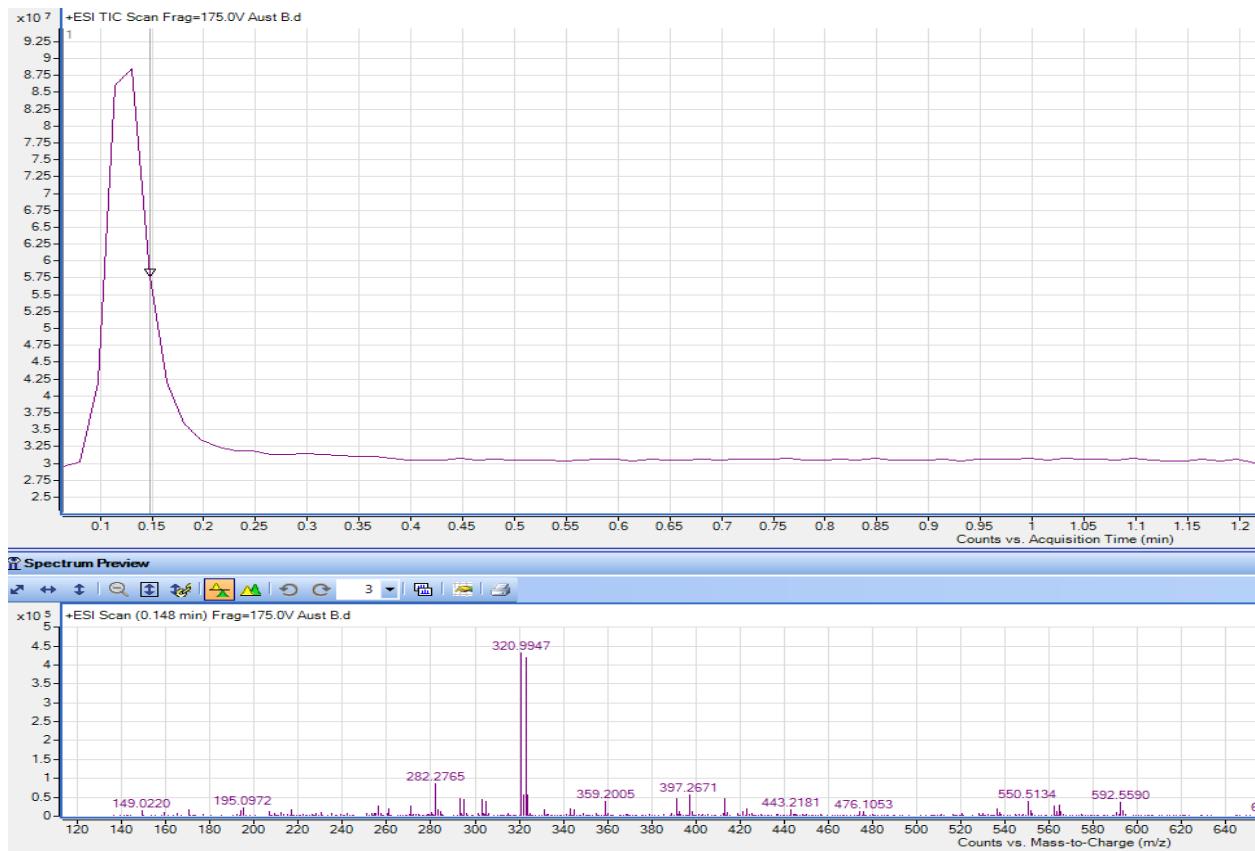
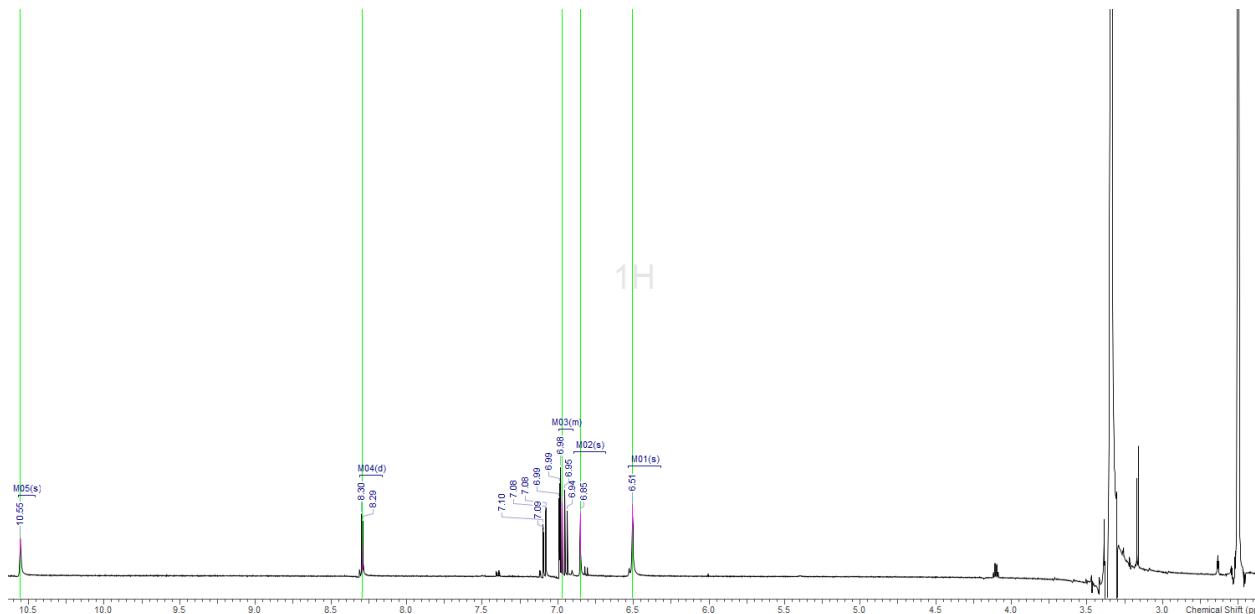


Figure S11. Australindolone B (2) HSQC NMR spectrum (500 MHz,  $\text{DMSO}-d_6$ )



**Figure S12.** Australindolone B (**2**) HRESIMS(+)



**Figure S13.** Australindolone C (**3**) <sup>1</sup>H NMR spectrum (500 MHz, DMSO-*d*<sub>6</sub>)

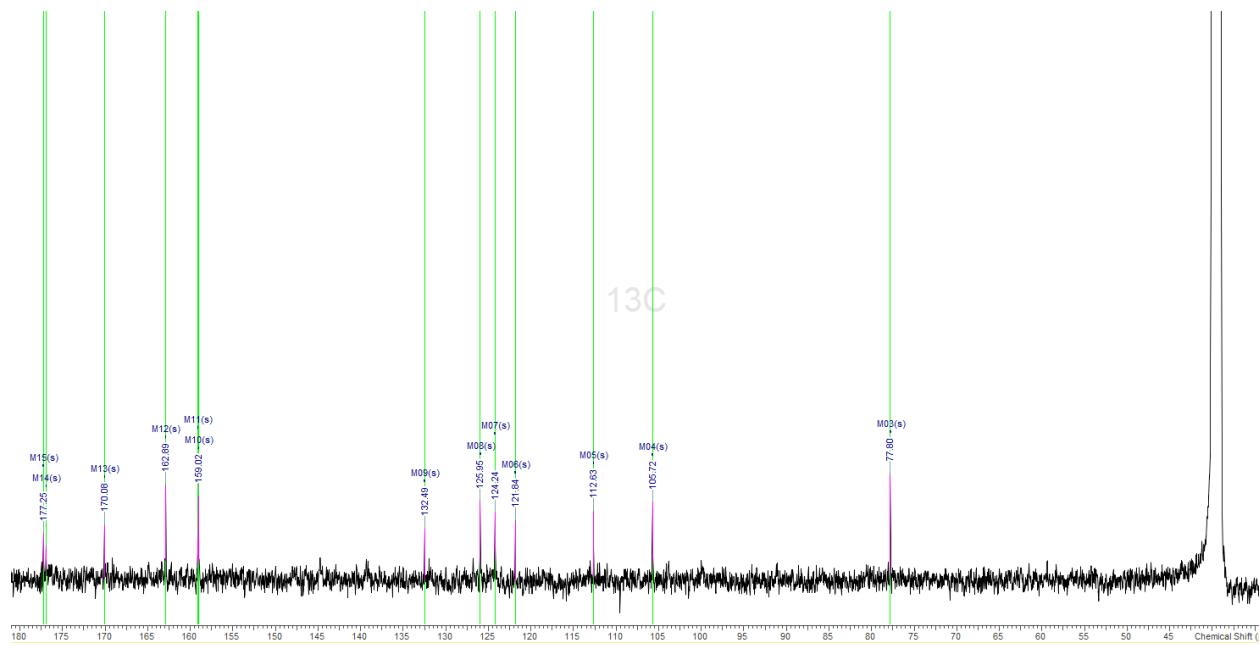


Figure S14. Australindolone C (3)  $^{13}\text{C}$  NMR spectrum (125 MHz,  $\text{DMSO}-d_6$ )

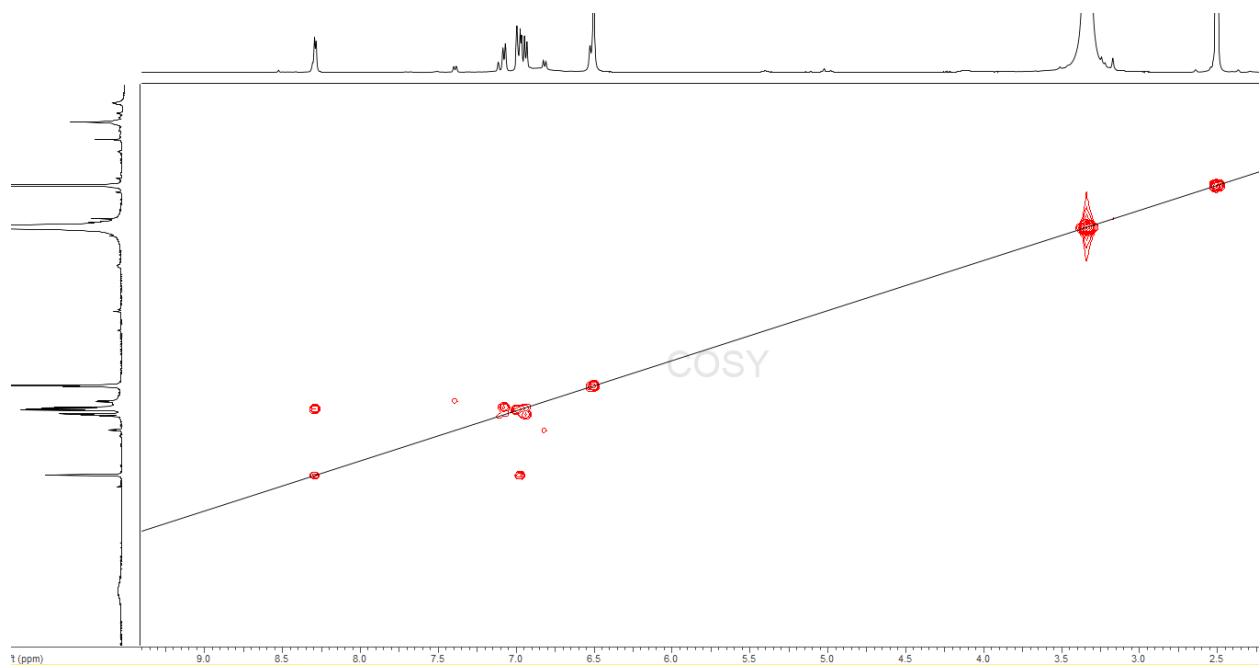


Figure S15. Australindolone C (3) COSY NMR spectrum (500 MHz,  $\text{DMSO}-d_6$ )

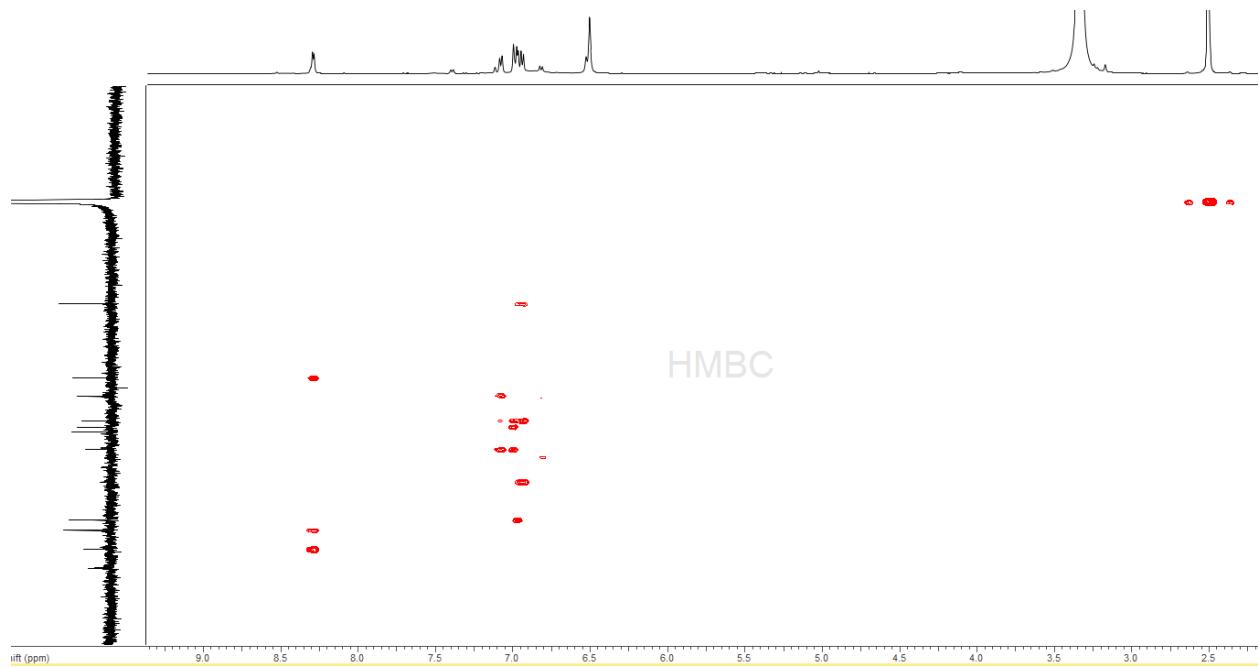


Figure S16. Australindolone C (3) HMBC NMR spectrum (500 MHz, DMSO-*d*<sub>6</sub>)

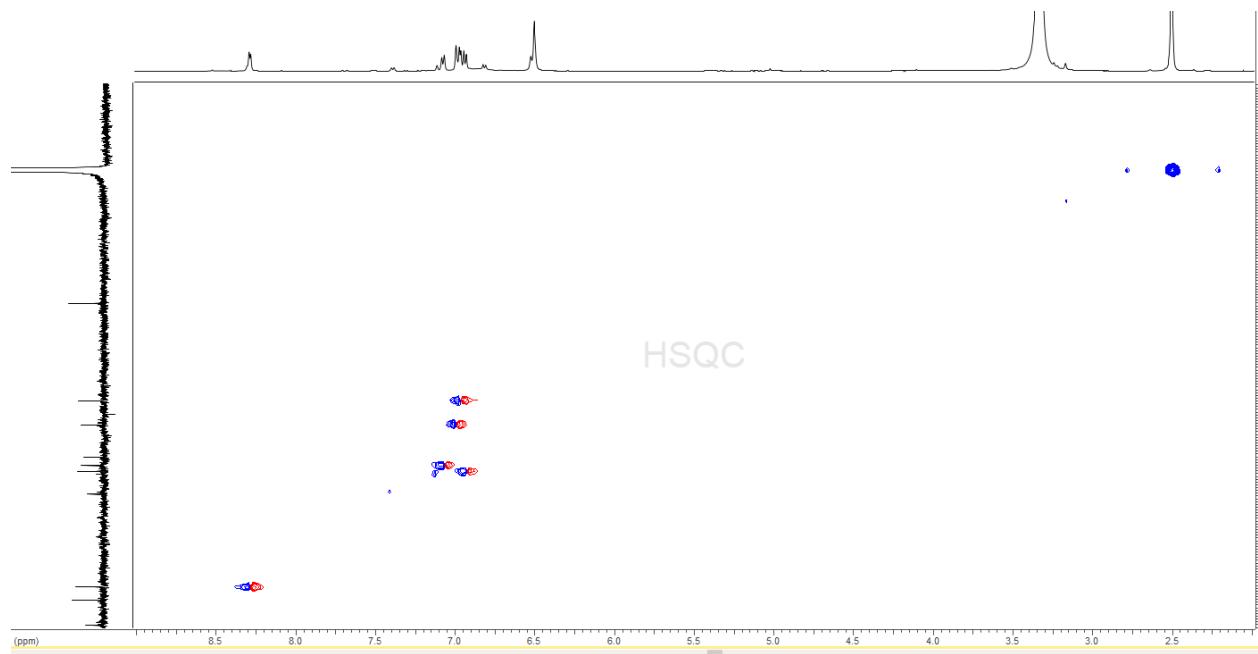


Figure S17. Australindolone C (3) HSQC NMR spectrum (500 MHz, DMSO-*d*<sub>6</sub>)

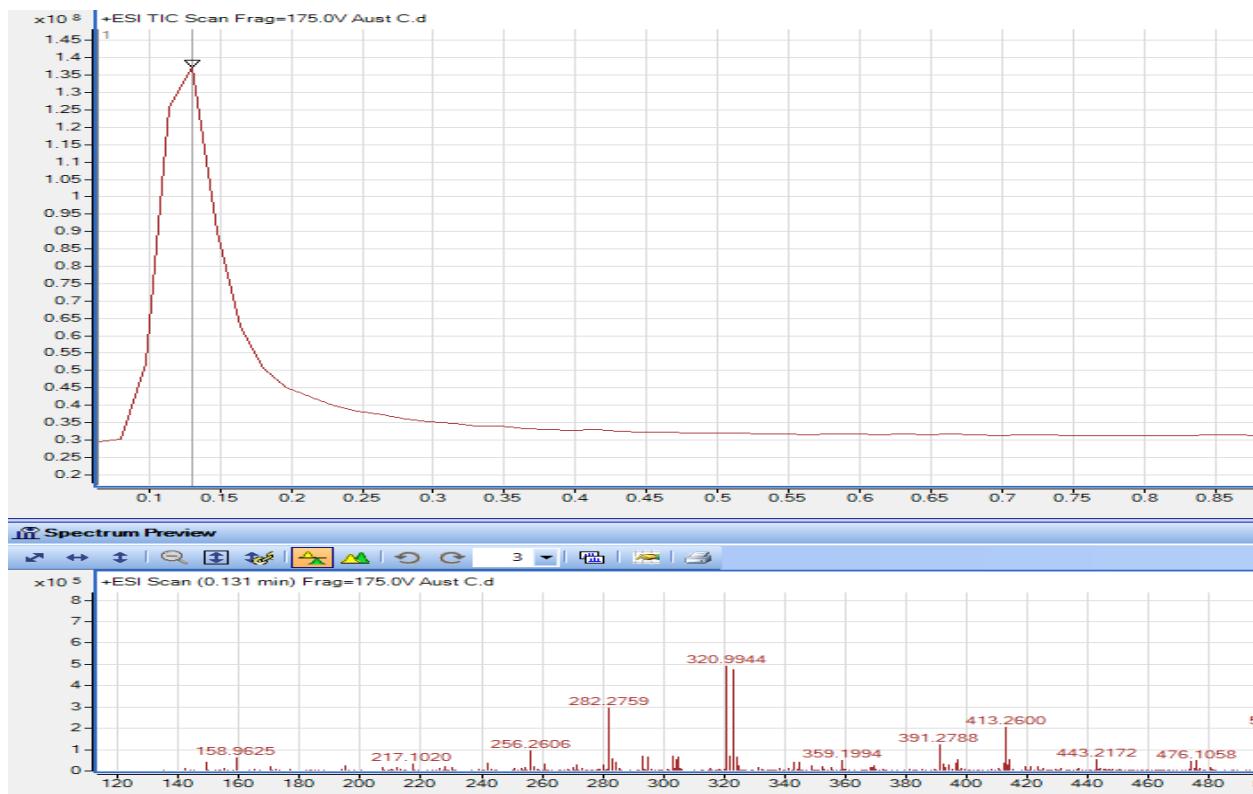


Figure S18. Australindolone C (3) HRESIMS(+)

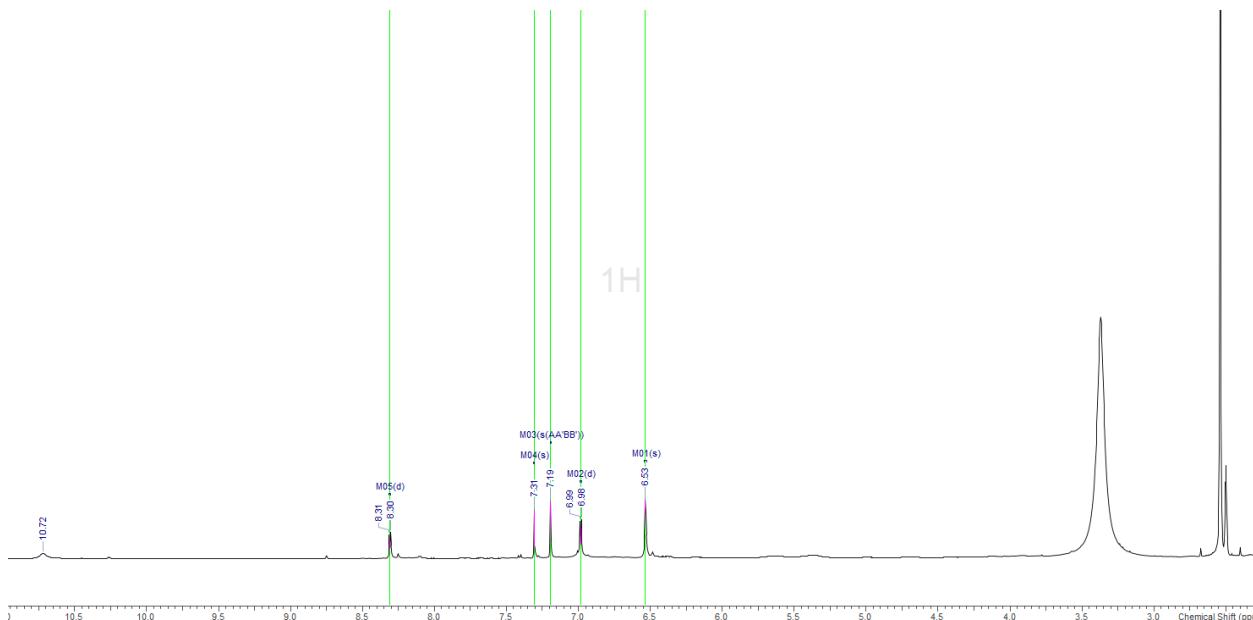
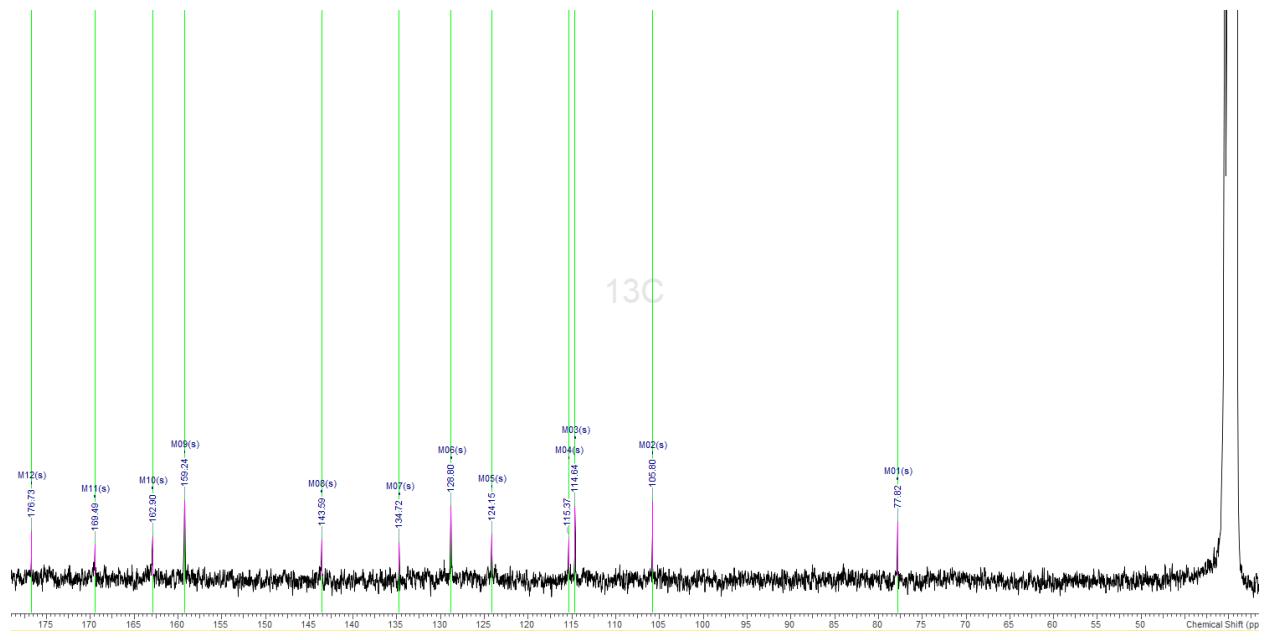
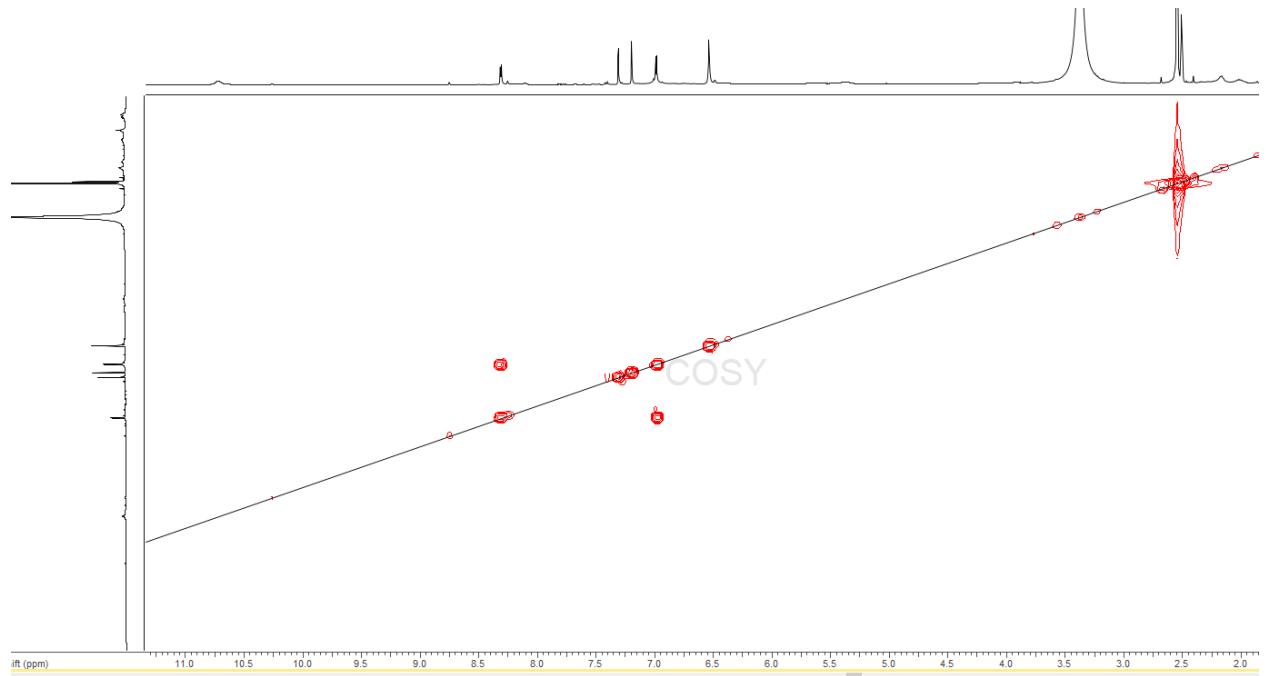


Figure S19. Australindolone D (4)  $^1\text{H}$  NMR spectrum (500 MHz,  $\text{DMSO}-d_6$ )



**Figure S20.** Australindolone D (**4**)  $^{13}\text{C}$  NMR spectrum (125 MHz,  $\text{DMSO}-d_6$ )



**Figure S21.** Australindolone D (**4**) COSY NMR spectrum (500 MHz,  $\text{DMSO}-d_6$ )

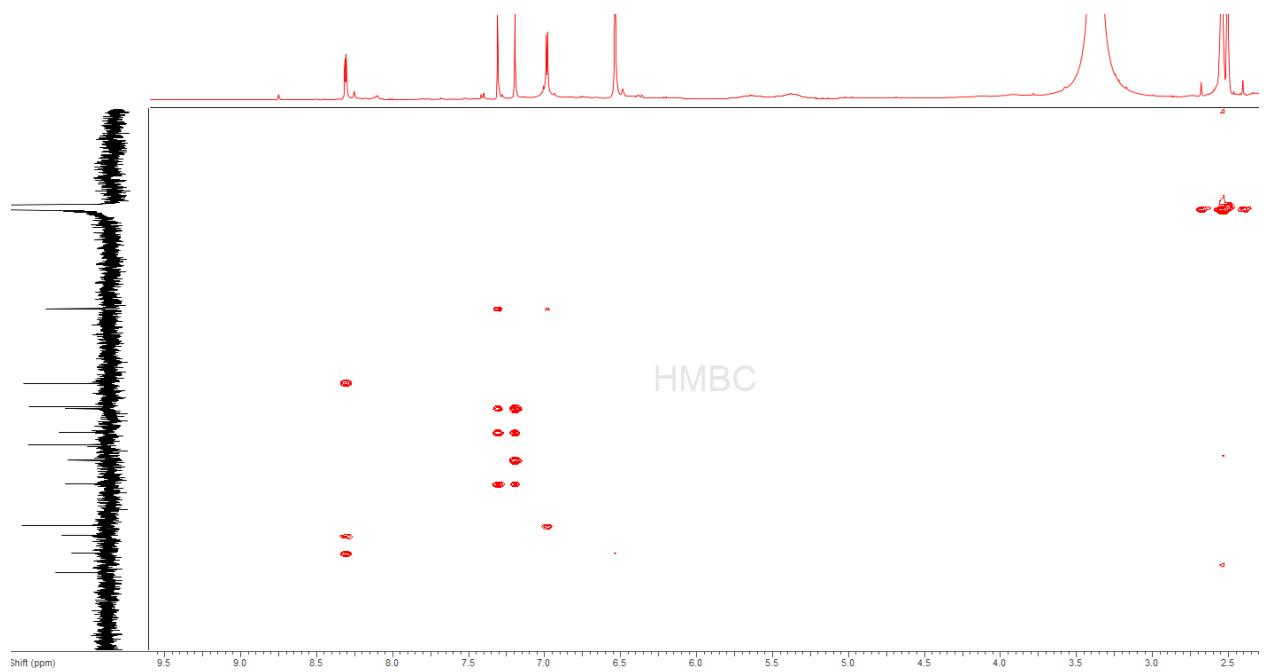


Figure S22. Australindolone D (4) HMBC NMR spectrum (500 MHz,  $\text{DMSO}-d_6$ )

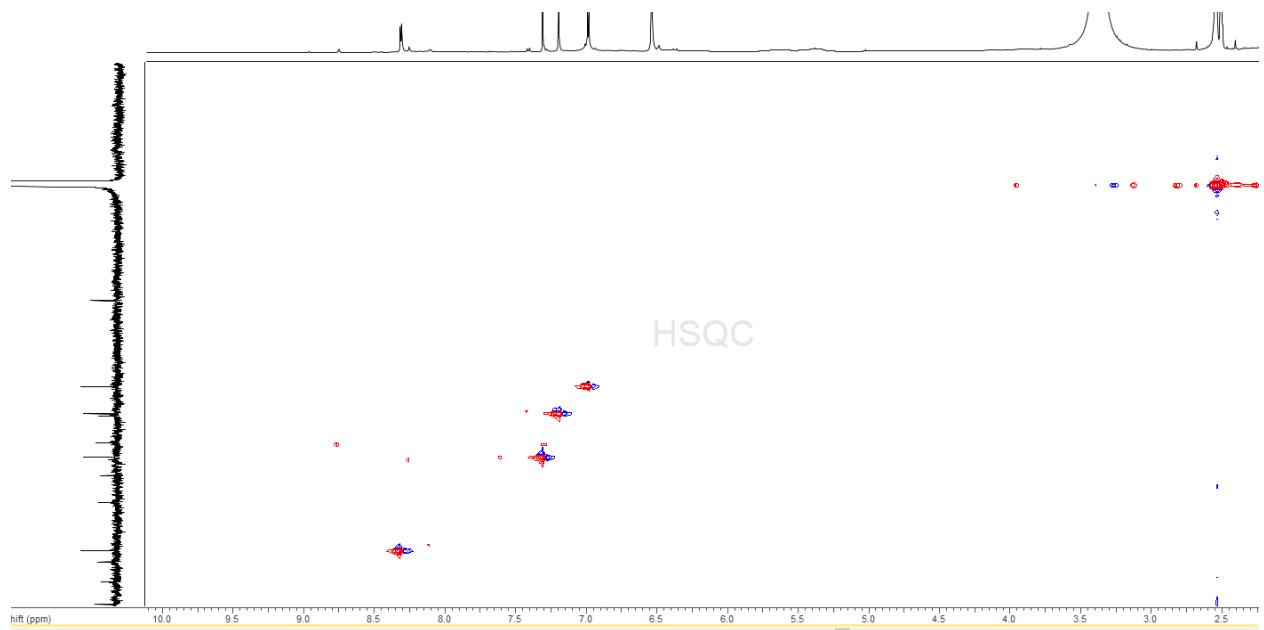
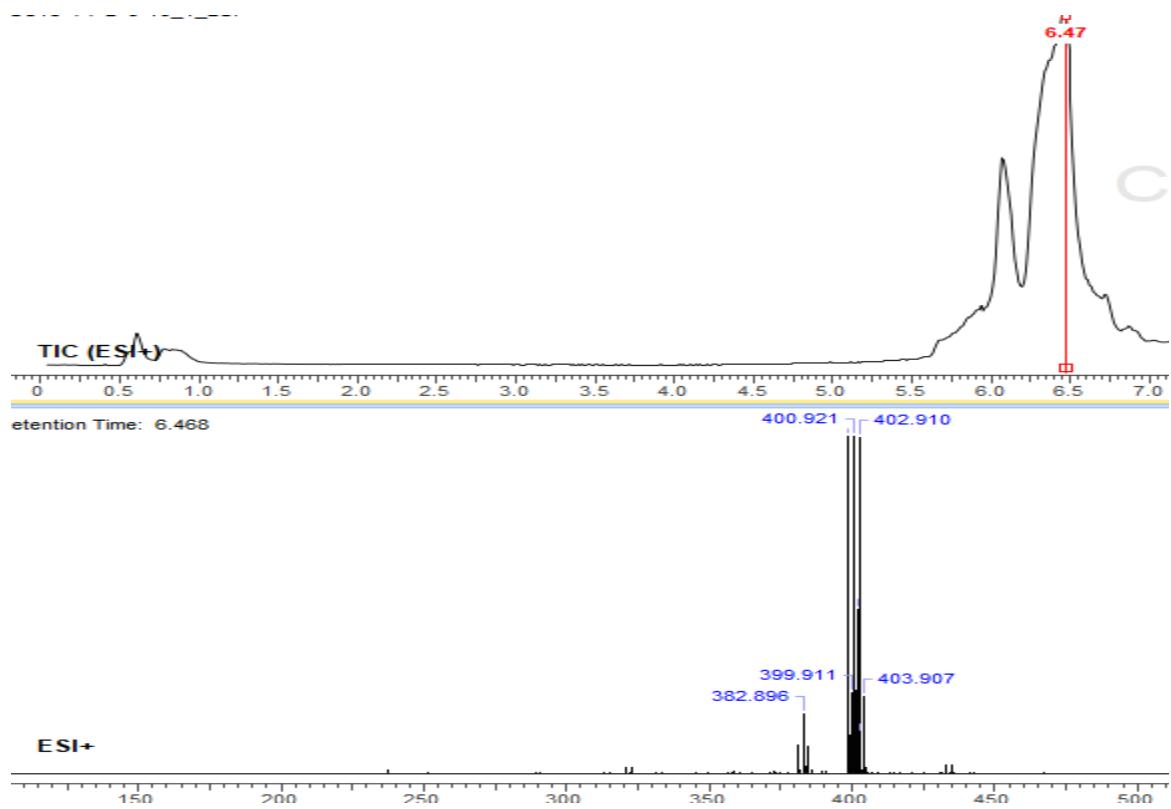


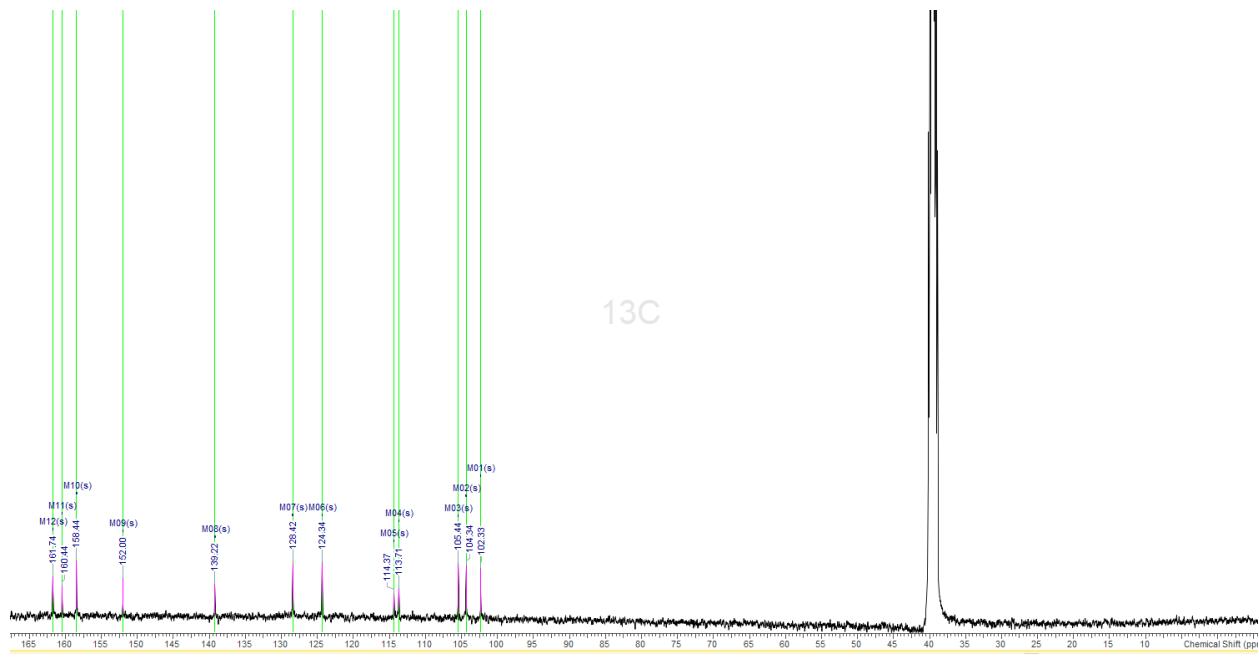
Figure S23. Australindolone D (4) HSQC NMR spectrum (500 MHz,  $\text{DMSO}-d_6$ )



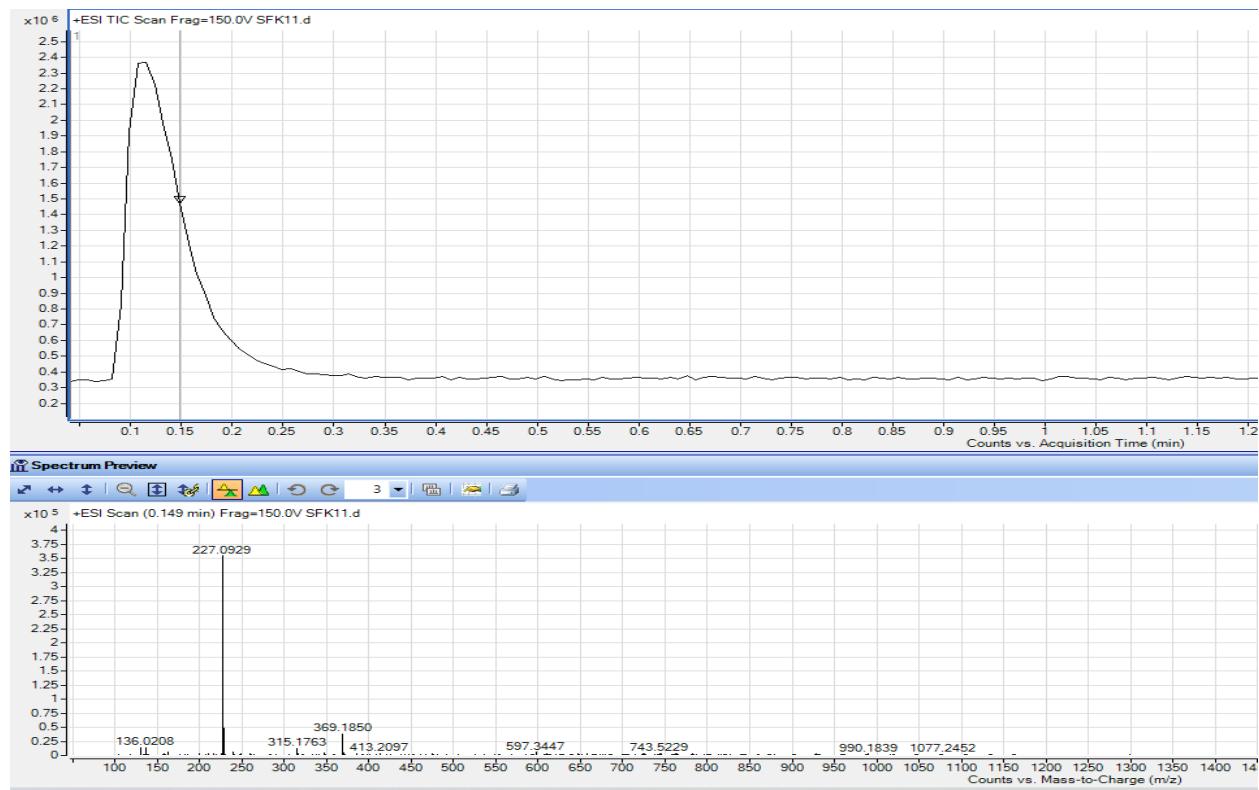
**Figure S24.** Australindolone D (4) HRESIMS(+)



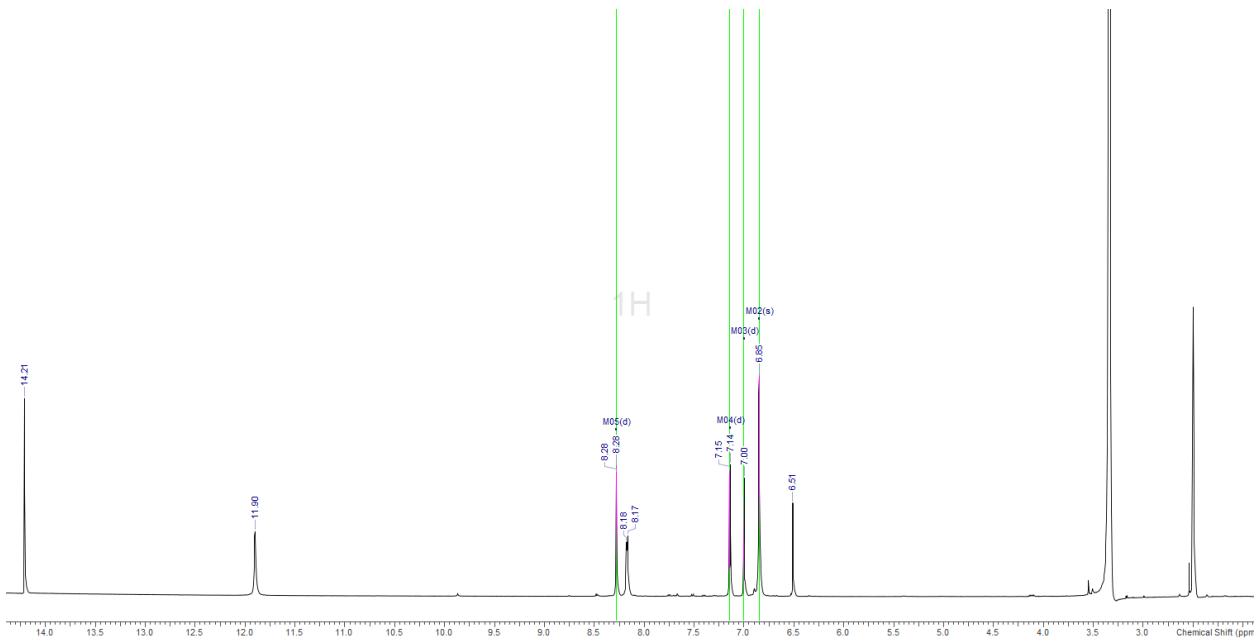
**Figure S25.** Meridianin A (5) <sup>1</sup>H NMR spectrum (400 MHz, DMSO-*d*<sub>6</sub>)



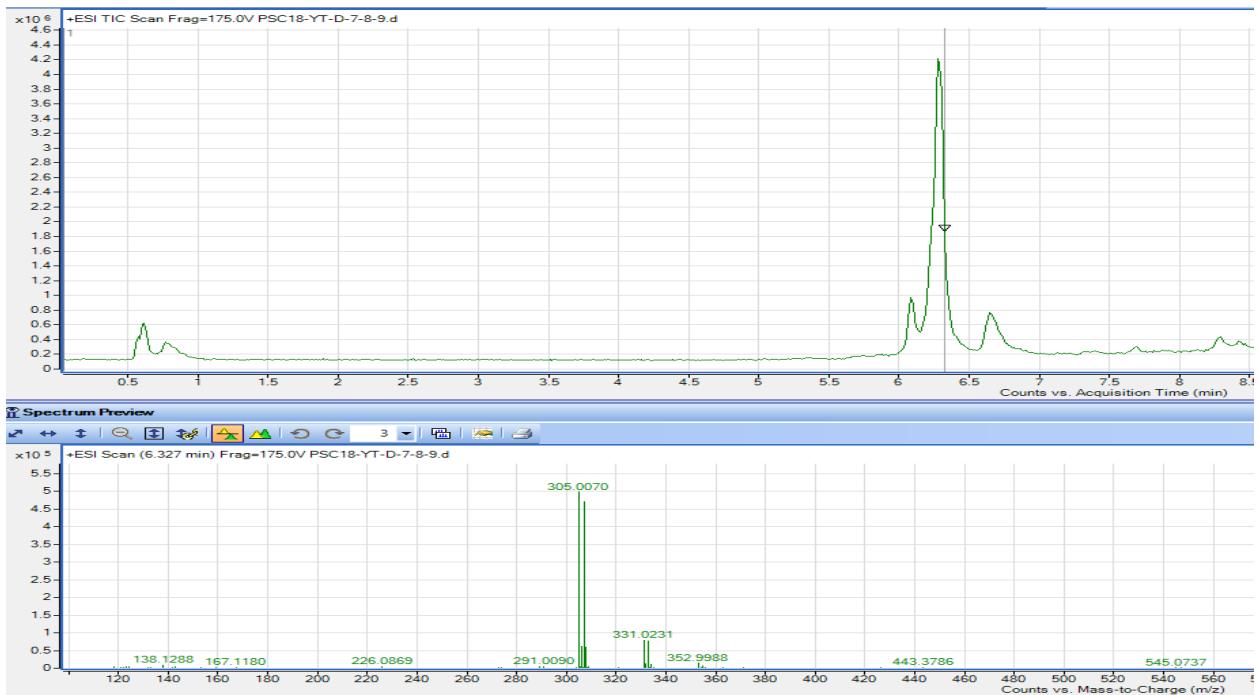
**Figure S26.** Meridianin A (5)  $^{13}\text{C}$  NMR spectrum (100 MHz, DMSO  $d_6$ )



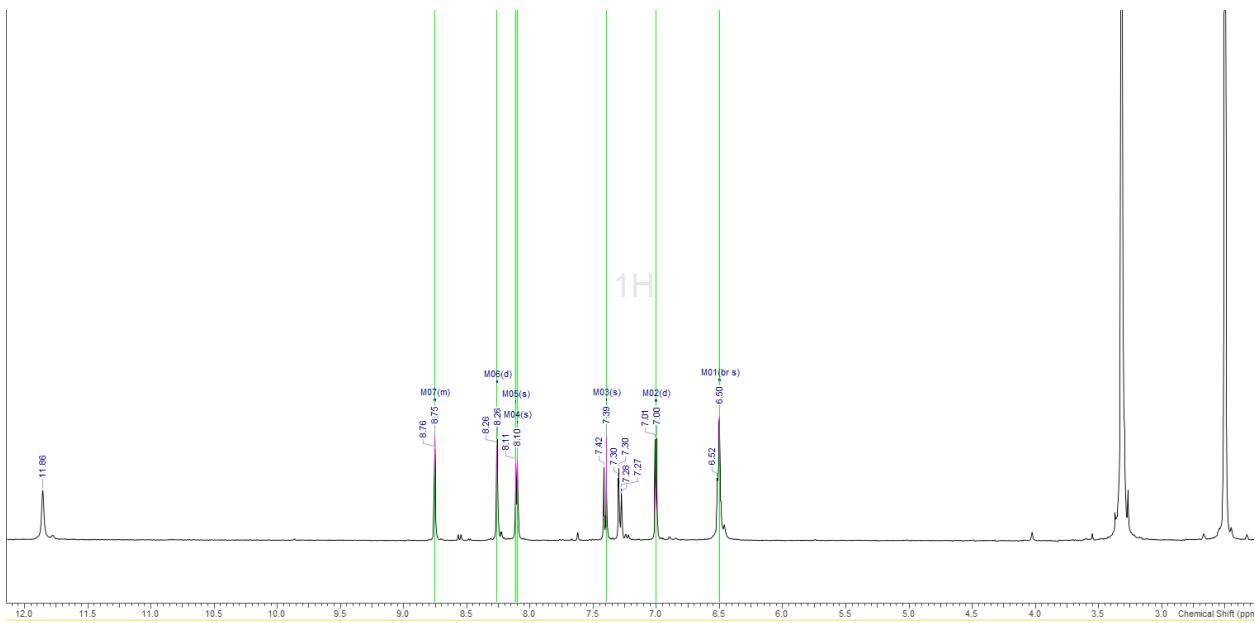
**Figure S27.** Meridianin A (5) HRSEIMS(+)



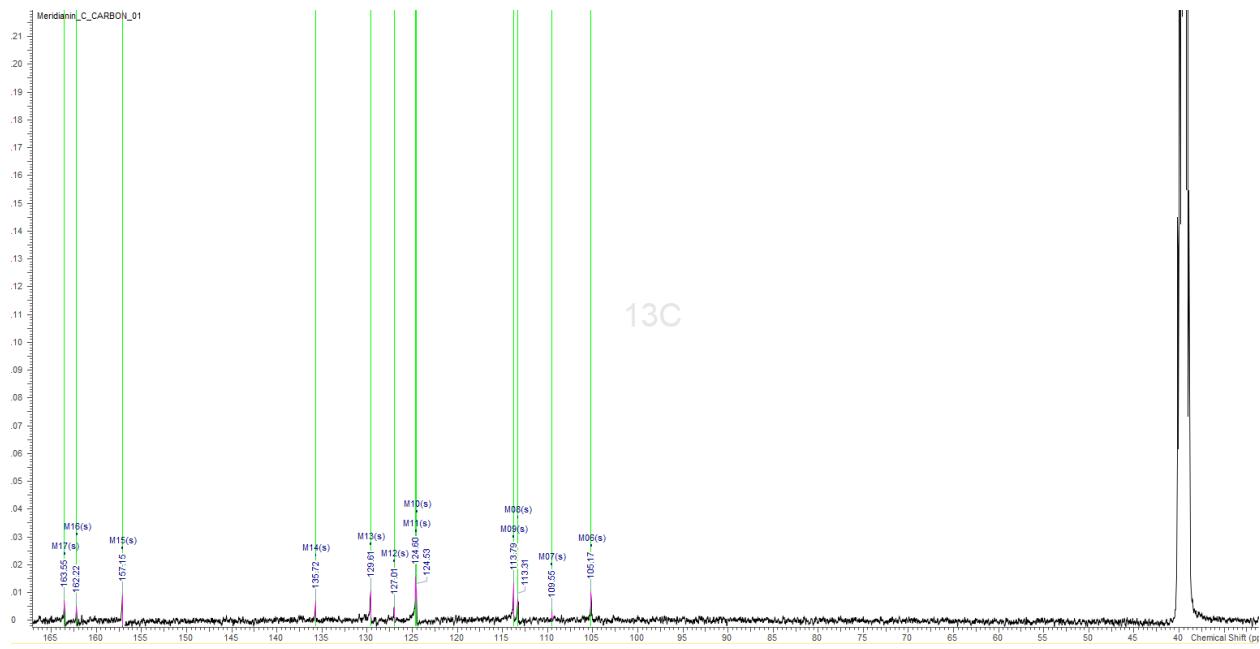
**Figure S28.** Meridianin B (6)  $^1\text{H}$  NMR spectrum (400 MHz,  $\text{DMSO}-d_6$ )



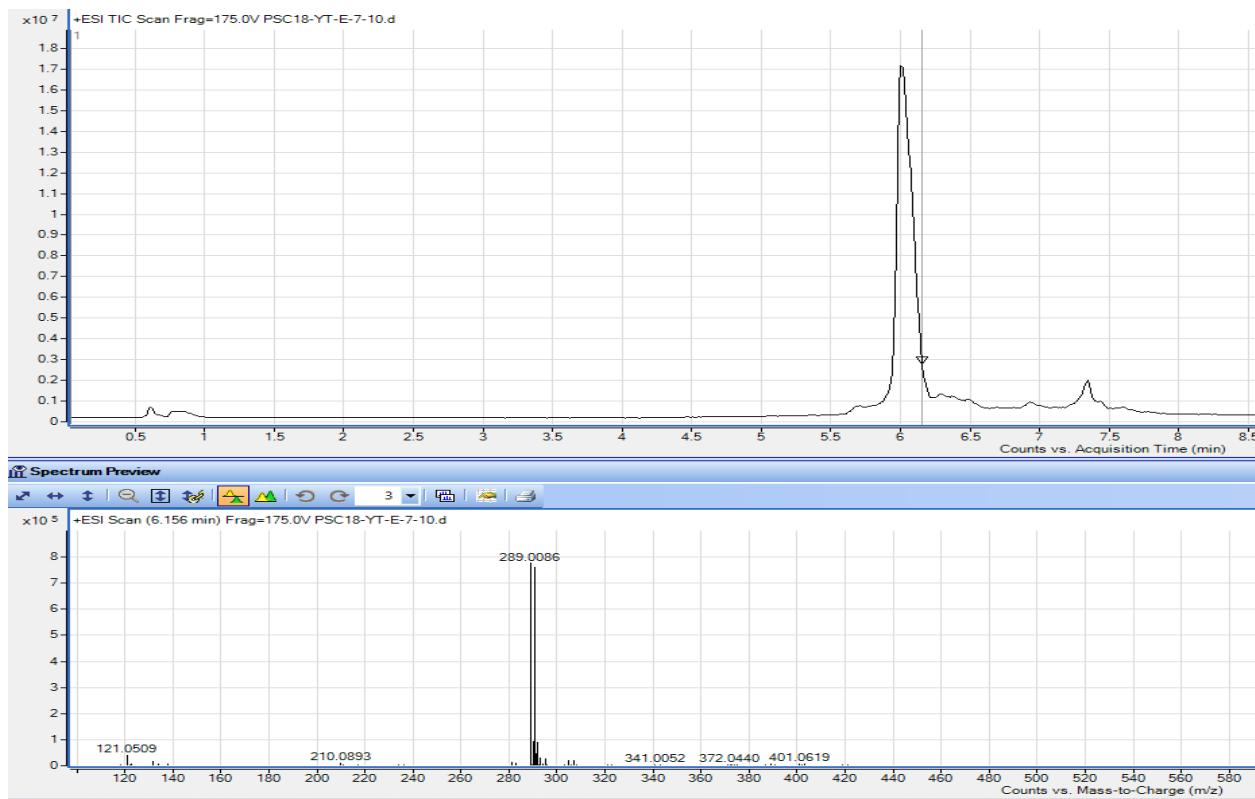
**Figure S29.** Meridianin B (6) HRESIMS



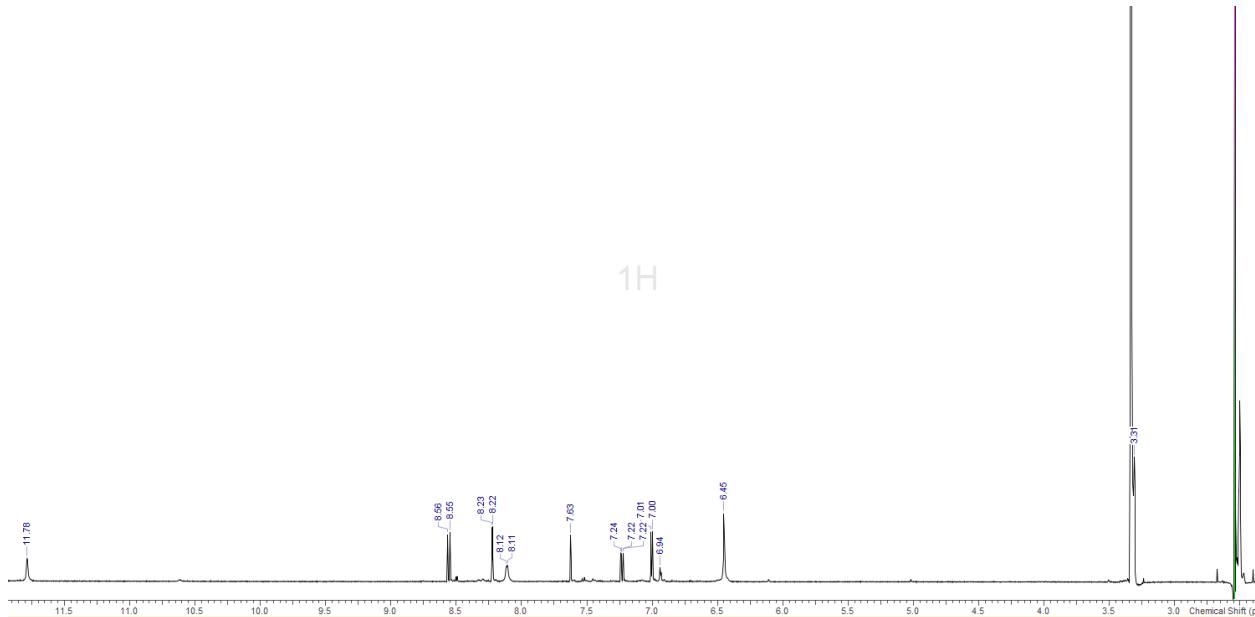
**Figure S30.** Meridianin C (7)  $^1\text{H}$  NMR spectrum (400 MHz,  $\text{DMSO}-d_6$ )



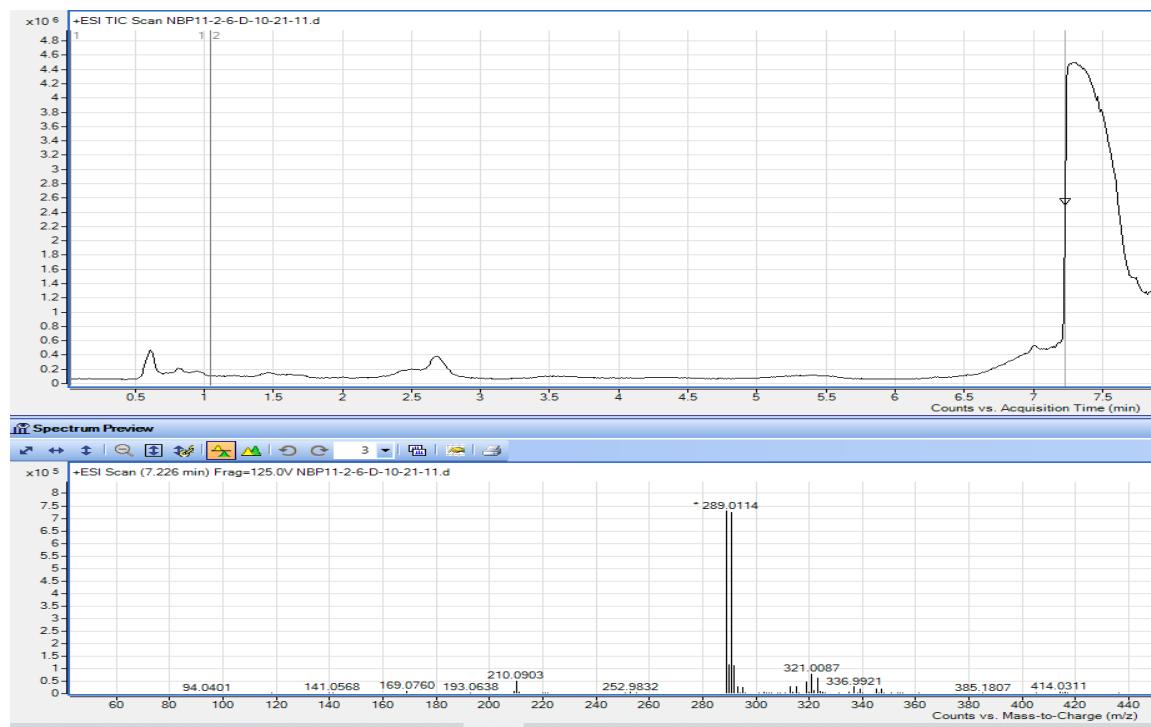
**Figure S31.** Meridianin C (7)  $^{13}\text{C}$  NMR spectrum (100 MHz,  $\text{DMSO}-d_6$ )



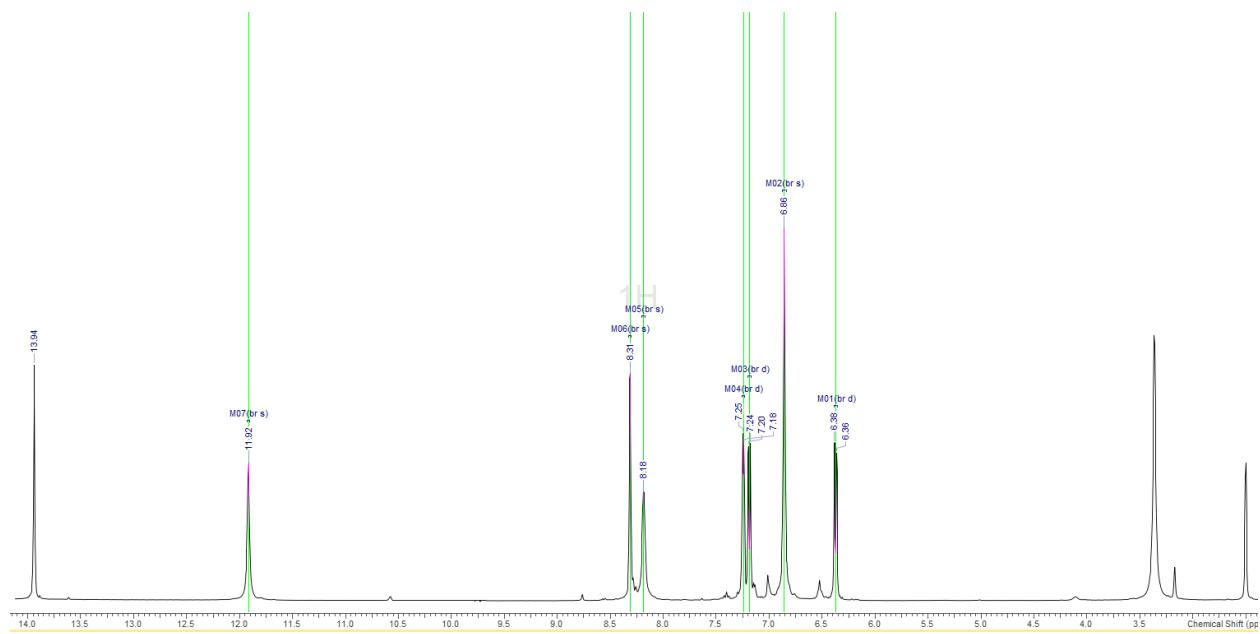
**Figure S32.** Meridianin C (7) HRESIMS(+)



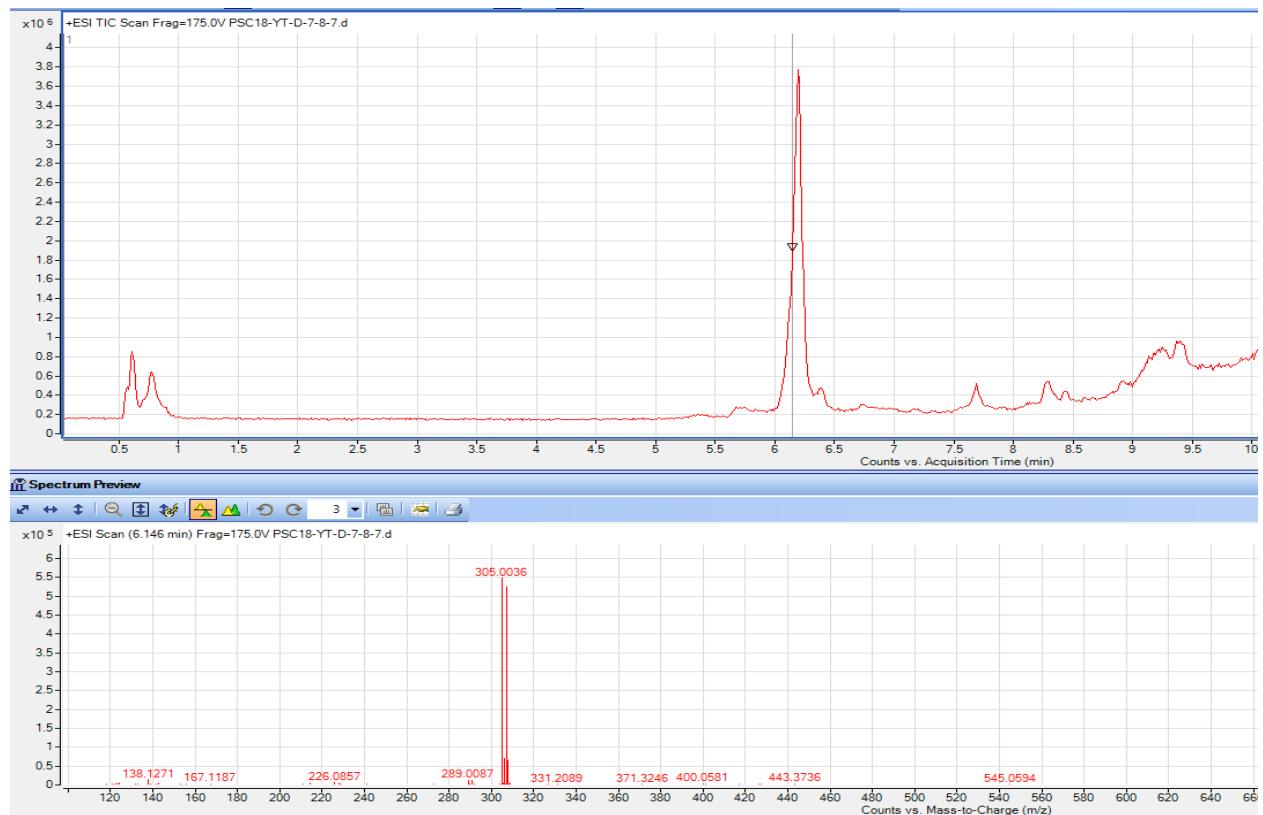
**Figure S33.** Meridianin D (8) <sup>1</sup>H NMR spectrum (400 MHz, DMSO-*d*<sub>6</sub>)



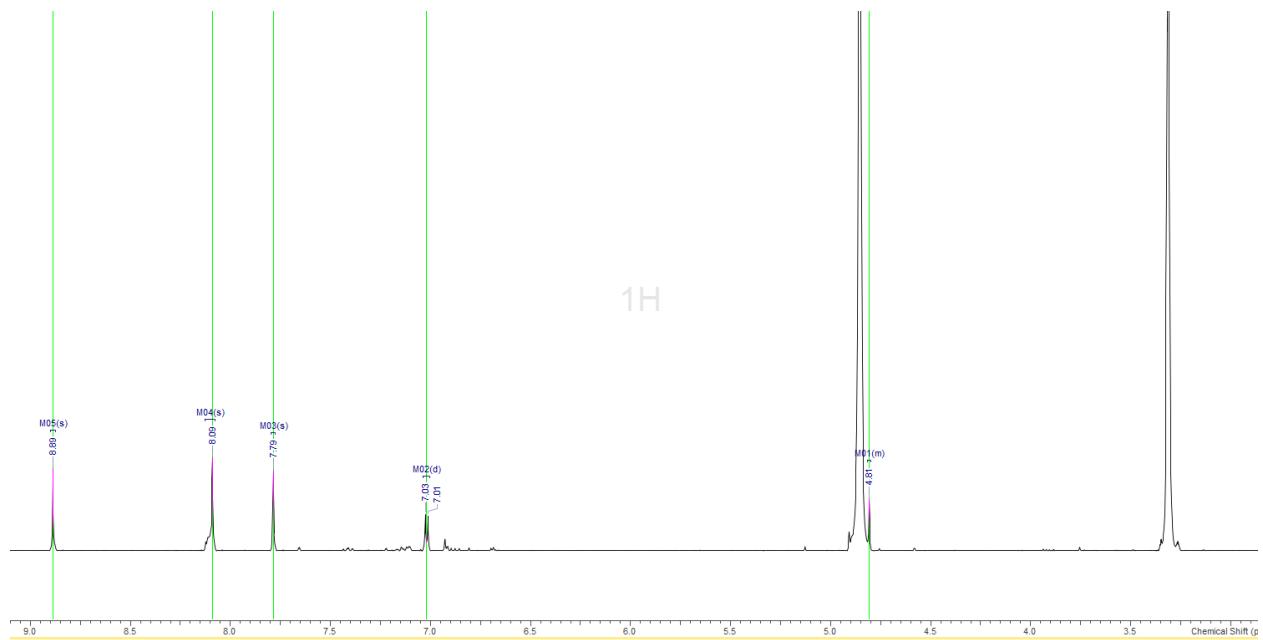
**Figure S34.** Meridianin D (8) HRESIMS(+)



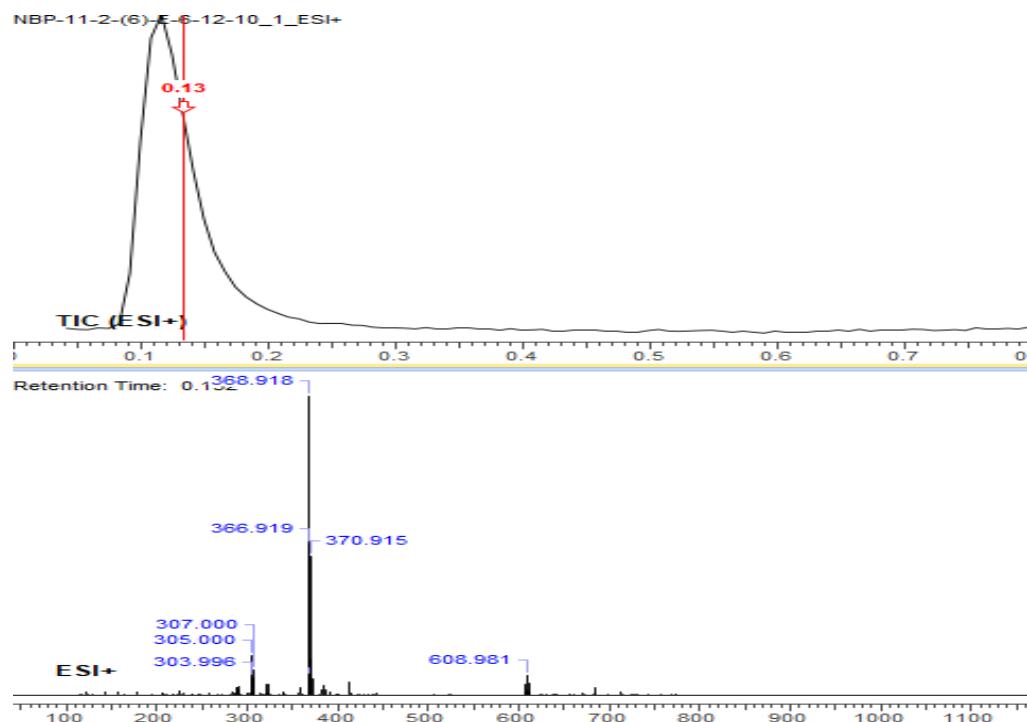
**Figure S35.** Meridianin E (9)  $^1\text{H}$  NMR spectrum (400 MHz,  $\text{DMSO}-d_6$ )



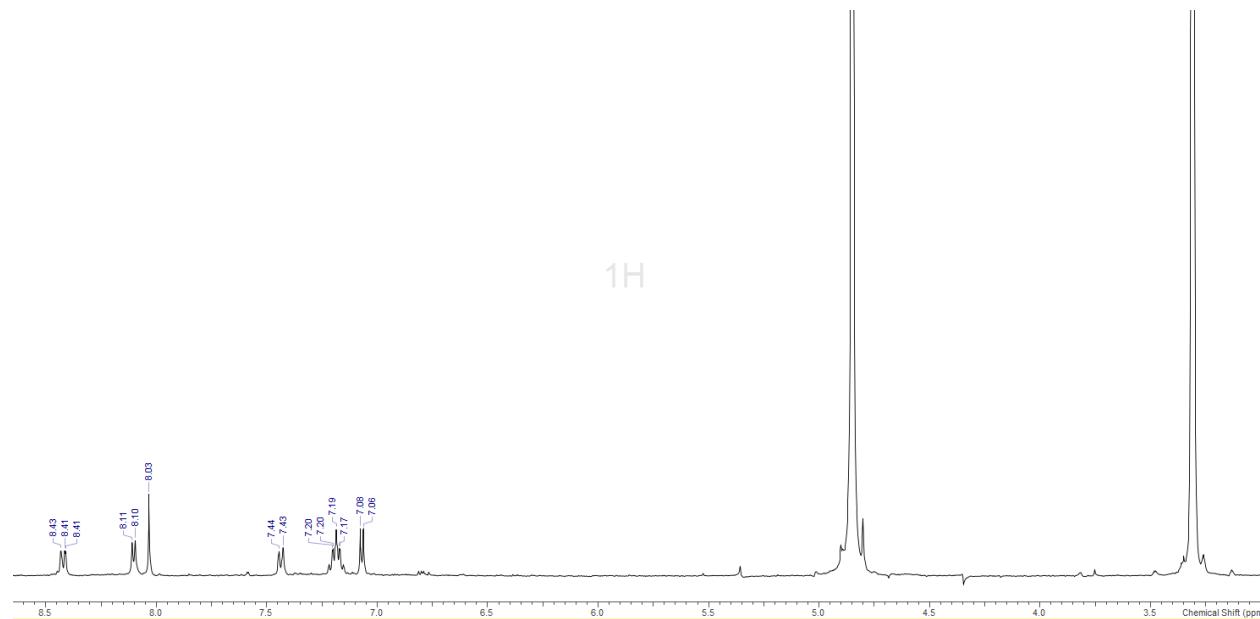
**Figure S36.** Meridianin E (9) HRESIMS(+)



**Figure S37.** Meridianin F (10) <sup>1</sup>H NMR spectrum (400 MHz, CD<sub>3</sub>OD)



**Figure S38.** Meridianin F (10) HRESIMS(+)



**Figure S39.** Meridianin G (11)  $^1\text{H}$  NMR spectrum (400 MHz,  $\text{CD}_3\text{OD}$ )

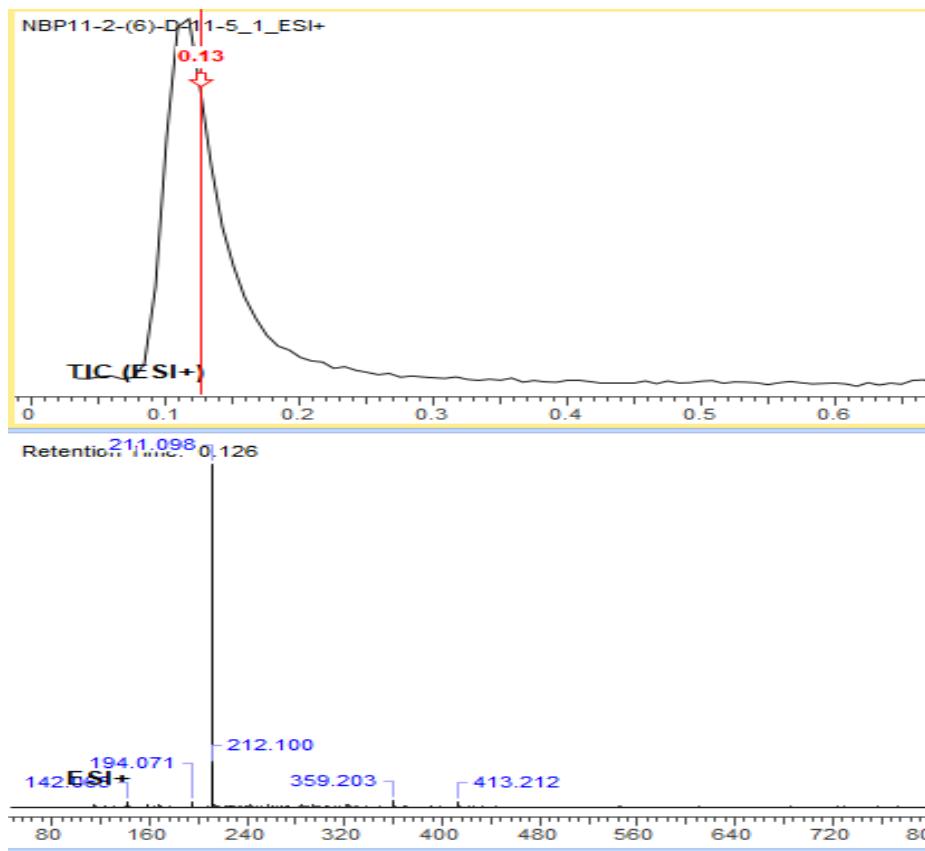


Figure S40. Meridianin G (11) HRESIMS(+)

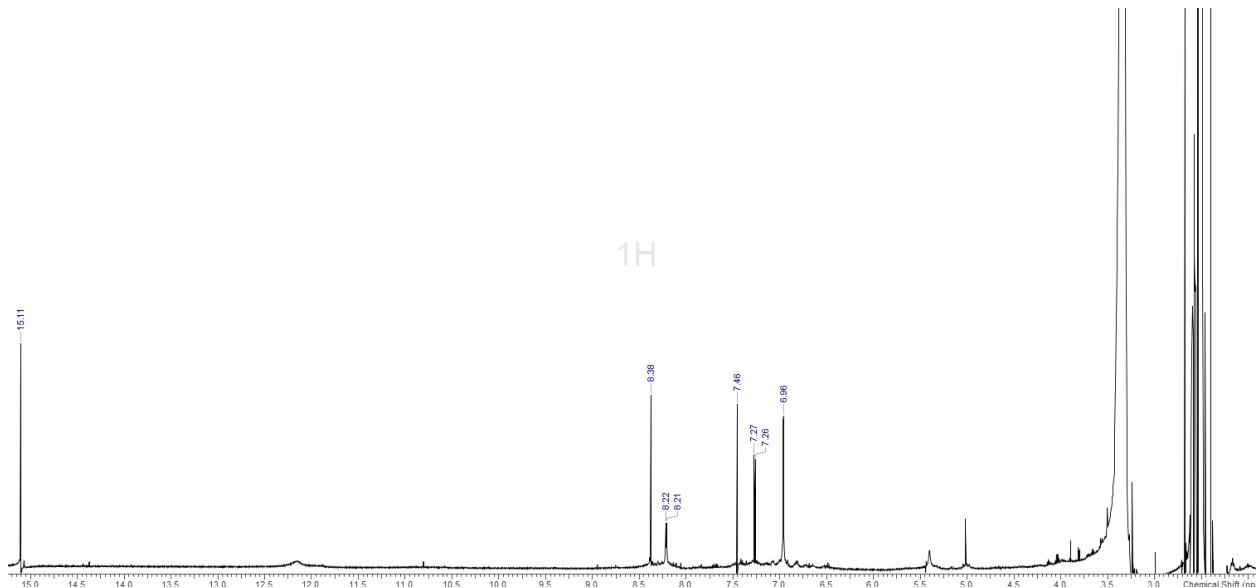


Figure S41. Meridianin H (12) <sup>1</sup>H NMR spectrum (500 MHz, DMSO-*d*<sub>6</sub>)

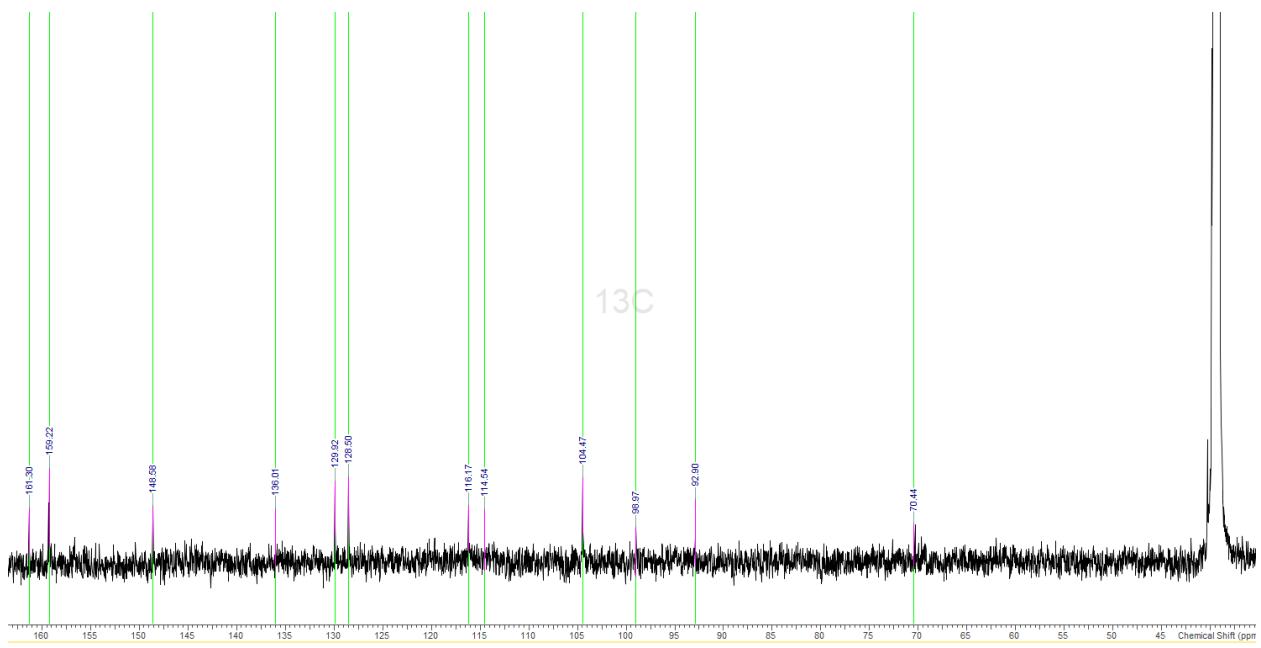


Figure S42. Meridianin H (**12**)  $^{13}\text{C}$  NMR spectrum (125 MHz,  $\text{DMSO}-d_6$ )

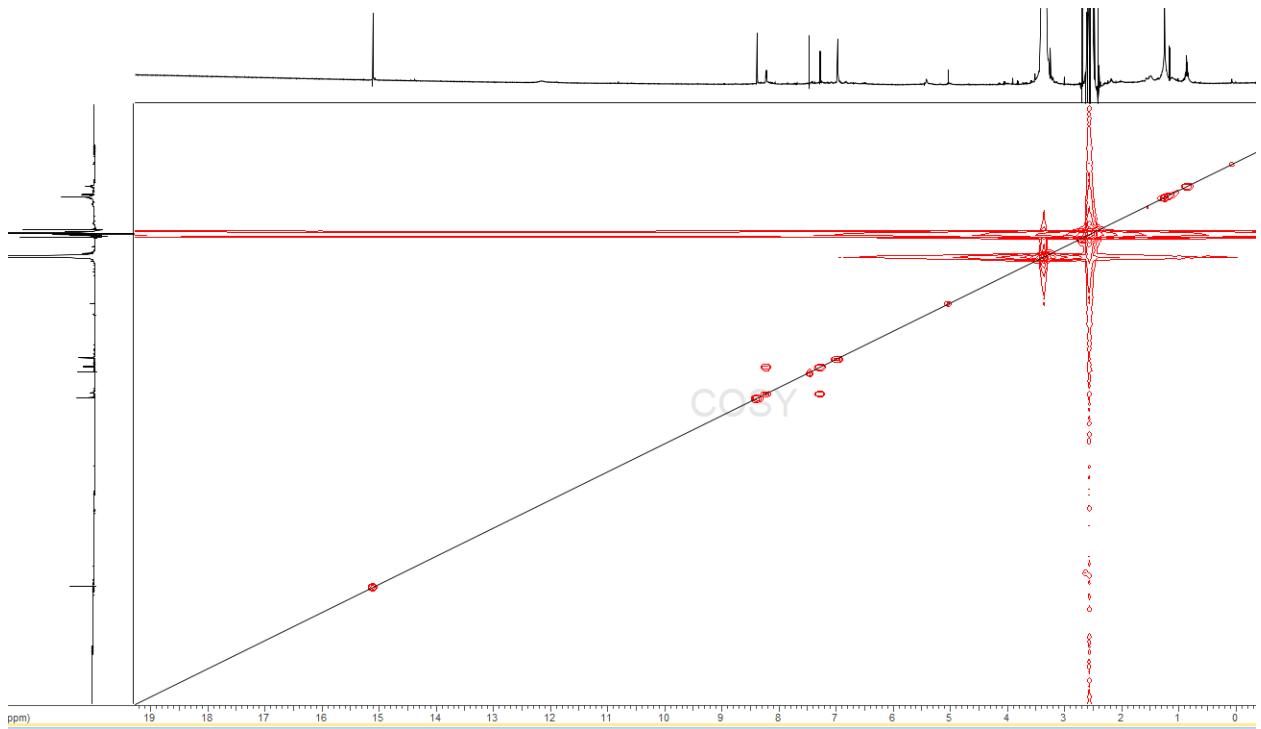
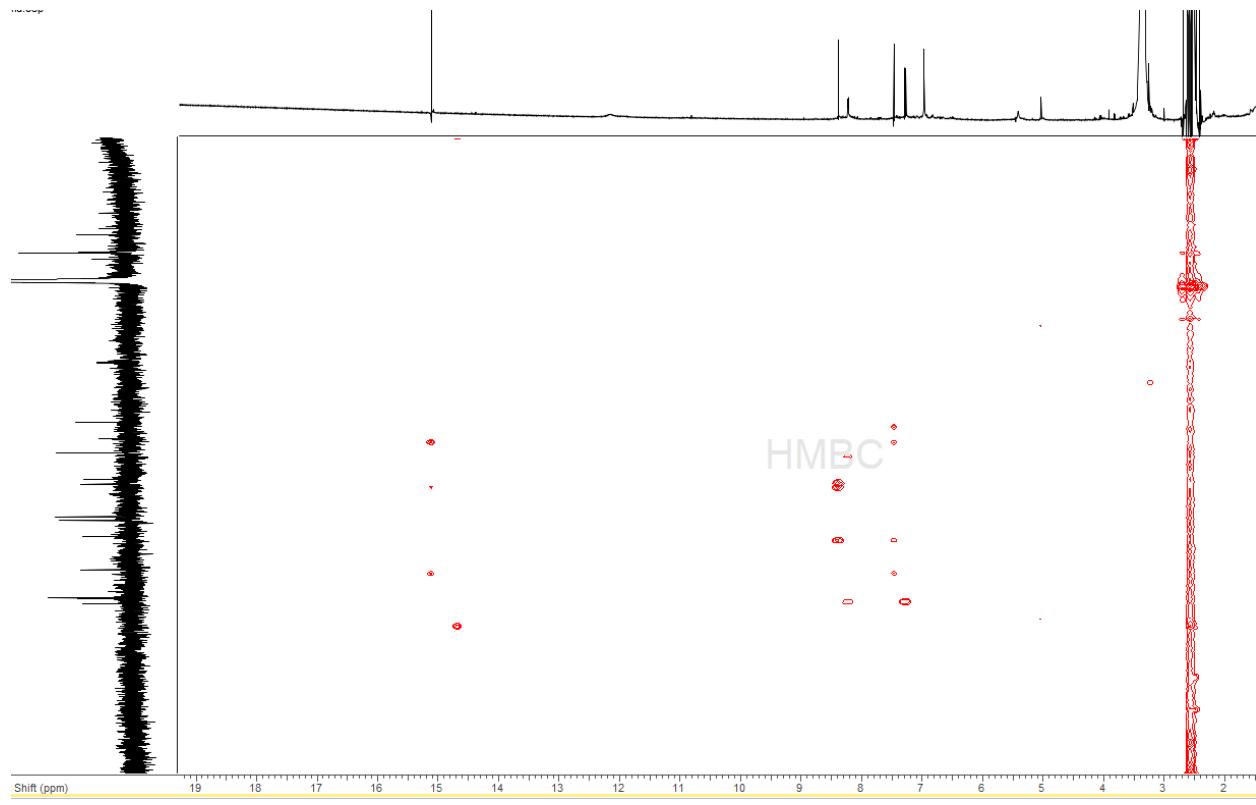
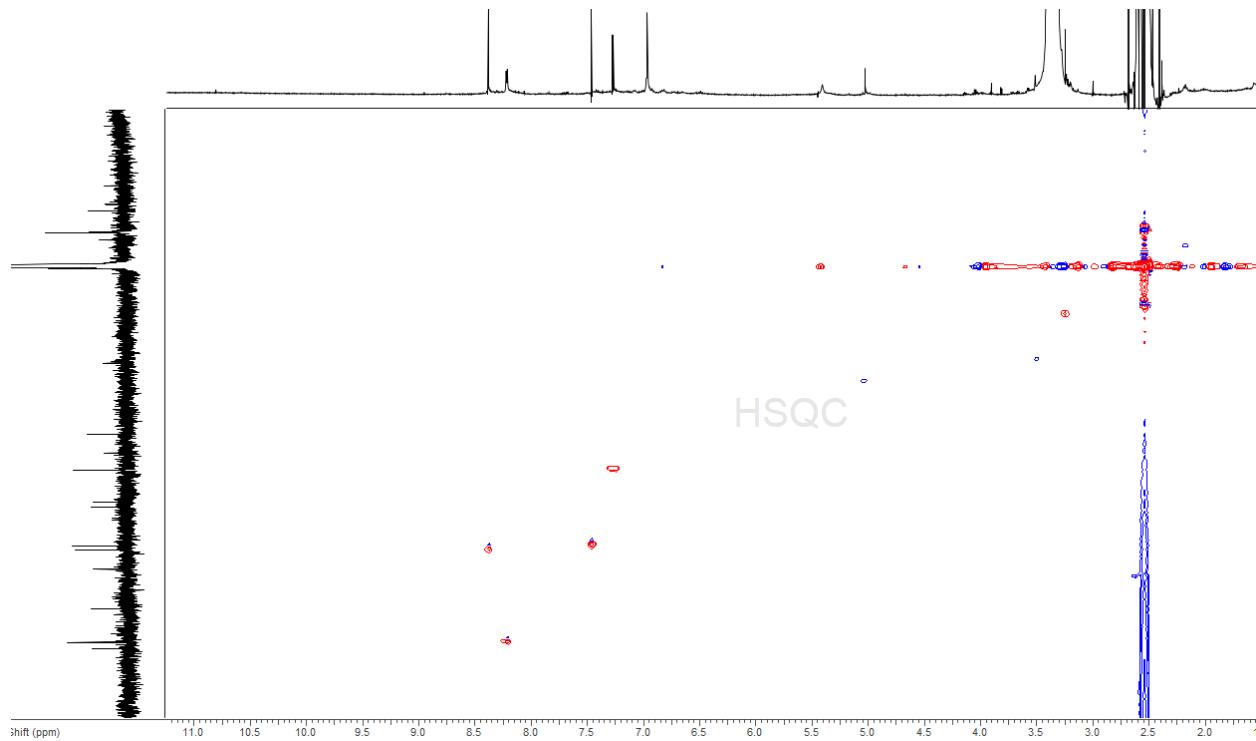


Figure S43. Meridianin H (**12**) COSY NMR spectrum (500 MHz,  $\text{DMSO}-d_6$ )



**Figure S44.** Meridianin H (**12**) HMBC NMR spectrum (500 MHz, DMSO-*d*<sub>6</sub>)



**Figure S45.** Meridianin H (**12**) HSQC NMR spectrum (500 MHz, DMSO-*d*<sub>6</sub>)

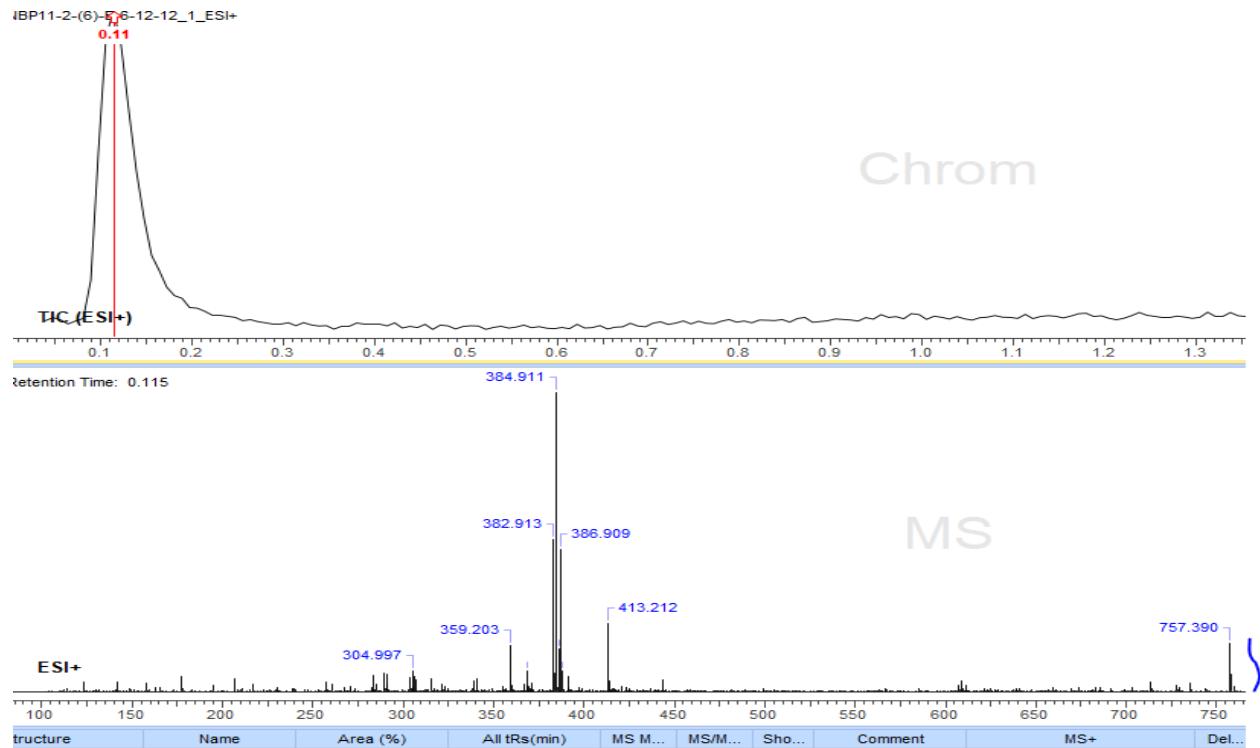


Figure S46. Meridianin H (12) HRESIMS(+)

**Table S1. Crystal data and structure refinement for australindolone B (2).**

Identification code	NBP13_9G_8
Empirical formula	C <sub>14</sub> H <sub>17</sub> BrN <sub>4</sub> O <sub>4</sub> S
Moiety formula	C <sub>12</sub> H <sub>9</sub> BrN <sub>4</sub> O <sub>2</sub> , (CH <sub>3</sub> ) <sub>2</sub> SO, H <sub>2</sub> O
Formula weight	417.29
Temperature/K	100
Crystal system	orthorhombic
Space group	Pbcn
a/Å	27.1828(8)
b/Å	8.5340(3)
c/Å	14.9595(5)
α/°	90
β/°	90
γ/°	90
Volume/Å <sup>3</sup>	3470.3(2)
Z	8
ρ <sub>calc</sub> g/cm <sup>3</sup>	1.597
μ/mm <sup>-1</sup>	4.599
F(000)	1696.0
Radiation	CuKα ( $\lambda = 1.54178$ )
2θ range for data collection/°	6.504 to 138.618
Index ranges	-32 ≤ h ≤ 32, -10 ≤ k ≤ 10, -17 ≤ l ≤ 17
Reflections collected	40465
Independent reflections	3224 [R <sub>int</sub> = 0.0554, R <sub>sigma</sub> = 0.0248]
Data/restraints/parameters	3224/552/349
Goodness-of-fit on F <sup>2</sup>	1.171

Final R indexes [ $I \geq 2\sigma(I)$ ]       $R_1 = 0.0724$ ,  $wR_2 = 0.1603$

Final R indexes [all data]       $R_1 = 0.0787$ ,  $wR_2 = 0.1631$

Largest diff. peak/hole / e Å<sup>-3</sup>      0.52/-0.71